NURSERY FERTILIZATION AND UNDERSTORY COMPETITION AFFECT SEEDLING GROWTH IN OIL SANDS RECLAIMED SOILS IN ALBERTA

by

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ABSTRACT

Revegetation in the oil sands mining areas in northern Alberta has often been difficult because of high mortality and slow growth of planted seedlings on reclaimed soils. Growth of the planted seedlings is limited by low nutrient and water availability in the soil on reclaimed sites. Management practices such as nursery fertilization to produce quality planting stock, field weed control and fertilization could help improve the early establishment of planted seedlings and reclamation success.

The main goal of this study was to examine the potential application of the nutrient loading technique (nursery fertilization) to enhance revegetation success in oil sands reclaimed soils. We hypothesized that i) nursery nutrient loading can build up nutrient reserves in the seedlings that would improve seedlings early growth after outplanting by increasing nutrient retranslocation within the seedlings and ii) weed control can improve seedlings growth by increasing nutrient availability in the soil in highly competitive reclamation sites. I studied the growth and biomass allocation, nitrogen (N) retranslocation within the seedling components and N uptake from the soil in nutrient-loaded (by exponential fertilization) seedlings planted on oil sands reclaimed sites in field experiments for two years. Nitrogen retranslocation and N uptake from the soil were traced using ^{15}N labeling and soil N availability was determined using foliar $\delta^{13}C$.The application of exponential fertilization to enhance revegetation on reclaimed mine sites is relatively new. The use of ^{15}N isotope to discriminate various N pools in new tissues and ^{13}C isotope to determine soil N availability and N uptake by nutrient loaded seedlings is the novel aspect of this study.

Seedlings were produced by loading of nutrients (essential macro and micro nutrients) with balanced fertilizers in nursery. In the first experiment, growth and N retranslocation in trembling aspen (*Populus tremuloides* Michx.) were examined on two reclaimed sites, one with peat mineral soil mix (PMM) and the other with LFH mineral soil mix (LFH) as cover materials. In the second and third experiments, growth and N retranslocation in jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* [Moench] Voss) seedlings were examined on the PMM site. The results showed that at the end of nursery production N reserve was increased in aspen and jack pine but not in white spruce seedlings by exponential fertilization. The growth, N retranslocation into new tissues from old tissues and N uptake from the soil in aspen seedlings in the field were increased by exponential fertilization and weed control. Weed competition for aspen growth was more prominent on the LFH site than on the PMM site. In the second experiment, exponential fertilization increased the growth, N retranslocation and N uptake from the soil in jack pine seedlings while weed control decreased N retranslocation and increased N uptake with no significant change in seedling growth. In both aspen and jack pine, exponentially fertilized seedlings allocated greater biomass into metabolically active tissues such as current – year leaf and stem than conventionally fertilized ones. In white spruce, exponential fertilization increased relative height growth but not absolute height, RCD and seedling component biomass. Weed control increased soil N availability, N uptake from the soil, N retranslocation and growth of white spruce seedlings in second year after outplanting.

We concluded that the effects of vegetation management practices on the growth and N retranslocation in planted seedlings varies with the species and cover soil type on reclaimed sites. Nursery nutrient loading has the potential to help enhance revegetation success by improving growth of aspen on both sites and jack pine seedlings on the PMM site, while weed control improves the growth of aspen seedlings planted on the LFH site and white spruce planted on the PMM site. Exponential fertilization was not able to build up nutrient reserves in white spruce seedlings at nursery production suggesting that further research on loading of nutrients into this species is needed.

PREFACE

This thesis is an original work conducted by Prem Pokharel. A version of chapter two of this thesis has been published as Pokharel, P. and Chang, S.X., "Exponential fertilization promotes seedling growth by increasing nitrogen retranslocation in trembling aspen planted for oil sands reclamation", *Forest Ecology and Management*, 372, 35-43. A version of chapter three of this thesis "Nursery nutrient loading increased growth and nitrogen retranslocation in jack pine (*Pinus banksiana* Lamb.) seedlings planted on an oil sands reclaimed soil" has been submitted to *Biology and Fertility of Soils* and is currently under review. A version of chapter four of this thesis "Weed control but not exponential fertilization increases nitrogen retranslocation and growth of white spruce seedlings on a reclaimed oil sands soil" has been submitted to *New Forests* and is currently under review. I was responsible for conducting the experiment, data collection and analysis as well as manuscript writing. Kwak, J.H.and Jamro, G.M. assisted with data collection from field and editing of manuscript. Choi W.J. assisted with editing of manuscript. Chang, S.X. was the supervisory author, contributed to the design of the research and edited the manuscripts.

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LIST OF SYMBOLS AND ACRONYMS

BI: biomass increment

C: carbon

$CaCl_2$: calcium chloride

CF: conventional fertilization

C:N ratio: carbon to nitrogen ratio

EC: electrical conductivity

EF: exponetial fertilization

HCl: hydrochloric acid

HI: height increment

KCl: potassium chloride

K_2SO_4: potassium sulphate

LFH: identifiable litter (L), fragmented and partially decomposed litter (F), and highly decomposed humus (H) material

m:v: mass to volume ratio

N: nitrogen

NaCl: sodium cholride

Na_2CO_3: sodium carbonate

NaOH: sodium hydroxide

NDFP: nitrogen derived from plnat

NDFS: nitrogen derived from soil

NH_4^+: ammonium

NO_3^-: nitrate

OB: overburden

OM: organic matter

PMM: peat mineral soil mix

RCD: root collar diameter

RCDI: root collar diameter increament

RWR: root weight ratio

SRL: specific root length

TC: total carbon

TN: total nitrogen

TOC: total organic carbon

TON: total organic nitrogen

TS: tailings sand

VSPs: vegetative storage proteins

CHAPTER 1 GENERAL INTRODUCTION

1. Research background

1.1 Oil sands reserve and mining disturbance in northern Alberta

Alberta's oil sands including the Peace River, Cold Lake and Athabasca River regions has the third largest oil reserves in the world, after Venezuela and Saudi Arabia. As of 2014, Alberta's oil sands proven reserves were 1.66 trillion barrels under 142,200 km^2 of land. Total oil sands production (mined and in situ) reached about 2.3 million barrels per day in 2014 (Government of Alberta, 2016). Oil sands are extracted either *in situ* (reserves below 100 meters) or via surface mining (up to 100 meters). Reserves shallow enough to surface mine (up to 100 meters) are found only within the Athabasca oil sands area. The Surface Mineable Area (SMA) is about 4,800 km^2 that lies in the boreal forest of Alberta (Fung and Macyk, 2000; Hrudey et al., 2010; Natural Regions Committee 2006). The oil sands mining activities in the northern Alberta are rapidly increasing and the area cleared or disturbed for oil sands mining is about 813 km^2 as of December 31, 2013 (Government of Alberta, 2014). The oil sands companies are required to work within a regulatory framework to conserve and reclaim their disturbed land under the Land Surface Conservation and Reclamation Act 1973 and the Environmental Protection and Enhancement Act 1992 (Government of Alberta, 1999). The main goal of reclamation of disturbed land is to convert it into the land of equivalent capability of predisturbance by building up of soil using mine residues that is capable of supporting stable plant and microbial communities (Rowland et al., 2009). The big challenge of oil sands reclamation in northern Alberta is its massive scale of disturbance and the reclamation complexities associated with soil forming, landscaping, cold climate and re-establishment of vegetation and microbial communities.

1.2 Land reclamation in oil sands region

During surface mining, all vegetation, peat and soil cover is removed and an overburden layers of 15 to 50 m is stripped away to extract bitumen from the oil sands. The mining activities leave the landscape with very large pits up to 100 m deep and several kilometers wide, dumps of mine tailings, and piles of overburden materials (Rowland et al., 2009). The final substrates for the reclamation are overburden, tailings sand (byproducts of mining activities), peat from the original stripped sites and neighboring undisturbed sites (Oil Sands Vegetation Reclamation Committee, 1998). After oil sands extraction, these pits are filled with overburden or tailings sands. Government of Alberta legislation requires that the disturbed land should be returned to the equivalent (or better) land capability to predisturbance. Equivalent land capability means the ability of the land to support various land used after conservation and reclamation is similar to the ability that existed prior to any activity being conducted on the land, but that the individual land used will not necessarily be identical (CEMA, 2006). Predisturbance land-use capabilities include timber harvesting, wildlife habitat, watershed functions, and wetlands, sources of traditional foods and medicinal plants, and recreation (Oil Sands Vegetation Reclamation Committee, 1998). In oil sands reclamation, the capability of the reclaimed land is determined by the Land Capability Classification System developed by the Cumulative Environment Management Association (CEMA, 2006). The disturbed land used for oil sands extraction is in boreal forest with uplands and wetlands. Provincial guidelines for oil sands reclamation require reestablishing commercial forest within the natural range of ecotypes found in the Central Mixed Wood Sub-Region of the Boreal Forest (Government of Alberta, 1999).

1.2.1 Reconstruction of functioning soil

In Alberta, reclamation to upland forest after oil sands extraction involves placement of overburden substrates or tailings sand in the mined pit to build the reconstructed soil profile, then reclamation cover materials are placed to help create soil environment capable of supporting stable plant and microbial communities (Table 1-1). Over the last 40 years, the most common prescription for reclamation materials is to use peat mineral soil mix (PMM) and LFH mineral soil mix (LFH). PMM is widely available in northern Alberta (Fenske, 2012) while LFH has limited availability to be used in large scale (CEMA, 2010). These two capping materials have different biological and chemical properties (Mackenzie and Quideau, 2012). PMM has low wind and water erosion potential and high available water holding capacity that can be important to improve water use efficiency early in reclamation (Fenske, 2012). PMM also has high content of recalcitrant carbon (C) resulting into high carbon to nitrogen (N) ratio (C:N) (Hemstock et al., 2010) and high pH (Jamro et al., 2015). But this material lacks plant propagules and seed bank materials which are conducive to re-establishment of vegetation in upland reclamation sites

(Mackenzie, 2006; Naeth et al., 2013). LFH mineral soil mix is considered good reclamation cover material as it contains plant propagules and seed banks, however its availability for large scale reclamation in northern Alberta is limited (Mackenzie, 2006; Mackenzie and Naeth, 2010). This material also has high organic matter, macro and micronutrients and has a C:N ratio and pH comparable to that of undisturbed upland forests and it is a good source of vascular and non vascular plant vegetative propagules and seeds for reclamation and can assist in natural recovery of vegetation after reclamation (Mackenzie, 2006; Mackenzie, 2012; Brown and Naeth, 2014).

1.2.2 Vegetation re-establishment on the reconstructed soil

One of the key components of reclamation is revegetation which is to provide barren land with a vegetation cover (Powter, 2002). Establishment of vegetation that replaces the original ground cover after disturbance can be a direct anthropogenic activity with seeding and transplanting or occur naturally. Natural re-establishment of plants, sometimes called natural recovery, excludes any anthropogenic involvement and deliberate introduction of propagules and seeds (Hrudey et al., 2010). The guidelines for vegetation re-establishment on the reclaimed landscapes are to determine the principal environmental gradients determining the natural vegetation composition (Oil sands Vegetation Reclamation Committee, 1998). An understanding of vegetation composition and environmental gradient helps in selecting understory and overstory species to be planted in the reclaimed sites (Table 1-2). Based on the moisture and nutrient gradients and other soil properties including pH, soil structure and consistency, electrical conductivity, and sodium absorption ratio, potential prescriptions have been developed for the plants best suited for different reclaimed sites. Current revegetation in the Athabasca Oil Sands Region is based on ecosite/site type or end land use approaches (CEMA, 2010). The ecosite/site type approach is based on selecting revegetation treatments by site conditions as determined by the Land Capability Classification System soil water and nutrient regimes and target ecosite/site type. The end land use approach is determined by target end land use and treatment methods to achieve stand objectives. Since vegetation develops along certain successional pathways, different plant species are recommended to represent different successional stages to ensure that, as succession proceeds, the later successional species would be available (Oil sands Vegetation Reclamation Committee, 1998).

Revegetation in oil sands reclaimed sites in northern Alberta historically focused on erosion control and the areas were seeded with grasses and legumes. Plant community development relied on planting desired species and encroachment of others from surrounding areas (CEMA, 2010). Revegetation techniques included seeding annual nurse crops of barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.) which are poor competitors and are readily invaded by local flora within the first few years (Oil Sands Revegetation Reclamation Committee, 1998). They also help in increasing soil organic matter. The root biomass of these grasses are also expected to provide erosion control, contribute to soil organic matter and help to develop favorable site conditions for trees, shrubs and forbs to establish from soil cover seed sources. The successful establishment of grass vegetation achieved the goal of erosion control and increasing organic matter but the growth of grasses also inhibits tree establishment by increasing competition for water, nutrients and light (Maundrell and Hawkins, 2004).

Native tree species of boreal forests including trembling aspen (*Populus tremuloides* Michx.), white spruce (*Picea glauca* (Moench) Voss), Jack pine (*Pinus banksian*a Lamb.), balsam poplar (*Populus balsamifera* L.) and black spruce (*Picea mariana* (P.Mill.) B.S.P. are planted on the reclaimed sites according to the environmental gradients and guidelines for reclamation to upland forest vegetation in the Athabasca oil sands region (CEMA, 2010). Nursery grown seedlings of these species are planted usually in the next available planting season of seeding of nurse crops. The most common shrub species planted on the reclaimed sites include pioneer species that help in N fixation such as green alder (*Alnus crispa* (Ait.) Pursh) and Canadian buffaloberry (*Shepherdia canadensis* (L.) Nutt.). Other shrub species planted on reclaimed sites are wild roses (*Rosa acicularis* Lindl. and *Rosa woodsia* Lindl.), pin cherry (*Prunus pensylvanica* L.f.), choke cherry (*Prunus virginiana* L.), and saskatoonberry (*Amelanchier alnifolla* (Nutt.) Nutt.) (Fung and Macyk, 2000).

1.2.3 Nutrient limitation and growth of planted seedlings on reclaimed soils

The establishment of a closed tree canopy which facilitates soil redevelopment through production and decomposition of litter (Klinka et al., 1990) is required to suppress the establishment of weedy forb and competitive grass species (Maundrell and Hawkins, 2004). Rapid establishment of the tree canopy is thus important to accelerate reclamation process. However, the survival and growth of planted tree seedlings is low in reclaimed soil because of various adverse characteristics inherent of reclaimed soils such as low nutrient availability, high pH, salinity, compaction and drought (Pinno et al., 2012; Lilles et al., 2009). Exposure to extreme temperatures is likely to be another issue for the survival and growth of planted seedlings.

Although foreign materials such as peat and LFH as sources of organic matter and nutrients are placed on reclamation substrates during the reclamation process, available nutrients are still low in reclaimed soils (Sloan and Jacobs, 2013; Duan et al., 2015; Kwak et al., 2015a). Stripping of top soil before oil sands extraction, storage of that stripped soil for long periods, mechanical mixing of the soil and handling during replacement, all lead to the loss of soil organic C and nutrients from the soil (Rowland et al., 2009). Nitrogen is the main limiting nutrient for plant growth in boreal forest soils in the oil sands region (Cheng et al., 2013), particularly in newly reconstructed ecosystems in the oil sands, where native N inputs are often lacking (Bradshaw, 1997). Net N mineralization and nitrification are typically slow in the boreal forest and peatland soils (Hemstock et al., 2010) which are the major sources of cover materials for oil sands reclamation. Low nitrification rates in boreal soils have been linked to low pH (Ste-Marie and Paré, 1999), anaerobic conditions, low substrate (ammonium) availability, and low nitrifying bacterial populations (Davidson et al., 1992). In reclaimed soils, low nitrogen mineralization is often caused by small microbial population size, reduced enzymatic activities and low microbial diversity (Kwak et al., 2015b). Since N availability in reclaimed soil is controlled by net nitrogen nitrification (Hemstock et al., 2010), low rate of net nitrification result in low available nitrogen in the soil.

1.3 Literature review

1.3.1 Nursery nutrient loading of seedlings to improve revegetation

The high cost of producing and planting bare root stocks in forest restoration led to the production of smaller containerized seedlings in nursery that are much more cost effective than bare root stock. But the nursery grown containerized seedlings pose a challenge of early establishment after outplanting in highly competitive sites because of their smaller size and low nutrient content (MacDonald and Weetman, 1993; Timmer and Aidelbaun, 1997). Loading of nutrients into the containerized seedlings during nursery production can help build nutrient reserves in the seedlings which could be beneficial for the survival and early growth after outplanting in nutrient deficient soil and weed prone sites (Timmer and Aidelbaun, 1997). Nutrient loading was defined by Timmer (1996) as fertilization in excess of the demand for current growth during nursery production to induce luxury uptake of nutrients characterized by increased internal concentration in plants without significantly changing the plant's total dry mass. Nutrient loading is a practice to increase the growth competitiveness of containerized seedlings in the field by building up of nutrient reserve in the seedlings at the end of nursery production. Exponential fertilization is one of the techniques of nutrient loading which is based on a principle of steady-state nutrition. Steady-state nutrition implies that the seedlings should grow with a constant internal nutrient concentration to be free from nutrient stress (Ingestad and Lund, 1986). Steady state nutrition in nursery grown seedlings can be achieved by providing nutrients at exponential rather than conventional (constant) rates (Timmer, 1997; Imo and Timmer, 1992; Timmer and Aidelbaum, 1996).

1.3.2 Conventional and exponential fertilization regime

In conventional regimes, fertilizer is either applied as a single fertilizer dose (rare practice) or at a rate based on constant feed model (more common practice). In this model, the quantity of nutrients to be added at each application time (N_t) is calculated as,

$$N_t = N_T (t^{-1})$$

Where N_T is the total nutrient amount assumed to be sufficient and t is the number of nutrients applications over the growing season.

In pure exponential fertilization model, nutrients are applied at exponentially increasing addition rates following the procedures described by Ingestad and Lund (1986), using the exponential function:

$$N_T = N_S (e^{rt}-1)$$

Where N_T is the total amount of nutrients required for the seedling over the entire growing season, N_S is the initial level of nutrients in the seedling at the start of fertilization and r is the relative addition rate required to increase N_S to a final level of $N_S + N_T$. After r is determined for the number of fertilizer applications (t) planned, Nt (the amount of nutrients to be added on a specific day) can be calculated from the equation:

$$N_t = N_S (e^{rt}-1) - N_{t-1}$$

Where N_{t-1} is the cumulative amount of nutrients added up to the last fertilization.

The exponential fertilization model has been further modified to ensure steady- state nutrient conditions by raising addition rates slightly at the start of the fertilization period so as to compensate for incomplete root exploitation during early growth. Nutrient compensation N_C can be calculated as,

$$N_C = N_0 (e^{rt}-1)$$

Where, N_0 is the final amount of N added over the compensation period. N_C corresponds to the difference between the last and the penultimate fertilizer application calculated from exponential fertilization model. This amount is subtracted from the final application to avoid possible bud damage before dormancy onset due to excess fertilization.

1.3.3 Growth and nitrogen retranslocation in nutrient-loaded seedlings

After outplanting containerized nursery-grown seedlings often suffer from nutrient deficiency because of slow regeneration and extension of the root system. Nutrient loading can help increase internal nutrient reserves of the seedlings; the reserves can then be reutilized in new tissue production through retranslocation that can reduce the risk of nutrient deficiency in the seedlings after outplanting (Chapin et al., 1990). The improved growth and nutrient responses in nutrient loaded seedlings following exponential fertilization regime have been confirmed in many coniferous and broadleaved species in green house and field experiments. Some of these experiments have used same amounts of nutrients in exponential fertilization as used in conventional fertilization regime but the trend is to use greater amount of nutrients in exponential than in conventional ones (Malik and Timmer, 1998; Imo and Timmer, 2001).

Exponentially fertilized seedlings of black spruce (*Picea mariana* [Mill.] B.S.P.) demonstrated more steady-state nutrition during the active growth than those fertilized conventionally in nursery production (Malik and Timmer, 1998). These seedlings also had improved growth, N uptake and retranslocation after transplanting on a high competition forest sites (Imo and Timmer, 2001). In Lutz spruce (*Picea* X *Lutzii* Littl.) seedlings planted for forest regeneration, nutrient loading increased new needle mass by 122 and 152% in low and high fertile sites, respectively and N retranslocation in the seedling was increased by nutrient loading (Jonsdottir et al., 2013). Nutrient loaded seedlings of Chinese-fir (*Cunninghamia lanceolate* (Lamb) Hook) planted in soils with different fertility class increased seedling biomass by 29% and N uptake by 44% (on average) than non-loaded seedlings. N retranslocation was also increased with higher nutrient reserves built up by nutrient loading during nursery culture (Xu and Timmer, 1999). In an experiment of optimizing nutrient loading through exponential fertilization in Chinese pine (*Pinus tabulaeformis* Carr.), nutrient-loaded seedlings with 80 mg N seedling^{-1} was found to maximize biomass accumulation and N content in nursery production and exhibited greatest survival rates among 10, 20, 40, 80, 100 and 120 mg N seedling^{-1} supply rates (Wang et al., 2015). Exponentially fertilized seedlings of western white pine (*Pinus munticola* Dougl.ex D. Don) receiving 45% less nutrients than that of conventionally fertilized ones yielded similar morphological characteristics and nutrient status at the end of greenhouse culture but increased root volume and ectomycorrhizal colonization in two years growth in the field (Dumroese et al., 2005). The effect of exponential fertilization in growth and nutrient status in hard wood species is not always significant. For instance, in holm oak (*Quercus ilex* L.) seedlings, exponential fertilization did not increase nutrient reserve at the end of nursery production (Oliet et al., 2009) but post-transplant root growth relative to shoot was greater in exponentially fertilized seedlings. However, exponential fertilization with an application rate higher than the conventional fertilization increased the internal nutrient reserve during nursery production and also increased new tissue biomass and N retranslocation after transplantation in trembling aspen (*Populus tremuloides* Michx.) (Hu et al., 2015).

Application of nutrient loading into seedlings has been shown to outperform conventional seedling stock on forest regeneration and restoration sites (Malik and Timmer, 1996; Timmer, 1996; Oliet et al., 2013). But the use of nutrient-loaded seedlings to mitigate the competitive effects of undersory vegetation on reclamation site is not well studied. In a mine land reclamation site in south-western Indiana, Salifu et al. (2009) used nutrient loading technique for forest restoration and found that nutrient-loaded seedlings of northern red oak (*Quercus rubra* L.) and white oak (*Q. alba* L.) increased survival, nutrient contents and dry mass compared to conventional seedlings. In oil sands reclaimed soils, nutrient-loaded trembling aspen seedlings outperformed conventional seedlings in terms of survival, growth and competitive ability (Schott et al., 2016).

Internal cycling of nutrients particularly N has been found to be a major source of nutrients used for the growth in both evergreen and deciduous plants (Millard, 1996). But there is a debate in the literature as to whether remobilization is governed by nutrient supply (Turner, 1977) or by the sink-strength of the current year's growth (Nambiar and Fife, 1987; Hikosaka, 2005; Fife et al., 2008). Munson et al. (1995) however, supports a hypothesis that the controlling factors of nutrient remobilization depends on the ecological niche of the species and its inherent physiological response to the increase in the availability of environmental resources. In seedlings soon after transplanting, new tissue growth occurs most likely at the expense of the stored nutrients (Proe and Millard, 1994; Miller, 1984) because of poor root-to-soil contact that limits the uptake of water and nutrients from the soil (Burdette, 1990). The amount of nutrient retranslocation from old to new tissues vary with time, quantity of nutrients reserve in the plant, soil nutrient availability, and sink demand (Nambiar and Fife, 1991; Millard, 1996; Salifu and Timmer, 2003a). For instance, 51 to 60% of N in old tissues in Chinese-fir (Xu and Timmer, 1999), 67% in black spruce (Malik and Timmer, 1998) and 72% in radiata pine (Nambiar and Bowen, 1986) was found to be retranslocated into new tissues.

2. Thesis Structure

The main goal of this study is to assess the effects of the nutrient loading technique to increase the survival and growth of planted seedlings in highly competitive and nutrient limited sites. The current work is a continuation of a project examining the potential application of nutrient loading to help improve revegetation success following reclamation in oil sands areas. In the first part of this project, Hu (2012) identified an optimum fertilizer rates to be used in nutrient loading of trembling aspen, jack pine and seedlings through modified exponential fertilization in a greenhouse experiment. The current study is mainly focused on assessment of growth and nutrient status of these seedlings in the field. Three separate field experiments were conducted to test the growth, N retranslocation and competitive ability of exponentially and conventionally fertilized seedlings of these three species. The reasons for maintaining three separate experiments for three species in this study are: 1) the rate of fertilizer used (for both conventional and exponential regimes) is different for three species, 2) experiment sites are different (in terms of cover materials used during reclamation and weed competition), 3) some of the parameters (such as growth and nutrients in the seedlings) analyzed are different, 4) the time of leaf and needle collection to determine N retranslocation is different for different species. The first experiment was conducted for trembling aspen on two reclamation sites (PMM and LFH) while second and third experiments were conducted for jack pine and white spruce seedlings on PMM site. A number of questions were formed to help direct the research in both nursery production and field experiments. These were:

- Do exponentially fertilized seedlings of trembling aspen, jack pine and white spruce improve survival and growth after outplanting to oil sands reclaimed soils?
- Does exponential fertilization increase competitive ability of the seedlings to grow in high competition reclaimed sites?
- How does exponential fertilization affect nutrient and biomass allocation in seedlings growing in the field?
- Does exponential fertilization in the nursery affect N retranslocation and N uptake from the soil when seedlings are growing in the field?
- Are growth and N retranslcoacation of seedlings different between PMM and LFH sites?
- What is the contribution of N retranslocation to meet the total N demand of new tissue growth in these seedlings? How does it vary with time?
- Do weed control and its interaction with exponential fertilization affect soil N availability, N uptake from the soil, N retranslocation and the growth of the planted seedlings?

The thesis consists of five chapters. Chapter one provides background information of the study and chapter five is about the summary of results, conclusions and further recommendation. Chapter two constitutes the manuscript entitled "Exponential fertilization promotes seedling growth by increasing nitrogen retranslocation in trembling aspen planted for oil sands reclamation", a version of this manuscript is published in *Forest Ecology and Management.* Chapter three constitutes the manuscript entitled "Nursery nutrient loading increased growth and nitrogen retranslcoation in jack pine (*Pinus banksiana* Lamb.) seedlings planted on an oil sands reclaimed soil", a version of this manuscript is under review in *Biology and Fertility of Soils*. Chapter four constitutes the manuscript entitled "Weed control but not exponential fertilization increases nitrogen retranslocation and growth of white spruce seedlings on a reclaimed oil sands soil”, which has been submitted to *New Forests*.

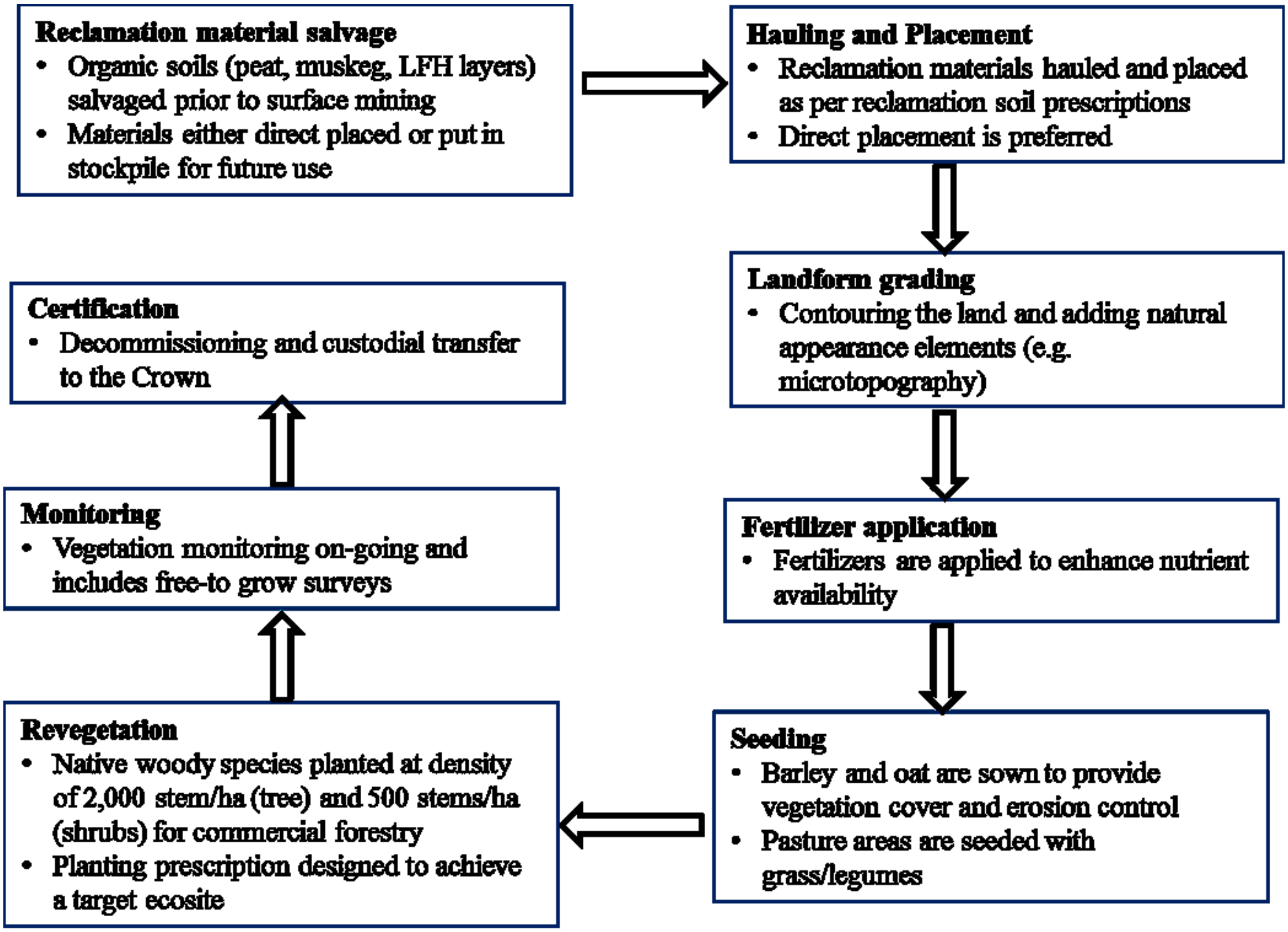

Figure 1-1 Reclamation process in oil sands region (modified from BGC Engineering Inc., 2010).

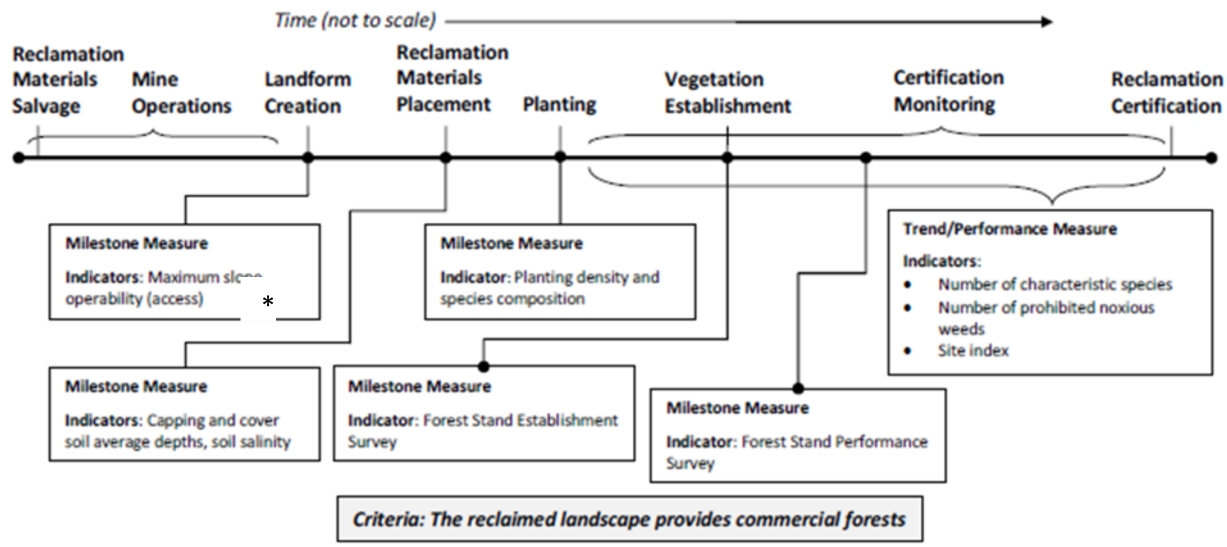

Figure 1-2 Land eclamation associated with the establishment of commercial forest stands (AESRD, 2013).

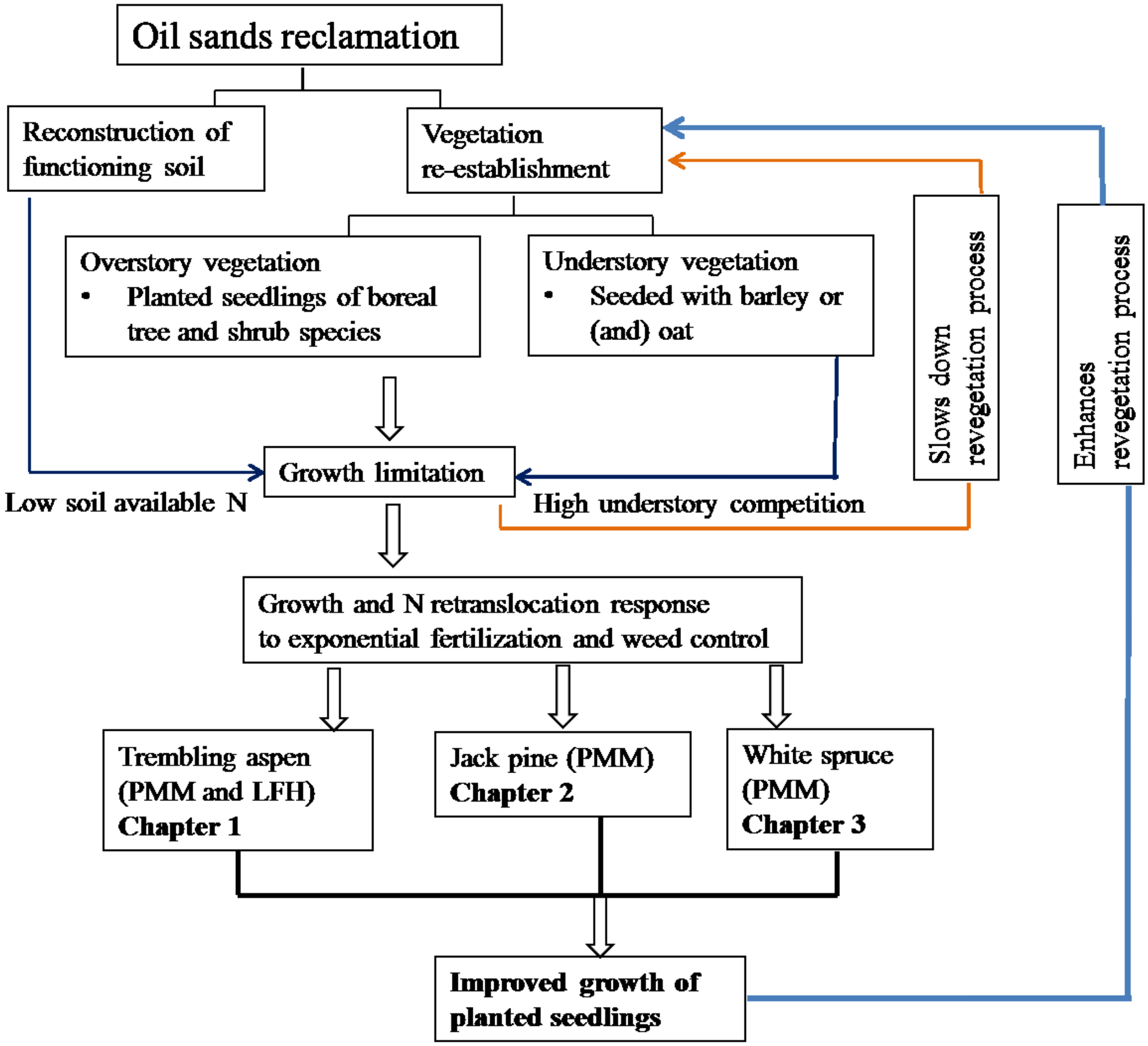

Figure 1-2 Flow chart for this study.

CHAPTER 2 EXPONENTIAL FERTILIZATION PROMOTES SEEDLING GROWTH BY INCREASING NITROGEN RETRANSLOCATION IN TREMBLING ASPEN PLANTED FOR OIL SANDS RECLAMATION

1. Introduction

Oil sands extraction by open-pit mining in northern Alberta has disturbed a large area of the boreal region (about 813 km^2 as of December, 2013; Government of Alberta, 2014), reclamation of these disturbed lands to equivalent capability similar to that existed pre-disturbance has been a priority for the industry and the provincial government. During open pit mining all vegetation, surface soil, overburden (nutrient poor layers of rock and soil below the surface soil) and oil sands are removed, leaving large pits (several kilometers wide and up to 100 m deep. A soil is reconstructed during reclamation of these pits after mineral extraction is complete using tailings sand (a by-product of bitumen extraction) or overburden substrates and placing cover soils on these substrates. Experience with revegetation in such reconstructed soils in reclamation is still rather limited (Macdonald et al., 2015). Success of revegetation in a reclaimed area largely depends on the establishment of understory vegetation and planted tree seedlings (Kimaro and Salifu, 2011) as most of the disturbed area was originally boreal forest. Reclamation success is often hindered by unsuitable growth conditions, such as low fertility and soil salinity (Duan et al., 2015), pH (Zhang et al., 2013; Jamro et al., 2014), and soil compaction (Jamro et al., 2015) that is associated with reconstructed soils in oil sands reclamation. Although the use of peat mineral soil mix (PMM) or LFH mineral soil mix (LFH) as capping materials placed over tailings sand or overburden substrates has been a common practice for improving soil quality (MacKenzie and Naeth, 2007; Rowland et al., 2009; Kwak et al., 2015), early establishment of planted seedlings can still be problematic. In addition to unfavorable physical/chemical site conditions, cover crops that are established on these reclaimed soils to control soil erosion and natural vegetation, which returns following disturbance, increase the competition with planted seedlings for resources such as light, water, and nutrients (Franklin et al., 2012). Field fertilization could alleviate nutrient deficiencies (Rowland et al., 2009; Duan et al., 2015), but may also encourage the growth of the understory vegetation, which subsequently intensifies competition for nutrients and other resources (Chang et al., 1996).

An alternative approach to improving revegetation success in heavily disturbed and competitive sites involves the use of quality seedling stock (Landhaeusser et al., 2012). The nutrient loading technique based on exponential fertilization (Timmer and Aidelbaum, 1996) is designed to increase the nutrient reserve in seedlings by inducing luxury nutrient consumption in nursery seedling production (Malik and Timmer, 1998). Nutrient-loaded seedlings have access to a larger nutrient reserve built up due to higher amounts of nutrients applied and can promote early seedling establishment in the field, resulting in superior survival, growth, and competitiveness over conventionally produced seedlings (Timmer and Munson, 1991; Timmer and Aidelbaum, 1996). Exponential nutrient fertilization has been used successfully to improve early establishment of seedlings of coniferous species such as black spruce (*Picea mariana* [Mill.] BSP) (Malik and Timmer, 1996; Imo and Timmer, 2001; Salifu and Timmer, 2003b), China-fir (*Cunninghamia lanceolata* [Lamb.] Hook.) (Xu and Timmer, 1999), and Lutz spruce (*Picea* × *lutzii* Little) (Jonsdottir et al., 2013). Outplanting experience with nutrient loaded deciduous species is limited (Birge et al., 2006), and non-existent with trembling aspen (*Populus tremuloides* Michx.), which is a widely distributed species across boreal North America, and is commonly used for land reclamation and commercial forestry (Pinno et al., 2012). Moreover, successful application of nutrient loading in aspen seedlings has not been tested in oil sands reclamation. However, nursery or greenhouse pot studies have been conducted on aspen (Hu, 2012 ; Schott et al., 2013; Hu et al., 2015). In the early stage after outplanting, seedlings can meet part of the sink demand for N that is required for new growth by retranslocating nutrients from old tissues; this has been demonstrated especially with evergreen species (Nambiar and Fife, 1991; Salifu and Timmer, 2003a). Quantification of N retranslocation from old to new tissues, which is referred to as N derived from plants (NDFP), versus N derived (or taken up) from the soil (NDFS) in newly transplanted seedlings are very important (Choi et al., 2005) for understanding which N pool is critical for promoting seedling growth (Salifu et al., 2009a). In this study, we determined the amount of N that was retranslocated to meet the sink demand in aspen seedlings after transplantation by using the ^{15}N tracing technique, which can more precisely discriminate the various N sources (such as NDFP) in the soil-plant system (Nommik, 1990; Proe and Millard 1994; Barraclough, 1995). Although several studies have quantified NDFP, which accounts for 40 to 100% of annual N demand in newly transplanted conifer seedlings (Nambiar and Fife, 1991; Millard, 1996; Salifu and Timmer, 2003a), the relative contributions made by NDFP and NDFS to new tissue growth in nutrient-loaded hardwood seedlings are poorly understood (Salifu et al., 2009a).

To demonstrate the potential use of nutrient-loaded seedlings of aspen for improving revegetation success in heavily disturbed sites, we studied the growth of aspen and N retranslocation within its tissues on newly reclaimed sites with both PMM and LFH as cover soils. The field experiment was conducted in the Oil Sands region of northern Alberta. The objectives of this study were to examine the growth performance and to determine N retranslocation response of exponentially and conventionally fertilized seedlings in competition with understory vegetation on reconstructed soils. In this study we tested the following hypotheses: i) exponentially fertilized aspen seedlings would have greater growth than conventionally fertilized ones after transplantation due to the greater N storage in the exponentially fertilized seedlings, ii) there would be a greater contribution of NDFP to new tissue growth in the exponentially than in the conventionally fertilized seedlings because of the greater nutrient reserve in the former, and iii) the effect of understory vegetation competition for nutrients would be lower with the exponentially fertilized than with the conventionally fertilized seedlings, its again due to greater nutrient retranslocation to new growth in the nutrient-loaded seedlings as weed competition will not directly affect nutrient retranslocation. Although all nutrients were loaded in the seedlings during nursery production, we focused on N in this study because N is usually the growth limiting nutrient in reclaimed soils in the Oil Sands (Rowland et al., 2009; Duan et al., 2015). Its internal cycling is similar to other mobile macronutrients (Nambiar and Fife, 1991). Thus, an improved understanding on the N nutrition has implications for the management of other mobile macronutrients.

2. Materials and Methods

2.1 Seedling nursery production and fertilization regimes

Containerized stocks of conventionally (C) and exponentially fertilized (E) trembling aspen seedlings were produced in 2013 at the Smoky Lake Forest Nursery (Smoky Lake, AB, Canada). In the conventional fertilization regime, an amount of total balanced fertilizer (equivalent of a seasonal total of 120 mg N per seedling) was applied for twelve weeks at a constant rate delivered weekly; this rate was based on the rate being used in the Smoky Lake

Nursery that had been found to be the optimum application rate (Hu, 2012). In the exponential fertilization regime, an amount of total balanced fertilizer (equivalent of a seasonal total of 240 mg N per seedling which is the optimum exponential fertilization rate identified in the nursery part of the study (Hu, 2012)) was delivered on a schedule based on the modified exponential model (Imo and Timmer, 1992; Birge et al., 2006). Details of the nursery culture of seedlings can be found in Hu (2012) and Hu et al. (2015). To determine NDFP and NDFS in seedlings after transplantation, labeled urea (60 atom%) was applied in solution to the seedlings in week eight during nursery seedling production. After twelve weeks of nursery culture, the seedlings were tested for hardiness, harvested, and kept in a cold storage (at -2 °C) until they could be taken to the field for transplanting.

Before transplanting, the nutritional status, size, and biomass of seedlings were determined to assess whether exponentially fertilized seedlings were successfully loaded with the required nutrients. To do that, five seedling samples of each of exponential and conventional fertilization from each of three storage boxes representing different replications used in nursery production were collected from cold storage just before transplantation. Individual seedlings were assessed for component biomass and composited together per box (each box represents a replication). For nutrient concentration analysis, five seedlings per box were composited together before analysis. Nutrient concentrations in the seedlings were analyzed following the procedure described below. Exponentially fertilized seedlings were confirmed to have a size and component biomass similar to that of conventionally produced seedlings but different N status prior to transplanting (Table 2-1).

2.2 Study site

The study was conducted at a reclamation site (56° 58' N and 111° 19' W) near Fort McMurray, in northern Alberta. The area was characterized by long cold winters and short warm summers, with mean annual temperature of 1 °C and precipitation of 418.6 mm (316.3 mm as rain and 102.3 mm as snow) between 1981 and 2010 (Environment Canada, 2015). The study area was located in the central mixedwood natural sub-region of the boreal forest and was dominated by trembling aspen, white spruce (*Picea glauca* [Moench] Voss) and balsam poplar (*Populus balsamifera* L.) in pure as well as in mixedwood stands (Fung and Macyk, 2000) before the forest was clearcut for oil sands mining. The natural ecosystems in the region have xeric to sub-hydric Brunisols, Luvisols and Gleysols in or Organic soils in hydric conditions (Oil Sands Vegetation Reclamation Committee, 1998). Details of basic properties of natural soils in surrounding boreal forest can be found in Jung and Chang (2012).

The research was conducted on two oil sands sites that had been reclaimed in 2008. These sites have 50 cm thick PMM and LFH as capping materials (cover soil) placed on overburden substrate and are hereafter referred as PMM and LFH sites, respectively. In 2009, both sites were seeded with barley (*Hordeum vulgare* L.) to stabilize soil and control erosion. The PMM and LFH sites have contrasting soil properties and competing understory vegetation. The PMM has higher carbon-to-nitrogen (C:N) ratio and available nitrogen (N) but lower bulk density than the LFH (Table 2-2). Percent cover by understory vegetation on the LFH was almost two times greater than that on the PMM site; aboveground biomass and N content of understory vegetation were also greater on the LFH site (Table 2-3).

2.3 Experimental design

The study used a completely randomized block design with two levels of nursery fertilization and two levels of competition control in a factorial experiment replicated in four blocks. The two fertilization regimes were exponential (E) and conventional fertilization (C) in nursery seedling production, and the two weed competition treatments were weed intact (control, +W) and weed removed (treated, -W). The weed removal treatment was maintained by periodically (every three weeks in active growing season) manual removal of weeds by cutting aboveground parts and pulling out the roots. Treatments were randomly allocated to the plots (5 × 5 m) within each block. Adjacent blocks were separated by a 2-m buffer and adjacent plots by a 1-m buffer. A total of 400 seedlings (2 fertilization regimes × 2 weed treatments × 25 seedlings per plot × 4 replications) were planted at 1 × 1 m spacing at each site. Nursery-produced seedlings were transplanted onto the study sites on 11 June 2014.

2.4 Seedling growth and understory vegetation assessment

The initial size of each seedling, including shoot height (37.2 ± 2.6 cm) and root collar diameter (RCD) (3.34 ± 0.4 mm), was measured at the time of transplanting to the field; final seedling size was measured at the end of the active growing season in August of both 2014 and 2015. Shoot height, RCD, and survival rate were determined for all 25 seedlings in each plot.

Shoot height was measured using a meter stick (to 1 mm accuracy) and RCD was measured using a vernier caliper (to 0.05 mm accuracy) in two directions perpendicular to one another at ground level, and the values were averaged to represent the RCD of each seedling. The growth in height and RCD were calculated as the difference between final seedling size at the end of each growing season and the initial size at transplanting. For understory vegetation assessment, two 50 × 50 cm quadrats were randomly established in each plot in August 2014 and 2015. Percent cover was determined in each quadrat for all vegetation and each life form of plant species, including shrubs, forbs, grasses, mosses and lichens.

2.5 Soil and plant sampling

Soil samples were collected from two depths (0-10 and 10-30 cm) in June 2014 using an auger from five randomly selected locations in each plot and mixed to obtain a composite sample for each layer. Bulk density in the 0-10 and 10-30 cm layers was determined by sampling of soil from three randomly selected locations in each plot using a steel corer with a volume of 98.12 cm3. Two (in the first growing season) and five seedlings (in the second growing season) were randomly selected from each plot and destructively harvested at the end of the active growing season in 2014 and 2015. The lower number of seedlings that were sampled at the end of the first growing season was to prevent too many seedlings from being lost to harvesting. The experiment was terminated at the end of the second growing season and, thus, a higher number of seedlings were sampled for analysis to improve their representativeness. At each sampling, seedlings were cut at the ground level, followed by excavation of the root system. After the plant material was brought back to the laboratory, the aboveground parts were separated into old stems, new stems, and leaves (being a deciduous species, the leaves were produced in the current growing season). The roots of each seedling were washed on to a sieve (0.5 mm mesh), most roots were recovered by carefully removing soil and peat from the root system, and as many broken roots that were retained on the sieve were manually collected as possible. Aboveground understory vegetation was collected in August 2014 using a 50 × 50 cm quadrate twice in each plot for the determination of its N content. Seedling component and understory vegetation samples were washed twice with distilled water and dried for 72 hours at 70 °C for determining dry mass and for chemical analyses.

2.6 Soil and plant analyses

Soil samples were air-dried at room temperature and ground to pass a 2 mm sieve; a subsample was further ground to powder using a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro, NJ) for analysis of total C and total N concentrations. Total C and total N were analyzed using the dry combustion method using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy). Soil pH was measured in a 0.01 mol L^{-1} $CaCl_2$ solution at a 1:2 (m:v) ratio using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) and electrical conductivity (EC) was measured after soil:water extraction at 1:1 (m:v) ratio using an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA) (Kalra and Maynard, 1991). For available N (NH_4^+-N and NO_3^--N) analysis, soil samples were extracted with 2 mol L^{-1} KCl and the extract was analyzed colorimetrically by the indophenol blue method for NH_4^+-N (Keeney and Nelson, 1982) and by the vanadium oxidation method for NO_3^--N (Miranda et al., 2001).

Component dry mass was measured for each seedling while plant component samples were composited per plot for nutrient analysis. Prior to chemical analysis, plant samples were ground to powder using the ball mill described above. The N concentration and ^{15}N abundance in the plant samples were analyzed using a stable isotope ratio mass spectrometer (Thermo Delta Plus XP IRMS, Waltham, MA, USA) linked to a CN analyzer (Costech 4010, Valencia, CA, USA) at the Stable Isotope Facility of the University of Wyoming (Laramie, WY, USA).

2.7 Data analyses

New tissues of seedlings were assumed to acquire N from two sources: N remobilized from old plant tissues or NDFP and N taken up from the soil or NDFS. The NDFP in new tissues represents N retranslocation and was calculated using the following mass balance method (Hauck and Bremner, 1976):

$$NDFP = TN\ [(A-B/(C-B)]$$

where TN is the total N content in new tissues calculated as the concentration (mgg^{-1}) multiplied by the biomass of the plant tissue, A is atom% ^{15}N in new tissues, B is the atom% ^{15}N in a natural standard (0.3663), and C is the atom% ^{15}N of the plant at transplantation which was calculated as the weighted mean of atom% ^{15}N of stems and atom% ^{15}N of roots.

NDFS was calculated as:

NDFS = TN-NDFP

Labeled N content per plant was calculated by summing the ^{15}N in different tissues.

Growth and nutritional data of seedlings before transplantation were analyzed by a one-way analysis of variance (ANOVA) and those from the field experiment by a two-way ANOVA to examine the statistical significance of a variable using the Proc MIXED model (SAS 9.2, SAS Institute, Cary NC). The linear model for the two-way ANOVA was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \tau_k + \varepsilon_{ijk}$$

where Y_{ijk} is a dependent variable, μ is the overall mean, α_i and β_j are the fixed effects of the ith and jth fertilization and weed removal treatments, respectively, $(\alpha\beta)_{ij}$ is the fixed effect of interaction of the two treatments, τ_k is the random effect of the kth block and ε_{ijk} is the random error within the experiment; estimation of the variance-covariance matrix involved the restricted maximum likelihood (REML) method.Correlation analyses were performed for height increment (HI), RCD increment (RCDI), biomass increment (BI) and nutrient concentrations in seedling components, understory vegetation biomass and N uptake, and soil properties. Prior to ANOVA and correlation analyses, data were tested for normality of distribution (Shapiro-Wilk test) in residuals using Proc UNIVARIATE. All data were confirmed to have normal distribution except NDFS on the PMM site. A log-transformation (base 10) was applied for the NDFS data to meet the assumption of normality. An $\alpha = 0.05$ was used to determine statistical significance in all analyses, and means of the variables were separated using the least significant difference (LSD) test.

3. Results

3.1 Seedling size and biomass

There were no significant interactions between nursery fertilization and weed control on seedling size or dry matter yield at both sites for both years (Table 2-4). At the end of the transplantation experiment (after the 2nd growing season), exponential fertilization significantly increased seedling size on both PMM and LFH sites (Tables 2-4 and 2-5). Exponential fertilization increased shoot height on the PMM site in 2014 and 2015 (P = 0.016 and 0.028, respectively) and on the LFH site in 2014 (P = 0.022). RCD increased on the PMM site in 2014 (P = 0.002) and on the LFH site in 2015 (P = 0.038). Weed removal increased seedling RCD in both years (P = 0.038 and 0.009, respectively) on the LFH site but not on the PMM site (Table 2-5).

Both treatments significantly increased component and total biomass of seedlings at the end of first and second growing seasons (Tables 2-4 and 2-5). Exponential fertilization increased new stem, old stem, root, and total biomass in the first growing season and leaf, new stem and total biomass in the second growing season (all $P < 0.05$) on the PMM site. On the LFH site, the increase (by 34%) was in new stem biomass (P = 0.03) during the first growing season and leaf (by 50%, P = 0.02), new stem (by 69%, P = 0.01), old stem (by 41%, $P < 0.01$) and total biomass (by 36%, $P < 0.01$) in the second growing season. Weed removal did not change component biomass, except 79% for new stem growth (P = 0.01) in the second growing season on the PMM site. Weed-removed plots yielded 92% ($P < 0.01$) more leaf biomass in the first growing season, and 48% (P = 0.03) and 77% (P = 0.01) more leaf and new stem biomass, respectively, in the second growing season compared to the weed-intact plots on the LFH site.

3.2 Biomass allocation to seedling components

Nursery fertilization and weed removal in the field significantly affected seedling component biomass partitioning (expressed as % of the total biomass of the seedling) at the end of the second growing season (Figure 2-1 and Table 2-6). Percent root biomass allocation was not affected by nursery fertilization or weed competition treatments on the PMM site (Figure 2-1a), but was greater in the C+W than in the other treatment combinations on the LFH site (Figure 2-1b). New stem biomass allocation was significantly increased by both exponential fertilization ($P < 0.01$) and weed removal ($P < 0.01$) on the PMM site and by weed removal (P = 0.03) on the LFH site. Although not significant, exponential fertilization also tended to increase percent leaf biomass allocation on both the PMM and LFH sites (P = 0.21 and 0.05, respectively).

3.3 Nitrogen concentration and total N of seedlings

The N concentrations of seedlings at the end of the first growing season did not differ among the treatments, except leaves on the PMM site, but were lower than those prior to outplanting in both exponentially and conventionally fertilized seedlings (Table 2-7). Exponential fertilization increased leaf N concentration by 23% ($P < 0.01$) at the end of first growing season on the PMM site. Although not significant ($P = 0.24$), weed removal treatment increased leaf N concentration by 14% on the LFH site.

In contrast to N concentration, total N in the seedlings was significantly increased by exponential fertilization on both the PMM and LFH sites (Table 2-7) and by weed removal only on the LFH site. Exponential fertilization increased N content in leaves by 37% ($P = 0.01$) and N content in the seedlings by 36% ($P = 0.01$), compared to conventional fertilization on the PMM site and 5% ($P = 0.74$) and 23% ($P = 0.04$), respectively, on the LFH site. Weed control treatment increased N content in leaves by 55% ($P < 0.01$) and N content in seedlings by 33% ($P = 0.01$) on the LFH site.

3.4 Relationship between seedling growth and N status, weed competition, and soil properties

RCD increment (RCDI) was correlated with foliar N concentration on the PMM site ($P = 0.04$). On the LFH site, RCDI was positively correlated with soil NH_4^+-N (P = 0.04), soil total C ($P = 0.01$), soil total N ($P = 0.03$), foliar N concentration ($P < 0.01$) and content (P < 0.01), and shoot:root ratio (S:R) ($P = 0.03$), and negatively correlated with understory aboveground biomass ($P = 0.03$) and understory aboveground N content ($P = 0.04$). The biomass increment (BI) was positively correlated with soil NH_4^+-N ($P = 0.02$), foliar N concentration ($P < 0.01$), and foliar N content ($P = 0.02$), new stems ($P = 0.04$) and old stems ($P < 0.01$) (Table 2-8) on the LFH site.

3.5 Nitrogen retranslocation and soil N uptake by seedlings

In comparison to N remobilized from old tissues, N uptake from the soil by aspen seedlings was much lower in the first growing season. On average, 80 (on the PMM site) and 73% (on the LFH site) of total N in new tissues was derived from old tissues during the first growing season (Figure 2-2). Although percent N retranslocation was not different among the treatments, total NDFP was significantly increased by both exponential fertilization and weed removal without a significant interaction among the treatments. In new tissues, NDFP in the exponentially fertilized seedling was 25 ($P = 0.02$) and 37% ($P < 0.01$) greater than that in conventionally fertilized seedlings on the PMM and LFH sites, respectively, while weed competition reduced NDFP by 37% ($P < 0.01$) on the LFH site. In contrast to NDFP, NDFS was increased only by weed removal on the LFH site but was not affected by the fertilization treatment on both sites. The increase in NDFS by weed removal was 158% ($P = 0.01$) on the LFH site.

4. Discussion

4.1. Exponential fertilization and weed removal increased aspen seedling growth

Exponential fertilization in nursery seedling production stimulated the early growth of aspen seedlings after they were outplanted, supporting our first hypothesis; this indicates that exponential fertilization can help facilitate revegetation in reclaimed soils with competing vegetation, as has been reported for other site conditions (Oliet et al., 2009; Salifu et al., 2009b). In a greenhouse experiment, Hu et al. (2015) also reported increased growth of aspen seedlings as a result of nutrient loading prior to planting in reclamation soil materials. The improved growth could be attributed to the nutrient reserve that was built up in exponentially fertilized seedlings (Timmer and Aidelbaum, 1996; Hu et al., 2015), as exponential fertilization in the nursery increased N content in stems and roots and built up the nutrient reserve in the seedlings prior to transplantation.

Although total biomass was significantly greater in exponentially fertilized seedlings, percent biomass allocation to roots was greater in conventionally fertilized seedlings two growing seasons after transplantation, suggesting nutrient stress in the reclaimed soil, as nutrient stress favors root growth that would increase the seedling's ability to exploit a larger volume of soil (Nambiar and Sands, 1993); plants investment of more photosynthate in root growth is an adaptation for increasing N uptake from the soil (Ingestad and Ågren, 1988). In contrast, greater allocation of biomass and N to metabolically active tissues such as new leaves and new stems in the exponential fertilization and weed removal treatment combination promotes carbon fixation by increasing the surface area and chlorophyll concentration of leaves (Gulmon and Chu, 1981), which in turn supports greater seedling growth. Competition from the understory vegetation was greater on the LFH than on the PMM site as reflected by the relatively higher percent cover and aboveground biomass of and N uptake by the understory vegetation (Table 2-2). Under field conditions, aspen seedlings exhibited a greater response to nursery fertilization than to weed management on the PMM site, while they responded to both treatments on the LFH site. Weed removal increased shoot height and seedling biomass on the LFH site by reducing the competition for resources such as light, moisture and nutrients. In our study, understory vegetation was cut short around seedlings in weed-intact plots to eliminate light competition; soil moisture content was not significantly different between weed intact and weed-removed plots (data not shown). Increased nutrient availability that was incurred by removing weeds likely contributed to greater seedling growth. Similar results regarding nutrient competition were observed by Imo and Timmer (2001) in black spruce in highly competitive forest sites. The negative effect of weed competition on seedling growth is well known and has been reported in many previous studies for coniferous species such as Monterey pine (*Pinus radiata* D. Don) (Woods et al., 1992), western red cedar (*Thuja plicata* Donn ex D. Don) (Chang et al., 1996), and white spruce (Matsushima and Chang, 2007; Man et al., 2008).

The effect of the fertilization treatment on N concentration disappeared at the end of the first growing season, despite the significant difference in N concentration in seedling components before outplanting. The greater reduction in N concentration in the exponentially fertilized seedlings in the field was caused by the dilution effect that was incurred by higher biomass production in these seedlings, suggesting short-term benefits of nursery nutrient loading in terms of N concentration, however the benefits of nutrient loading in biomass production were maintained during field growth. The greater reduction in N concentration in exponentially fertilized seedlings is consistent with the findings of Jonsdottir et al. (2013) for Lutz spruce and Heiskanen et al. (2009) for Norway spruce (*Picea abies* [L.] Karsten). Although foliar N concentration in exponentially fertilized seedlings was higher than that in conventionally fertilized seedlings on the PMM site, foliar N concentrations in all treatments were below the optimal level (30-40 mg g^{-1}) for aspen growth (Hansen, 1994); therefore, supplementary fertilization may be required in reclaimed soils (Sloan and Jacobs, 2013), particularly when the soil nutrient availability cannot meet the demand of the outplanted trees as the trees grow older where nutrient limitation can mean the complete failure of the revegetation or reclamation effort (Duan et al., 2015). The significantly positive relationship between seedling growth and available soil N implies that the reduced availability of soil N in recently reclaimed and highly competitive LFH sites was limiting seedling growth. Furthermore, the significant correlation between growth and foliar N in seedlings on the LFH site further suggests the relevance of weed removal treatment in increasing foliar N and growth of aspen seedlings on the reclaimed soil.

4.2. Exponential fertilization and weed removal increased NDFP and NDFS

The high rate of N retranslocation (80 and 73% of total sink demand of new tissues on the PMM and LFH sites, respectively) in aspen is similar to that reported for black walnut (*Juglans nigra* L.) (Salifu et al., 2009a), in which NDFP accounted for 68 to 83% of the total N demand. However, only 32% of total N demand by new tissue was met by NDFP in northern red oak (*Quercus alba* L.) (Salifu et al., 2008), indicating that hardwood species vary widely in terms of the contribution of NDFP in meeting the total sink demand. The greater contribution of NDFP compared to NDFS in aspen seedlings suggests the importance of internal nutrient cycling to meet sink demand of new growth during early establishment after outplanting. The difference in percent NDFP contribution between aspen and oak can perhaps be attributed to the main functional types of woody plants such as broadleaved deciduous and evergreens (Villar-Salvador et al., 2015).

The increased NDFP in exponentially fertilized seedlings, owing to the greater N reserve in the seedlings prior to outplanting, supports our second hypothesis. In our study, exponential fertilization increased nutrient reserves without increasing seedling size in nursery production (Hu, 2015), suggesting that N retranslocation during field growth may be determined by the nutrient reserve but not by seedling size. The difference in net N retranslocation between exponentially and conventionally fertilized seedlings was likely determined by the amount of readily available N in the seedlings. Exponentially fertilized seedlings may have larger pools of less diluted, more readily available and mobile nutrients in the form of non-functional amino acids and proteins as a result of luxury consumption of nutrients (Malik and Timmer, 1998; McAlister and Timmer, 1998). Low N uptake from the soil compared to N remobilized in the early stage of seedling establishment after outplanting may be caused by restricted root placement and poor root-soil contact, which limits water and nutrient uptake from the soil (Burdett, 1990), or by salinity (Frota and Tucker, 1978) that contributes to reduced permeability of roots, and which subsequently decreases nutrient uptake in a reclaimed soil. The increase in N uptake from the soil on the LFH site by weed removal was linked with increased soil-available N with reduced understory competition (Woods et al., 1992).

The greater NDFP during field growth of exponentially fertilized seedlings was linked to the increase in plant biomass production, especially in new tissues. The availability of N for remobilization was more important than N uptake from the soil for the formation of new tissues in aspen seedlings during early establishment. Growth had been reported to be sustained by internal reserves in the plant rather than by N uptake from the soil in early years of outplanting for several plant species (Millard and Neilsen, 1989; Munson et al., 1995; Millard, 1996). The fact that the seedlings having higher growth were associated with greater N retranslocation in exponentially fertilized seedlings suggests that current growth was strongly affected by N retranslocation in the planted aspen seedlings.

Nitrogen retranslocation in seedlings growing in less stressed environments (i.e., weed removal plots on the LFH site) was greater than those growing in the stressed environment (i.e., weed-intact plots). Greater N uptake from the soil was linked to the higher growth of seedlings in weed-removed plots. Presumably, stronger growth sinks caused by greater new tissue formation in aspen seedlings on weed-removed plots increased the need for internal N redistribution (Nambiar and Fife, 1991; Proe and Millard, 1994), supporting the N retranslocation driven by sink demand hypothesis. This result, however, contradicts the findings for black spruce of Malik and Timmer (1998), who observed higher nutrient retranslocation in weed-intact than in weed-removed plots. They pointed out that nutrient conservation could be one of the reasons for having less N retranslocation in black spruce seedlings growing in weed-removed plots.

5. Conclusions

We conclude that N remobilization is important in meeting the nutrient requirement in the new growth in the early establishment of aspen seedlings in reclaimed soils, given that three-quarters of the demand for N by the new growth was derived from old tissues after the seedlings were transplanted. Since N retranslocation was dependent on nutrient pool size in the seedlings prior to transplantation, this study also demonstrated the significance of building up of nutrient reserves in the seedlings during nursery culture for the nutrition of seedling after transplantation. Yet the nutrient pool size before transplanting had little influence on N uptake from the soil. Exponential fertilization promoted the growth of seedlings after transplant in the field by increasing N retranslocation, demonstrating its potential use in helping to improve revegetation success in heavily disturbed and competitive sites. The effect of understory removal was site-specific, with significant improvement in growth, N retranslocation and uptake from the soil on the LFH site (a site with more severe weed competition), but the effect was less significant on the PMM site. Our study only tested the field growth performance of exponentially fertilized seedlings of aspen for two growing seasons; the utility of the nutrient loading technique needs to be operationally tested for the longer term and applied in future land reclamation practices once operationally tested to improve the success of land reclamation using trembling aspen in the boreal region. Our two-year data did not show any nutrient-loading effect on seedling survival rate and as such nutrient-loading may not be effective in improving all aspects of seedling performance such as survival and frost resistance after outplanting.

Table 2-1 Biomass and N status (mean ± SE, n=3) of seedlings before transplanting.

Seedling component	Dry mass (g seedling^{-1})		N concentration (mg g^{-1})		N content (mg seedling^{-1})		^{15}N content (mg seedling^{-1})		Atom% ^{15}N	
	C	E	C	E	C	E	C	E	C	E
Stem	1.54 (0.17)	1.82 (0.15)	21.64 b (1.09)	27.20 a (0.33)	33.02 b (2.43)	49.66 a (4.09)	0.49 (0.02)	0.61 (0.04)	1.53 (0.14)	1.25 (0.06)
Root	1.55 (0.28)	1.72 (0.08)	24.83 b (1.22)	34.15 a (0.58)	38.26 b (5.40)	58.80 a (3.77)	0.50 (0.08)	0.64 (0.02)	1.30 (0.03)	1.09 (0.05)
Stem+root	3.09 (0.56)	3.54 (0.06)	23.03 b (0.26)	30.60 a (0.51)	71.28 b (7.70)	108.47a (0.31)	0.99 (0.10)	1.25 (0.01)	1.41 (0.06)	1.16 (0.01)

Abbreviations: C = conventional, E = exponential.
Different letters in the same row indicate significant differences between fertilization treatments for a particular parameter at $P < 0.05$.

Table 2-2 Chemical and physical properties (mean ± SE, n=16) of peat mineral soil mix (PMM) and LFH mineral soil mix (LFH) used in the study sites.

Soil	Depth (cm)	pH	EC (ds m^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Bulk density (g cm^{-3})
PMM	0-10	6.85 (0.05)	1.88 (0.08)	98.2 (8.7)	3.1 (0.4)	33.3 (1.5)	3.36 (0.34)	5.02 (0.80)	0.58 (0.07)
	10-30	7.10 (0.05)	1.86 (0.13)	109.2 (13.9)	2.7 (0.3)	41.2 (2.0)	1.78 (0.47)	2.65 (0.47)	0.45 (0.05)
LFH	0-10	6.57 (0.07)	2.02 (0.16)	80.7 (9.3)	4.9 (0.7)	17.6 (1.3)	7.35 (0.82)	8.03 (0.44)	0.77 (0.07)
	10-30	6.71 (0.06)	1.63 (0.16)	78.8 (7.5)	3.5 (0.3)	21.4 (1.7)	5.78 (0.41)	5.02 (0.73)	0.84 (0.10)

Abbreviations: EC = electrical conductivity, C:N = carbon to nitrogen ratio.

Table 2-3 Percent cover, aboveground biomass and N uptake (mean ± SE, n=4) by understory vegetation on the peat mineral soil mix (PMM) and LFH mineral soil mix (LFH) sites.

Site	Fertilization treatment	% cover	Biomass (g m^{-2})	N uptake (g m^{-2})
PMM	Conventional	37.2 (5.3)	245.1 (28.4)	3.25 (0.4)
	Exponential	41.6 (4.3)	237.8 (14.4)	2.88 (0.5)
LFH	Conventional	76.0 (6.4)	347.3 (49.3)	6.02 (0.6)
	Exponential	75.7 (1.9)	294.5 (18.3)	4.70 (0.5)

Table 2-4 ANOVA table for height, RCD (root collar diameter) and biomass of seedlings as affected by nursery fertilization and field weed control treatments over two growing seasons.

Parameters	PMM						LFH					
	Fertilization (F)		Weed (W)		F × W		F		W		F× W	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
2014												
Height	**7.73**	**0.016**	**7.04**	**0.021**	0.33	0.578	**7.00**	**0.022**	0.02	0.878	0.01	0.932
RCD	**12.42**	**0.004**	0.51	0.487	2.67	0.128	3.05	0.108	**5.50**	**0.038**	0.28	0.605
Leaf biomass	2.14	0.169	<0.01	0.963	0.52	0.484	0.43	0.524	**25.66**	**<0.001**	3.80	0.748
New stem biomass	**17.24**	**0.001**	0.15	0.705	2.71	0.125	**5.80**	**0.033**	0.87	0.368	0.31	0.589
Old stem biomass	**8.36**	**0.013**	1.21	0.292	0.33	0.577	2.88	0.115	0.29	0.600	1.27	0.282
Root biomass	**6.62**	**0.024**	1.69	0.218	0.20	0.661	3.90	0.071	3.98	0.063	0.20	0.659
Total biomass	**9.31**	**0.010**	1.00	0.337	0.17	0.683	3.69	0.078	4.19	0.063	0.68	0.425
2015												
Height	**6.21**	**0.028**	1.27	0.281	0.03	0.867	3.54	0.086	**5.94**	**0.033**	0.31	0.588
RCD	4.01	0.068	0.78	0.394	0.18	0.680	**5.53**	**0.038**	**9.96**	**0.009**	1.16	0.305
Leaf biomass	**6.67**	**0.024**	2.25	0.159	1.22	0.291	**6.16**	**0.028**	**5.75**	**0.033**	0.52	0.483
New stem biomass	**16.58**	**0.001**	**8.42**	**0.013**	1.67	0.220	**8.02**	**0.015**	**9.24**	**0.010**	0.01	0.939
Old stem biomass	4.01	0.068	0.08	0.780	0.09	0.771	**9.88**	**0.008**	0.75	0.404	1.97	0.185
Root biomass	1.27	0.282	0.74	0.405	0.13	0.724	3.55	0.083	2.33	0.152	0.55	0.472
Total biomass	**5.31**	**0.039**	1.39	0.261	0.45	0.512	**10.99**	**0.006**	4.54	0.054	0.47	0.505

Abbreviations: PMM = peat mineral soil mix, LFH = LFH mineral soil mix.
Values in bold indicate significance at $P < 0.05$.

Table 2-5 Survival, height, RCD (root collar diameter) and biomass of seedling components (mean ± SE, n=8) over two growing seasons after transplanting.

Year	Site	Treatments	Survival (%)	Height (cm)	RCD (mm)	Leaf	New stem	Old stem	Root	Total
						g seedling^{-1} of biomass				
		Fertilization								
2014	PMM	C	94.0	42.33 b	4.97 b	1.4	0.52 b	2.22 b	1.96 b	6.11 b
		E	92.7	46.94 a	5.34 a	1.82	1.06 a	3.61 a	3.13 a	9.63 a
		Weed control								
		+W	92.0	46.84 a	5.12	1.62	0.77	2.65	2.25	7.29
		-W	94.7	42.44 b	5.19	1.61	0.82	3.18	2.84	8.44
		Fertilization								
	LFH	C	93.0	41.36 b	5.03	1.39	0.56 b	3.01	2.01	6.94
		E	88.0	45.41 a	5.38	1.5	0.75 a	3.82	2.54	8.62
		Weed control								
		+W	90.0	43.26	4.97 b	0.99 b	0.61	3.28	2.01	6.89
		-W	91.0	43.5	5.44 a	1.90 a	0.69	3.54	2.55	8.68
2015	PMM	Fertilization								
		C	88.0	50.85 b	6.37	1.63 a	0.65 a	4.39	4.36	11.02 a
		E	85.5	56.88 a	6.98	2.77 b	1.48 b	5.46	4.99	14.86 b
		Weed control								
		+W	87.5	55.23	6.54	1.87	0.76 a	4.85	4.44	11.96
		-W	86.0	52.5	6.81	2.53	1.36 b	5.01	4.91	13.55
	LFH	Fertilization								
		C	82.0	49.03	6.21 b	1.95 a	1.01 a	4.74 a	4.33	12.05 a
		E	77.7	54.04	6.69 a	2.93 b	1.71 b	6.70 b	5.09	16.44 b
		Weed control								
		+W	81.5	48.29 b	6.12 b	1.97 a	0.98 a	5.5	4.4	12.86
		-W	78.5	54.79 a	6.77 a	2.91 b	1.74 b	5.94	5.03	15.62

Abbreviations: PMM = peat mineral soil mix, LFH = LFH mineral soil mix, C = conventional, E = exponential, +W = weed intact, -W = weed removed.

Different letters in the same column indicate significant differences between fertilization regimes and between weed control treatments in each year at $P < 0.05$.

Table 2-6 Effects of nursery fertilization and weed removal on percent biomass allocation to seedling components (mean ± SE, n = 4) in second growing season.

Treatments	PMM site				LFH site			
	Leaf	New stem	Old stem	Root	Leaf	New stem	Old stem	Root
C + W	14.61 (1.65)	4.33 (0.55)	42.41 (2.63)	38.63 (2.48)	12.19 (2.31)	6.29 (0.43)	38.92 (1.70)	42.59 (0.53)
C - W	15.04 (1.38)	7.09 (0.38)	38.50 (2.07)	39.36 (2.21)	18.04 (1.11)	10.62 (1.88)	39.03 (1.19)	32.30 (1.21)
E + W	16.11 (1.01)	7.56 (1.18)	39.70 (1.06)	36.08 (1.84)	16.22 (1.43)	8.93 (0.75)	44.39 (0.77)	30.45 (1.34)
E - W	19.86 (0.92)	11.76 (0.51)	34.73 (1.28)	33.62 (1.34)	18.82 (1.18)	11.84 (1.58)	36.96 (1.53)	32.37 (1.71)
ANOVA (*P* > F)								
Fertilization (F)	0.053	**<0.001**	0.159	0.101	0.212	0.224	0.297	**0.022**
Weed (W)	0.812	**<0.001**	0.062	0.717	**0.039**	**0.033**	**0.036**	0.093
F*W	0.283	0.404	0.813	0.507	0.392	0.646	**0.032**	**0.021**

Abbreviations: PMM = peat mineral soil mix, LFH = LFH soil mineral soil mix, C + W = conventional fertilization + weed intact, C – W = conventional fertilization + weed removed, E + W = exponential fertilization + weed intact, E – W = exponential fertilization + weed removed

Values in bold indicate significance at *P* < 0.05.

Table 2-7 Effects of nursery fertilization and field weed control treatments on total N concentration (mg g^{-1}) and N content (mg $seedling^{-1}$) in seedlings (mean ± SE, n=8) post-transplanting..

Site	Treatments	New leaf		New stem		Old stem		Root		Total	
		TN	N content	TN	N content	TN	N content	TN	N content	TN	N content
PMM	Fertilizer										
	C	11.49 b	16.10 b	10.45	5.43	5.26	10.30 b	6.38	14.59 b	7.98	46.42 b
	E	14.17 a	25.74 a	8.02	8.44	5.11	19.32 a	6.36	19.18 a	7.56	72.69 a
	Weed control										
	+W	12.85	21.37	10.23	7.46	5.42	13.20	6.81	15.12	8.21	57.15
	-W	12.83	20.47	8.24	6.42	4.94	16.41	5.91	18.66	7.33	61.95
LFH	Fertilizer										
	C	13.18	19.47	9.72	5.47 b	6.48	19.24 b	8.38	18.37 b	9.81	62.73 b
	E	13.77	20.5	11.48	8.29 a	8.16	29.77 a	9.44	23.55 a	8.82	82.11 a
	Weed control										
	+W	12.48	12.41 b	10.04	5.95	7.11	23.22	7.83	16.45	8.51	58.04 b
	-W	14.47	27.55 a	11.15	7.81	7.53	25.96	9.99	25.46	10.11	86.79 a

Abbreviations: PMM = peat mineral soil mix, LFH = LFH mineral soil mix, TN = nitrogen concentration, C = conventional, E = exponential, +W = weed intact, -W = weed removed.

Different letters in the same column indicate significant differences between fertilization regimes and between weed control treatments at $P < 0.05$

Table 2-8 Pearson correlation coefficients and probability for relationship between height increment (HI), root collar diameter increment (RCDI), and biomass increment (BI) and soil properties, understory vegetation competition, and N status of seedlings.

Site	Parameter		NH_4^+-N	NO_3^--N	TC	TN	EC	UB	UN	LTN	LN	NSN	OSN	S:R
PMM	HI	r	0.24	0.25	-0.05	-0.01	0.06	0.27	0.21	0.38	0.28	0.28	0.19	0.3
		P	0.35	0.33	0.85	0.98	0.83	0.43	0.550	0.14	0.38	0.27	0.47	0.25
	RCDI	r	0.05	0.03	-0.04	-0.03	-0.05	-0.33	-0.31	**0.51**	0.17	0.26	0.39	0.24
		P	0.83	0.89	0.86	0.91	0.84	0.34	0.38	**0.04**	0.52	0.31	0.12	0.35
	BI	r	-0.12	-0.21	-0.21	-0.18	-0.42	-0.28	-0.41	**0.60**	0.38	0.21	0.40	-0.01
		P	0.67	0.42	0.42	0.49	0.10	0.42	0.24	**0.01**	0.15	0.43	0.13	0.97
LFH	HI	r	0.31	0.48	0.45	0.37	**0.59**	-0.59	-0.51	**0.59**	**0.56**	0.32	-0.08	0.18
		P	0.28	0.09	0.11	0.20	**0.03**	0.07	0.13	**0.02**	**0.02**	0.23	0.77	0.51
	RCDI	r	**0.54**	0.43	**0.65**	**0.57**	0.48	**-0.67**	**-0.63**	**0.76**	**0.69**	**0.57**	**0.52**	**0.55**
		P	**0.04**	0.12	**0.01**	**0.03**	0.08	**0.03**	**0.04**	**<0.01**	**<0.01**	**0.02**	**0.04**	**0.03**
	BI	r	**0.60**	0.47	0.48	0.46	0.12	-0.56	-0.58	**0.62**	**0.57**	**0.57**	**0.59**	0.47
		P	**0.01**	0.07	0.06	0.07	0.65	0.09	0.07	**<0.01**	**0.02**	**0.02**	**0.01**	0.06

Abbreviations: PMM = peat mineral soil mix, LFH = LFH mineral soil mix, TC = total carbon, TN = total nitrogen, EC = electrical conductivity, UB = understory vegetation biomass, UN = understory vegetation N uptake, LTN = leaf N concentration, LN = leaf total N content, NSN = New stem total N content, OSN = Old stem total N content, and S:R = shoot to root biomass ratio.
Values in bold indicate significance at $P < 0.05$.

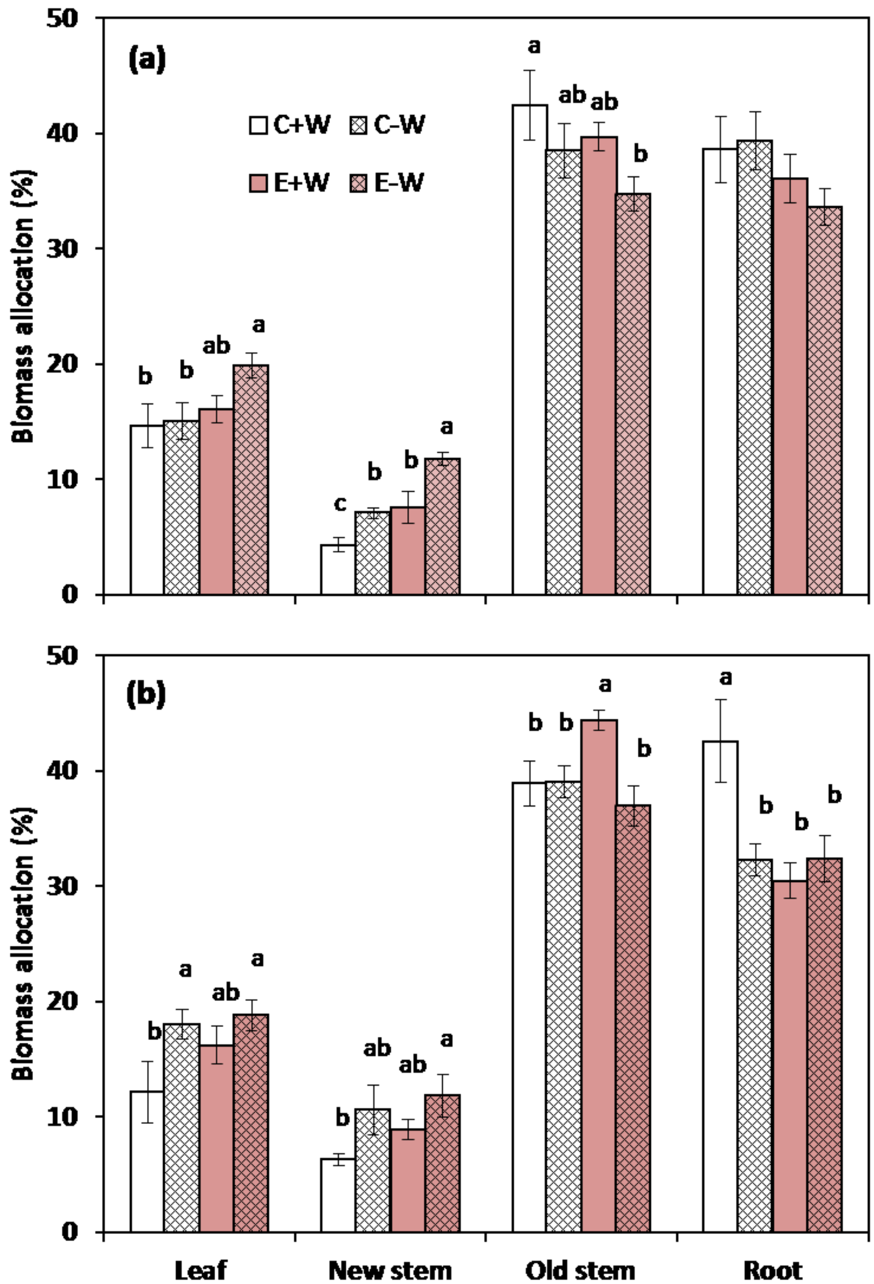

Figure 2-1 Percent biomass allocation to components of seedlings on a) the PMM (peat mineral soil mix) and b) the LFH (LFH mineral soil mix) sites in the second growing season. Different letters represent significant differences among treatments within each seedling component at $P < 0.05$. Vertical bars are standard errors of the means (n = 4).

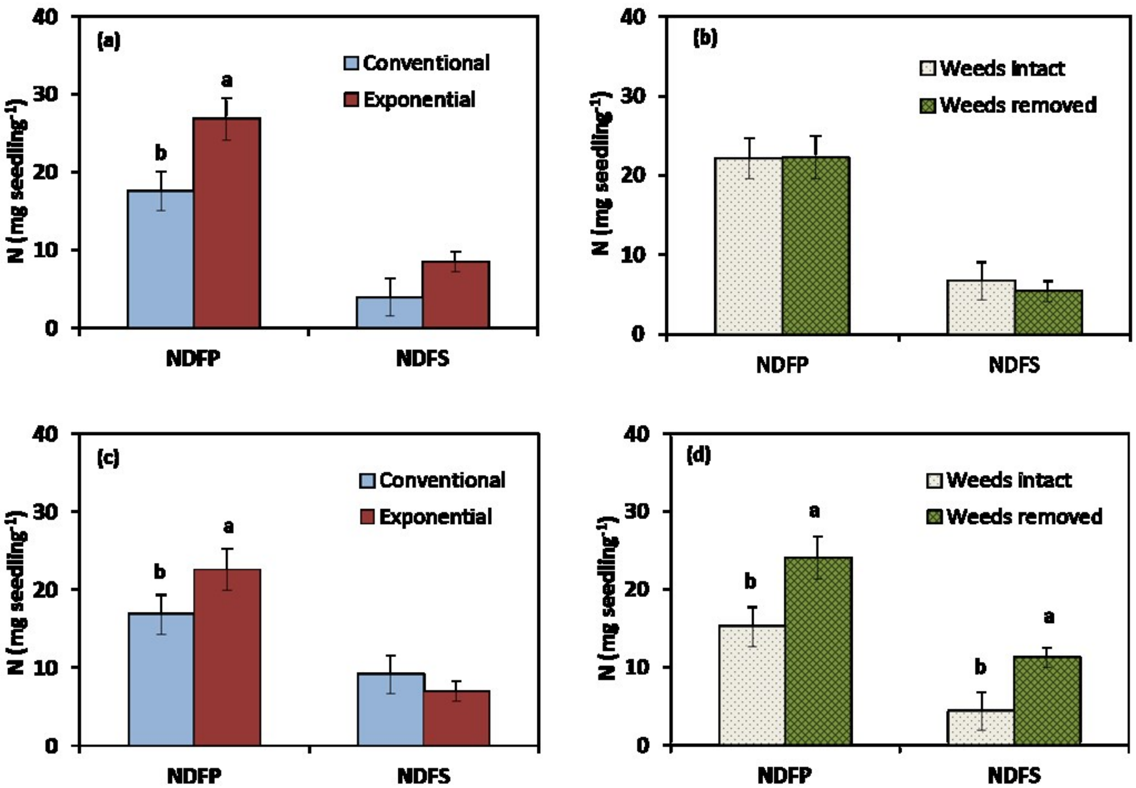

Figure 2-2 Effects of nursery fertilization and weed control after field outplanting on a) nitrogen derived from plant (NDFP) and nitrogen derived from the soil (NDFS) in seedlings on the PMM site as affected by nursery fertilization, b) NDFP and NDFS in seedlings on the PMM site as affected by weed competition, c) NDFP and NDFS in seedlings on the LFH site as affected by nursery fertilization, and d) NDFP and NDFS in seedlings on the LFH site as affected by weed competition. Different letters represent significant differences between the nursery fertilization or weed control treatments at $P < 0.05$. Vertical bars are standard errors of the means (n = 8).

CHAPTER 3 NURSERY NUTRIENT LOADING INCREASED GROWTH AND NITROGEN RETRANSLOCATION IN JACK PINE (PINUS BANKSIANA LAMB.) SEEDLINGS PLANTED ON AN OIL SANDS RECLAIMED SOIL

1. Introduction

Managing competing understory vegetation has been an integral part of forest management in many parts of the world. Forest vegetation management primarily uses herbicides to control competing vegetation and has been proven effective in improving growth and survival of planted seedlings (Woods et al., 1992; Wagner et al., 2006). Improved growth in many coniferous species such as jack pine (*Pinus banksiana* Lamb) (Longpre et al., 1994), Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Eissenstat and Mitchell, 1983), and Radiata pine (*Pinus radiata* D. Don) (Woods et al., 1992) has been attributed to reduction in competition for nutrients in low fertility soils. Fertilizer application is routinely used to increase productivity of many coniferous species including Douglas-fir, ponderosa pine (*Pine ponderosa* Dougl. ex P. Laws. & C. Laws), and western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) (Rose and Ketchum, 2002). However, broadcast fertilization does not only provide nutrients to the target plants but also to the understory vegetation that can further intensify understory vegetation competition for nutrients in the soil (Chang et al., 1996). These management practices are often criticized because of the negative effect of herbicides on the environment and biodiversity and potential increase of understory vegetation competition caused by fertilization.

An alternative approach of improving the growth of planted seedlings in competitive and nutrient deficient environments is to use quality seedling stock produced in nurseries; nutrient loading during nursery production is one of the techniques that can be used to produce quality seedling stocks (Imo and Timmer, 2001). The nutrient loading technique based on exponential fertilization is designed to increase the nutrient reserve in seedlings by inducing luxury nutrient consumption during nursery production (Malik and Timmer, 1998), while conventional nursery production involves a constant rate of fertilizer application which disregards the exponential growth of seedlings, resulting in toxicity at the beginning and deficiency at the later stage of nursery seedling production (Timmer, 1997). Nutrient-loaded seedlings can improve the survival, growth, and competitiveness over conventionally produced seedlings (Timmer and Munson, 1991; Timmer and Aidelbaum, 1996) because of the greater nutrient reserve in the seedling in the former. Nutrient loading has improved early establishment of seedlings of many coniferous species such as Chinese-fir (*Cunninghamia lanceolata* [Lamb] Hook) (Xu and Timmer, 1999), black spruce (*Picea mariana* [Mill.] BSP) (Malik and Timmer, 1996; Imo and Timmer, 2001; Salifu and Timmer, 2003b), and Lutz spruce (*Picea glauca × lutzii* Little) (Jonsdottir et al., 2013) in forest restoration. However, the use of nutrient loading in mine reclamation is limited to few broadleaved species such as trembling aspen (Schott et al., 2016; Pokharel and Chang, 2016) and northern red and white oak (Salifu et al., 2009). The study of growth performance of nutrient loaded seedlings of coniferous species in the oil sands reclaimed sites is still lacking.

In oil sands reclamation, cover soil materials such as peat mineral soil mix (PMM) and LFH mineral soil mix (LFH) that are significant sources of nutrients are placed over overburden or tailings sand substrates to improve the physical, chemical and biological properties of reconstructed soils (Jamro et al., 2014; Kwak et al., 2015). However, growth of planted seedlings in these reconstructed soils is often affected by low N availability (Duan et al., 2015) and competition of weeds that often colonize disturbed areas. In addition, cover vegetation grown on reclaimed soils to control erosion can also cause competition with planted seedlings. Although, fertilization is recommended to increase available N in reclaimed soils (Sloan and Jacobs, 2013; Duan and Chang, 2015), broadcast fertilizer use can intensify vegetation competition (Roth and Newton, 1996; Imo and Timmer, 1999). Nutrient loading has been shown to improve the growth of seedlings in adverse environments (Imo and Timmer, 2001), including recently reconstructed soils in oil sands reclamation (Pokharel and Chang, 2016) without intensifying vegetation competition. Application of control release fertilizer to root zone has also shown positive results in increasing soil N availability to the planted seedlings without increasing understory competition (Sloan and Jacobs, 2013).

New root and shoot growth in a newly transplanted seedling occurs most likely at the expense of the stored nutrients (Millard, 1996) because of poor root-to-soil contact that limits the uptake of water and nutrients from the soil (Burdette, 1990). Internal cycling of nutrients, an important mechanism for early establishment of seedlings, can contribute a large proportion of nutrients required to support new growth (Miller, 1984) particularly with evergreens (Nambiar and Fife, 1991; Salifu and Timmer, 2003a). Determining the quantity of nutrient retranslocated from old to new tissues in seedling is therefore important to improve our understanding of which nutrient pool (such as the nutrient derived from plant or the soil) supports the early growth of planted seedlings. The amount of N retranslocation from old to new tissues in coniferous species varies widely with time, quantity of N reserve in the plant, soil N availability and sink demand (Nambiar and Fife, 1991; Millard, 1996; Salifu and Timmer, 2003a). For example: 51 to 60% of N in old tissues retranslocated in Chinese-fir (Xu and Timmer, 1999), 67% in black spruce (Malik and Timmer, 1998) and 72% in radiata pine (Nambiar and Bowen, 1986); however, there is no data for jack pine. Moreover, N retranslocation in plants has often been determined by N budget studies which have issues of impreciseness (Millard, 1996), while the ^{15}N tracing technique can more precisely discriminate the various N pools (such as the N derived from plant (NDFP) and N uptake from soil (NDFS)) in the soil-plant system (Nommik, 1990; Proe and Millard, 1994; Barraclough, 1995).

The objective of this study was to examine if nursery exponential fertilization was effective for improving jack pine seedling growth planted on reconstructed oil sands soils. In this study, we tested the following hypotheses: i) exponentially fertilized jack pine seedlings would have better growth than conventionally fertilized ones after transplantation because of the higher nutrient reserve in the former, ii) there would be a greater contribution of N retranslocation in new tissue growth in the exponentially fertilized than in the conventionally fertilized seedlings again because of the higher nutrient reserve in the former, and iii) weed removal would decrease N retranslocation in exponentially fertilized seedlings due to the greater N uptake from the soil. In this experiment, we considered only N because N is usually the growth limiting nutrient in reclaimed soils in the oil sands (Rowland et al., 2009; Duan et al., 2015) and internal cycling of N is similar to other mobile macronutrients (Nambiar and Fife, 1991).

2. Materials and Methods

2.1 Nursery production of seedling

Jack pine seedlings were produced at Smoky Lake Forest Nursery, Alberta, Canada, using conventional and exponential fertilization regimes in 2013. In the conventional fertilization regime, an amount of balanced fertilizer (equivalent to a seasonal total of 200 mg N per seedling) was applied at a fixed rate delivered weekly for 20 weeks based on the optimum application rate used by the Smoky Lake Forest Nursery. In the exponential fertilization regime, an amount of balanced fertilizer (equivalent to a a seasonal total of 500 mg N per seedling) was delivered weekly for 20 weeks following an exponential fertilization schedule based on the optimum rate determined for jack pine in the nursery part of the study of Hu (2012); the weekly rate of fertilizer application was calculated following a modified exponential model (Birge et al., 2006; Imo and Timmer, 1992). Urea labeled with ^{15}N at 60 atom% was applied to the seedlings in week 15 during nursery seedling production to allow us to discriminate various N pools such as NDFP and N NDFS into new tissues of seedlings after transplantation. Seedlings were harvested from nursery at week 22, after proper hardening by inducing short days and lowering temperature in the greenhouse, and kept in a cold storage (at -2 °C) until the seedlings were taken to the field for transplanting. To determine biomass and N status of seedlings at the time of transplanting, five seedlings each of exponential and conventional fertilization from each of three storage boxes representing different replications used in nursery production were collected from the cold storage. Seedlings were composited per replication for conventional and exponential fertilization treatments. Biomass, N concentration and atom% ^{15}N of seedling components were analyzed following the procedure (same for the samples from the field experiment) described below.

2.2 Study site

The study area is located near Fort McMurray, in northern Alberta. The area is characterized by long cold winters and short warm summers with mean annual temperature of 1 °C and mean annual precipitation of 418.6 mm for the period of 1981 to 2010 ((Environment Canada, 2015). The study area had trembling aspen (*Populus tremuloides* Michx.), white spruce (*Picea glauca* [Moench] Voss, B.S.P.) and balsam poplar (*Populus balsamifera* L.) as dominant species in pure as well as in mixed-wood stands (Fung and Macyk, 2000) before the forest was clear-cut for oil sands mining. The region is characterized by Brunisols, Luvisols and Gleysols in xeric to sub-hydric or Organic soils in anaerobic and hydric conditions (Oil Sands Vegetation Reclamation Committee, 1998) in the natural ecosystem. The field experiment was established on a reclamation site (56° 58' N and 111° 19' W) in the oil sands region. The site was reclaimed with overburden in 2008 with 50 cm thick PMM as cover soil placed on an overburden substrate and then seeded with barley to stabilize the soil and control erosion in 2009. The basic soil properties are given in Table 2-1.

2.3 Experimental design

The study used a randomized complete block design with a 2 (exponentially vs conventionally fertilized seedlings) × 2 (weed-intact vs weed-removed) factorial experiment, replicated in five blocks. Weed treatments were maintained by periodic clipping of aboveground parts and pulling out the roots of weeds in every three weeks in active growing season. We used the following treatment codes in this experiment: C - Conventional fertilization with weed-intact (Control), W - Conventional fertilization with weed-removed, E – Exponential fertilization with weed-intact and E+W – Exponential fertilization with weed-removed. Treatments were randomly assigned to the plots (each plot was 7 × 7 m in size) within each block and the seedlings were planted at a 1 × 1 m spacing. A total of 980 seedlings (2 fertilization regimes × 2 weed treatments × 49 seedlings per plot × 5 replications) were planted for this experiment. Nursery-produced seedlings were thawed for a week before the seedlings were transplanted to the study site on June 12, 2014.

2.4 Soil sampling and analyses

In June 2014, soil samples were collected from two depths (0-10 and 10-30 cm) from five randomly selected locations in each plot using an auger and mixed to obtain a composite sample for each depth. Bulk density samples were collected from three randomly selected locations in each plot using a steel corer (with a volume of 98.12 cm3). Soil samples were air-dried at room temperature and ground using a ball mill (Mixer Mill MM 200, Thomas Scientific, Swedesboro NJ). The total carbon (TC) and total nitrogen (TN) concentrations of the soil samples were analyzed by the dry combustion method using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy). Soil pH was measured in 1:2 (m:v) in a 0.01 mol L^{-1} $CaCl_2$ solution using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA) and electrical conductivity (EC) was measured after 1:1 (m:v) soil: water extraction using an AP75 portable conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA). Soil samples were extracted with 2 mol L^{-1} KCl and analyzed for NH_4^+-N and NO_3^--N concentrations colorimetrically by the indophenol blue and the vanadium oxidation methods, respectively.

2.5 Plant size measurement and chemical analyses

The initial size (shoot height and root collar diameter (RCD)) of each seedling was measured at the time of transplanting to the field; final seedling size was measured at the end of the active growing season in September 2014. Shoot height was measured using a meter stick (to 1 mm accuracy) and RCD was measured using a Vernier caliper (to 0.05 mm accuracy) in two directions perpendicular to one another at the ground level, and the values were averaged to represent the RCD of each seedling. Height and RCD growth were calculated as the difference between final seedling size at the end of the growing season and the initial size at the time of transplanting. To measure foliar and root size and determine biomass and N content, seedling samples were destructively harvested in September 2014. Each seedling was cut at the ground level and then the root system of each seedling was excavated by digging a soil pit of 40 cm depth and 40 cm diameter.

For understory vegetation assessment, two 50 × 50 cm quadrats were randomly established in each plot in August 2014. Percent cover was determined in each quadrat for all vegetation. Aboveground understory vegetation within the quadrat was clipped to determine the biomass and N concentration as described below.

Each harvested seedling was then separated into needles and stems of the current (new tissues) and previous year's growth (old tissues) after the plant material was brought back to the laboratory. Soil and peat from the root system were carefully removed and the roots were washed on a sieve (0.5 mm mesh). The majority of the roots were recovered and broken roots retained on the sieve were collected manually as much as possible. Seedling components were quickly rinsed twice with distilled water and dried for 72 hours at 70° C, composited per plot for biomass, and foliar and root size measurement, and nutrient analyses.

Foliage and root size were measured using WinSeedle and WinRhizo image analysis softwares, respectively (Regent Instruments, Quebec, Canada) coupled with a flatbed scanner. Root weight ratio (RWR) was calculated as the ratio of root biomass to the total biomass of seedling and specific root length (SRL) was calculated as the ratio of root length to the root biomass. Plant samples were ground using the ball mill described above. The N concentration and ^{15}N abundance in the ground samples were analyzed using a stable isotope ratio mass spectrometer (Thermo Delta Plus XP IRMS, Waltham, MA, USA) linked to a CN analyzer (Costech 4010, Valencia, CA, USA) at the Stable Isotope Facility at the University of Wyoming, Laramie, WY, USA.

2.6 Data analyses

The internally remobilized N from old plant tissues and the N taken up from the soil are the main sources of N for new tissues in seedlings in the field. The NDFP in new tissues represents N retranslocation and was calculated using the following mass balance method (Hauck and Bremner, 1976):

$$NDFP = TN \times (A\text{-}B/C\text{-}B)$$

Where TN is the total amount of N in new tissues calculated as the concentration (mg g^{-1}) multiplied by plant tissue dry weight, A is atom% 15N in new tissues, B is the atom% ^{15}N in a natural standard (0.3663), and C is the atom% ^{15}N of plant at transplantation which was calculated as the weighted mean of atom% ^{15}N of needles, stems and roots.

NDFS was calculated as:

$$NDFS = TN\text{-}NDFP$$

A two-way analysis of variance (ANOVA) was used to analyze the effect of the treatments on the growth and nutrition of the seedlings in the field experiment using the Proc MIXED model (SAS 9.2, SAS Institute, Cary NC). The linear model for the analysis was:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + (\beta\tau)_{jk} + \varepsilon_{ijk}$$

where $Y_{ij}k$ is a dependent variable, μ is the overall mean, αi is the random effect of block, β_j and τ_k are the fixed effects of fertilization and weed removal treatment, respectively, $(\beta\tau)_{jk}$ is the fixed effect of interaction of the two treatments, and ε_{ijk} is the random error within the experiment. A linear regression was conducted to determine the relationship between NDFP and seedling growth. Data on plant parameters collected before transplantation were analyzed by a one-way ANOVA using the following linear model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} is a dependent variable, μ is the overall mean, α_i is the fixed effect of the fertilization treatment and ε_{ij} is the random error within the experiment. Prior to ANOVA and regression analyses, data were tested for normality of distribution (Shapiro-Wilk test) in residuals using Proc UNIVARIATE. A log-transformation (base 10) was applied for NDFP, NDFS, current-year shoot, root and total biomass of seedlings to meet the assumption of normality. An $\alpha = 0.05$ was used to determine statistical significance in all analyses. Vector analyses were used to assess the growth and nutritional status of seedlings before and after transplantation.

3. Results

3.1 Growth and N status of seedlings

Exponential fertilization in nursery significantly increased the N concentration and content but not the biomass of seedlings before transplantation (Table 3-2). The N concentration of needles, stems and roots of exponentially fertilized seedlings were about two-fold ($P = 0.009$, 0.001 and 0.024, respectively) that of conventionally fertilized ones. Exponential fertilization increased the N content by 85% ($P = 0.004$) in needles, by 79% ($P = 0.038$) in stems and by 26% ($p = 0.266$) in roots. Initial height and RCD of seedlings measured before transplanting were not different between the fertilization treatments (Figure3-1).

In the field experiment, seedling size (including height and RCD) and biomass at the end of the first growing season were significantly increased by exponential fertilization but not by weed removal. Height and RCD of exponentially fertilized seedlings were 11 % ($P = 0.003$) and 22% ($P < 0.001$), respectively, greater than conventionally fertilized seedlings (Figure 3-1). Exponential fertilization increased current-year shoot biomass by 121 % ($P < 0.001$), root biomass by 33% ($P = 0.006$) and total biomass of seedlings by 51% ($P < 0.001$) (Figure 3-1C and 3-1D).

Similar results were observed in the N status of seedlings at the end of first growing season (Table 3-3). Exponentially fertilized seedlings had greater N concentrations in old needle ($P = 0.005$), old stem ($P= 0.012$) and root ($P= 0.005$) than conventionally fertilized seedlings, but there was no significant effect of weed removal on N concentrations in old tissues. Both (exponential fertilization and weed removal) treatments did not affect N concentrations in new tissues (current-year needle and stem). A significant interaction (P = 0.039) between the treatments was observed for root N concentration. Exponential fertilization significantly increased N content in current-year needles by 138% (P < 0.001), current-year stems by 103% (P = 0.007), old stems by 58% (P = 0.001), and roots by 72% (P = 0.001). Weed removal had no significant effect on N content of seedlings.

Biomass and N allocation (expressed as % of the total in the seedling) into seedling components at the end of the first growing season were also affected by exponential fertilization but not by weed removal (Figure 3-2). Exponentially fertilized seedlings allocated a greater percentage of biomass to current-year tissues (needles and stems, P < 0.001 and 0.007, respectively) than conventionally fertilized seedlings but the percent biomass allocation to roots, old needles and old stems were greater (P = 0.001, < 0.001 and < 0.001, respectively) in conventionally than in exponentially fertilized seedlings (Figure 3-2A). Weed removal did not have a significant effect on percent biomass allocation. Percent N allocation to different parts such as current-year needles, old needles and old stems were similar to that of percent biomass allocation (Figure 3-2B). In current-year needles, exponential fertilization had greater percentage of N allocation than in conventional fertilization while percent N allocation in old needles was greater in conventional fertilization. Weed removal had no significant effect on percent N allocation in seedling components except the greater percent N allocation (P = 0.03) in old stems in weed-removed plots.

Needle and root size of seedlings were affected by both treatments (Figure 3-3). Exponential fertilization significantly increased length of current-year needle by 67% (P = 0.003) and old needle by 15% (P < 0.001), decreased RWR (P = 0.002) and SRL (P = 0.006). However, the effects of weed removal on needle length, RWR and SRL were not significant.

3.2 Nitrogen retranslocation and N uptake from the soil

Both exponential fertilization and weed removal significantly affected the amount of N retranslocation from old to new tissues and N uptake from the soil (Figure 3-4). Exponential fertilization increased the amount of N retranslocation in the seedlings by 147% (P < 0.001)) and N uptake from the soil by 175% (P = 0.012). Weed removal reduced the amount of N retranslocation (P = 0.046) and marginally increased N uptake from the soil (P = 0.077). There was no significant interaction between nursery fertilization and weed removal on N retranslocation and N uptake from the soil. In weed-intact plots, NDFP accounted for 82% of total sink demand of current-year needles in exponential fertilization but only 70% in conventional fertilization.

Regression analyses indicated that growth of seedling in the first year after outplanting was related to NDFP (Figure 3-5). The NDFP and height growth ($r^2 = 0.81$, $P < 0.001$), RCD growth ($r^2 = 42$, $P = 0.002$) and biomass growth ($r^2 = 0.63$, $P < 0.001$) was positively correlated.

4. Discussion

Conventional and exponential fertilization in nursery produced seedlings that were morphologically similar but exponentially fertilized seedlings had greater N concentration and content (measured before seedling transplantation), indicating that exponential fertilization lead to development of greater nutrient reserve during nursery production, confirming the nursery study of Hu (2012). The exponentially fertilized seedlings thus had more internally stored nutrients that could be used for early growth after transplanting. Nursery fertilization practices can strongly influence the attributes of coniferous seedlings such as seedling size, root/shoot ratio and internal nutrient storage (Salifu and Timmer, 2003b; Islam et al., 2006) that can affect seedling performance after outplanting (Grossnickle, 2012). In our experiment, conventional fertilization in the nursery achieved seedling quality targets but there was lower nutrient concentration due to growth dilution, suggesting that nutrient stress occurred at the end of nursery culture (Timmer, 1997). But exponential fertilization allowed luxury consumption to occur and that maximized N content and concentration without inducing toxic building up of nutrients in the seedling (Salifu and Timmer, 2003b). Seedling nutritional status can be easily interpreted by foliar vector analysis (Timmer and Ray, 1988; Teng and Timmer, 1990). Vector analysis of dry mass, N concentration and content in needles (Figure 3-6a) before transplant demonstrated that the exponentially fertilized seedlings were in luxury consumption of N (Salifu and Timmer, 2003b) resulting in higher amount of N stored in the seedling, most likely as reserves of non-functional amino acids and proteins (Chapin et al., 1990) that are more readily available for translocation after field planting. Although the vector monogram of roots showed that the relative biomass of roots was lower in exponential fertilization regime (as indicated by the shift in Figure 3-6b), absolute root biomass of exponentially fertilized seedling was not different from that of conventional ones (Table 3-1) indicating that no toxicity occurred in exponential fertilization during nursery production of seedlings.

In the field experiment, survival of seedling was 94% (on average) in the first year of outplanting and was not affected by both treatments. Greater height, RCD and biomass in exponentially fertilized seedlings at the end of the first growing season indicate that exponential fertilization improved seedling growth, supporting our first hypothesis. Improved growth of exponentially fertilized seedlings can be attributed to the greater nutrient reserve built up in these seedlings during nursery production. The access of seedlings to the greater amount of stored nutrients in early growth is especially important to overcome transplanting stress during the critical stage of establishment (Grossnickle, 2012) immediately after outplanting to the field because the poor root-soil contact restricts the uptake of water and nutrients from the soil. The transplanting stress can be severe and persists for a long time limiting the growth of seedlings in soils with low nutrient availability such as in reclaimed soils.

As compared to the N concentration of seedling components before outplanting, the seedlings at the end of the first growing season had much lower N concentrations regardless of the treatment, indicating that the growth of seedlings was limited by N availability. The greater reduction in N concentration in the exponentially fertilized seedlings was caused by dilution due to greater biomass production. Vector analysis (Fig 6c and 6d) also indicates that dilution effect occurred in exponentially fertilized seedlings growing with or without weed competition. The lower foliar N concentration in all seedlings (regardless of the treatment) than the optimum level and the low available N (nitrate level below 28 kg ha^{-1}) at our study site suggest that additional silvicultural treatments such as control release fertilizers applied directly to the root zone (Jacobs et al., 2005; Sloan and Jacobs, 2013; Sloan et al., 2016) should be used to improve seedling growth in reclaimed soils.

Weed competition has been found to reduce growth of aspen seedlings planted on reclaimed soils (Hu et al., 2015; Pokharel and Chang, 2016), longleaf pine (*Pinus palustris* Mill.) in an abandoned land (Ramsey et al., 2003) and black spruce in a mixed-wood forest (Imo and Timmer, 2001). In contrast, the effect of weed competition on the growth of jack pine seedlings was not significant in this study; however, the result is consistent with Hu et al. (2015) on white spruce, indicating that weed competition was not limiting the growth of jack pine seedlings in this reclaimed soil. In our study site with PMM as the cover soil, understory vegetation competition was relatively low as compared to that of a LFH site (Pokharel and Chang, 2016), as indicated by the low percent cover (48 %), biomass (215 g m^{-2}) and N content (3.5 g m^{-2}) in aboveground understory vegetation in this study. However, it should be noted that jack pine is a shade intolerant species that may be severely affected when the understory vegetation overtops the jack pine seedlings (Hosie, 1979).

Exponentially fertilized seedlings allocated more biomass and N to metabolically active tissues such as current-year needles and stems than conventionally fertilized seedlings; the result is consistent with Malik and Timmer (1998) on black spruce. Greater allocation of biomass and N to metabolically active tissues enhances plant photosynthetic and competitive capacity (Cuesta et al., 2010). Larger needles in exponentially fertilized seedlings mean greater light absorption and higher rate of photosynthesis and increased seedling growth. In exponentially fertilized seedlings more than 50% of the total N was in the current-year shoots, indicating that current-year shoot is the main sink for the N remobilized and N taken up from the soil. Although, the contribution of stored N to the growth of new fine roots has been found to be low in coniferous species (Villar-Salvador et al., 2015), exponentially fertilized seedlings with higher nutrient reserves were found to produce greater root biomassthan in conventionally fertilized ones (Figure 1). A strong link between increased photosynthetic capacity in current year needles and the growth of new roots (Villar-Salvador et al., 2015) may be the reason for the improved growth of roots in exponentially fertilized seedlings. In contrast, conventionally fertilized seedlings allocated a greater percent of biomass to roots than to current-year needles, had greater RWR and SRL, consistent with Brown and Higginbotham (1986) on white spruce, indicating that there was nutrient stress in the soil (Malik and Timmer, 1998) for planted jack pine seedlings. Greater allocation of biomass to roots allows seedlings to exploit a greater volume of soil for increasing N uptake from the soil (Nambiar and Sands, 1993).

In this study, NDFP accounted for 59 to 82% of the total sink demand for N from the new growth, illustrating the importance of N retranslocation in meeting the N requirement in early growth of these seedlings. During early establishment of seedlings, the demand of nutrients required for optimum growth could not be met by the nutrients taken up from the soil because of nutrient limitation in the soil and low capacity of seedlings to take nutrients and water from the soil. When plant N demand was not met by the N uptake from the soil, new growth strongly relied on remobilized N (Millard and Proe, 1993) but the amount of N remobilized depends on internal nutrient reserve and biophysical factors such as weed competition and N availability in the soil (Malik and Timmer, 1998). In our study, the amount of N retranslocated in exponentially fertilized seedlings was two and half times greater than in conventionally fertilized ones, supporting our second hypothesis. The greater amount of N retranslocated in exponentially fertilized seedlings was associated with greater N reserve in these seedlings.The greater the amount of stored N in the seedling, the greater the amount of N remobilization that can occur (Millard, 1996). It also supports the contention that N reserve in the seedling is a key factor driving retranslocation (Millard and Proe, 1993; Malik and Timmer, 1998). Building up of stored nutrients is therefore critically important for seedlings planted on soils with low nutrient availability. Exponential fertilization also increased the amount of N uptake from the soil. The improvement in N uptake may be linked to the greater root production that enabled exponentially fertilized seedlings to exploit more volume of the soil.

Weed removal decreased N retranslocation in exponentially fertilized seedlings and increased N uptake from the soil, supporting our third hypothesis. Malik and Timmer (1998) also observed higher N retranslocation and lower N uptake in weedy plots in both nutrient-loaded and non-nutrient-loaded black spruce seedlings growing on boreal mixedwood sites. But in *Pinus halepensis* Mill. growing on poorly developed soils (Entisol), weed competition did not change N retranslocation but reduced N uptake from the soil (Cuesta et al., 2010). They also found that remobilized N was the main source of N for new growth in seedlings competing with weeds (consistent with our result), while N uptake from the soil was the main source of N for seedlings growing without weeds (in contrast with our result). The fact that exponentially fertilized seedlings retranslocated more N growing under a competitive environment in our study suggests that nutrient retranslocation in jack pine seedlings was an adaptive strategy for nutrient conservation (Malik and Timmer, 1998). The increase in N uptake by these seedlings in weed-removed plots was due to increased N availability in the soil as a result of reduced competition from the understory vegetation.

5. Conclusions

We conclude that loading jack pine seedlings with nutrients by exponential fertilization in the nursery production stage is an alternative approach to field fertilization in the reclamation of severely disturbed landscapes. Exponential fertilization was effective in increasing the nutrient reserve in jack pine seedlings in nursery production and in improving seedlings early growth after outplanting. The new growth of jack pine seedlings was positively affected by the N stored in and remobilized from old tissues. Increasing the nutrient reserve and N retranslocation by exponential fertilization demonstrates its implication to improve early growth of planted seedlings. Nutrient loading has the potential to improve the success of planted jack pine seedlings establishment in oil sands reclamation. Further study on the relative contribution of N remobilized into new tissues from different organs such as needles, stems and roots at different stages of growth would provide greater insight into the significance of exponential fertilization in the early establishment of planted jack pine seedlings in land reclamation in the oil sands. This study was limited to a one-year field experiment, benefits of exponential fertilization in jack pine seedlings to improve growth need to be monitored for a longer term before the technique is operationally applied in future land reclamation.

Implications for practice

Exponential fertilization in jack pine can increase nutrient reserve in the seedlings in nursery production and improve seedling growth after outplanting on reclaimed soils. The N retranslocation and N uptake from the soil after outplanting can be increased by exponential fertilization in jack pine that help in early establishment of seedlings in nutrient poor sites. Exponential fertilization can be used for achieving revegetation success in reclamation of disturbed lands with a forest as the end land use. Weed removal does not increase growth and N retranslocation site with PMM as a cover material in the oil sands region.

Table 3-1 Chemical and physical properties of the soil at the study site (mean ± SE, n = 16).

Depth (cm)	pH	EC (ds m^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Bulk density (g cm^{-3})
0-10	6.85 (0.05)	1.42 (0.11)	83.53 (7.99)	3.07 (0.33)	29 (2.16)	5.59 (0.54)	3.81 (0.59)	0.55 (0.04)
10-30	6.96 (0.06)	1.44 (0.13)	76.99 (10.14)	2.62 (0.42)	39 (6.63)	4.00 (0.33)	3.01 (0.47)	0.52 (0.04)

Abbreviations: EC = electrical conductivity, C:N = carbon to nitrogen ratio.

Table 3-2 Biomass and nitrogen (N) status (mean ± SE, n = 3) of seedlings before transplanting.

Seedling parts	Dry mass (g seedling^{-1})		N concentration (mg g^{-1})		N content (mg seedling^{-1})		Atom % ^{15}N	
	C	E	C	E	C	E	C	E
Needle	5.73 (0.58)	5.53 (0.42)	14.52 b (2.51)	26.96 a (0.86)	80.24 b (1.19)	148.84 a (17.12)	0.95 b (0.12)	1.19 a (0.21)
Stem	2.14 (0.33)	1.70 (0.22)	8.40 b (1.19)	17.99 a (0.38)	17.19 b (0.70)	30.69 a (4.38)	0.79 (0.06)	1.13 (0.21)
Root	3.59 (0.92)	2.22 (0.32)	11.09 b (2.10)	20.95 a (1.87)	36.14 (5.23)	47.58 (5.09)	0.95 (0.02)	0.80 (0.11)
Needle + Stem + root	11.46 (1.37)	9.45 (0.96)	11.97 b (1.35)	23.92 a (0.51)	133.57 b (4.04)	225.11 a (18.63)	0.92 (0.08)	1.21 (0.08)

Abbreviations: C = conventional, E = exponential.
Different letters in the same row indicate significant differences between fertilization treatments for a particular parameter.

Table 3-3 Effects of nursery fertilization and weed removal on nitrogen (N) concentration (mg g^{-1}) and N content (mg $seedling^{-1}$) in seedlings (mean ± SE, n = 5) after outplanting.

Treatments	**N concentration**					**N content**					
	Needle		Stem		Root	Needle		Stem		Root	Total
	Current-year	Old	Current-year	Old		Current-year	Old	Current-year	Old		
C	13.61 (0.96)	8.76 (0.65)	7.51 (0.51)	3.52 (0.13)	8.11 (0.55)	52.98 (6.11)	39.28 (2.81)	17.28 (2.70)	13.63 (0.53)	29.49 (1.57)	152.66 (11.31)
W	13.27 (1.47)	7.70 (0.65)	7.56 (0.36)	3.49 (0.18)	6.55 (0.31)	48.12 (7.74)	36.60 (6.00)	15.46 (1.31)	14.82 (0.71)	25.94 (1.89)	140.96 (11.38)
E	12.99 (0.83)	11.11 (0.54)	7.47 (0.49)	4.19 (0.31)	8.75 (0.63)	120.55 (16.69)	47.49 (2.90)	33.58 (6.92)	21.44 (2.05)	44.35 (3.79)	267.41 (25.01)
E+W	13.89 (1.24)	10.80 (1.35)	7.57 (0.72)	4.51 (0.46)	9.95 (0.86)	119.87 (24.32)	49.17 (6.98)	32.75 (7.84)	23.58 (3.52)	51.27 (9.24)	276.64 (49.44)
ANOVA (p > F)											
Fertilization (F)	0.998	**0.005**	0.978	**0.011**	**0.004**	**<0.001**	0.055	**0.007**	**0.001**	**0.001**	**<0.001**
Weed (W)	0.811	0.437	0.894	0.6402	0.779	0.861	0.922	0.811	0.435	0.747	0.966
F*W	0.596	0.674	0.961	0.571	**0.039**	0.894	0.669	0.929	0.822	0.324	0.721

Abbreviations: C = control, W = weed removal, E = exponential fertilization, E+W = exponential fertilization + weed removal.

Values in bold indicate significance at $p < 0.05$

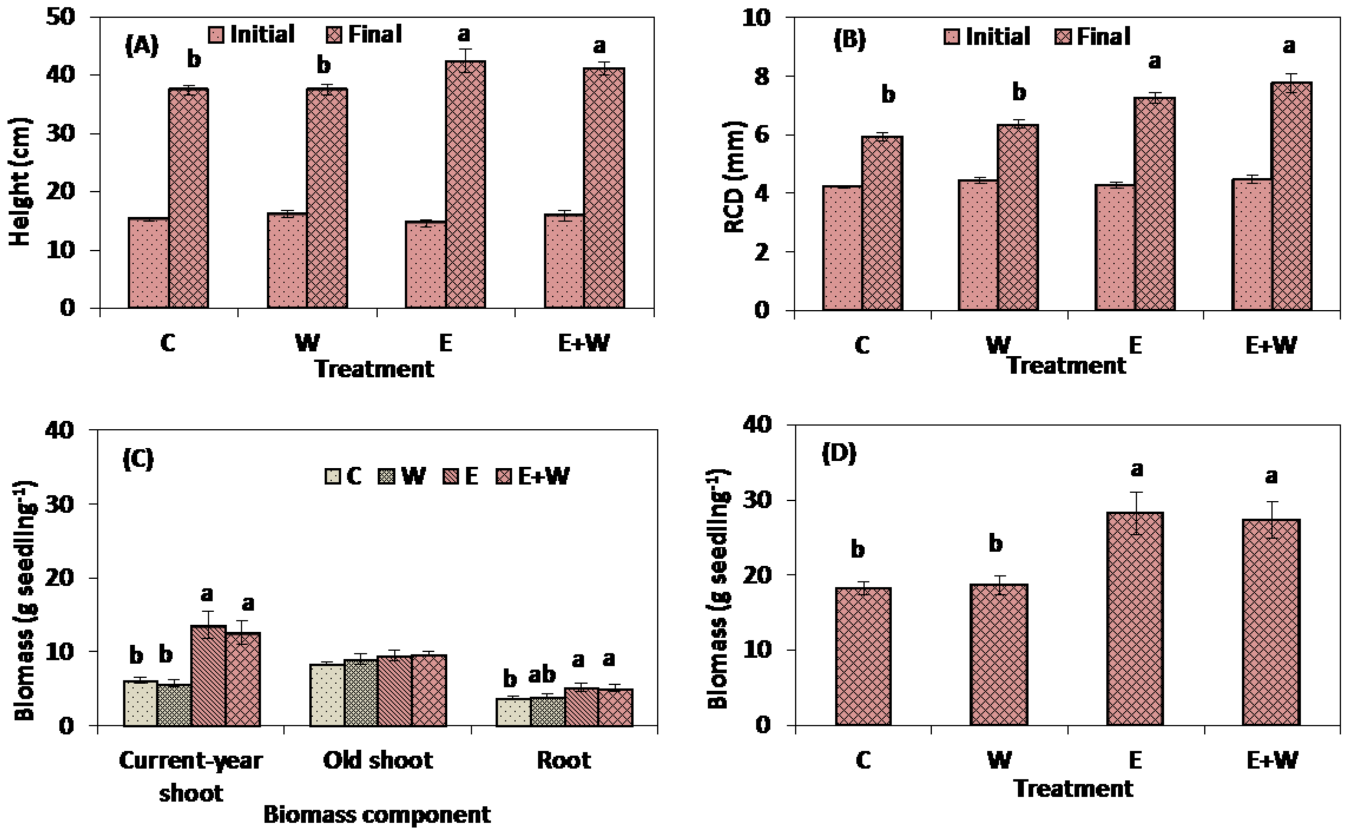

Figure 3-1 Seedling size and biomass: A) Initial (at transplanting) and final height (at the end of the growing season), B) Initial (at transplanting) and final root collar diameter (RCD) (at the end of the growing season), C) component biomass (at the end of the growing season), and D) total biomass (at the end of the growing season). The abbreviations used are: C: control, W: weed removal, E: exponential fertilization and E+W: exponential fertilization + weed removal. Different letters represent significant differences among treatments at $p < 0.05$. Vertical bars are standard errors of means ($n = 5$).

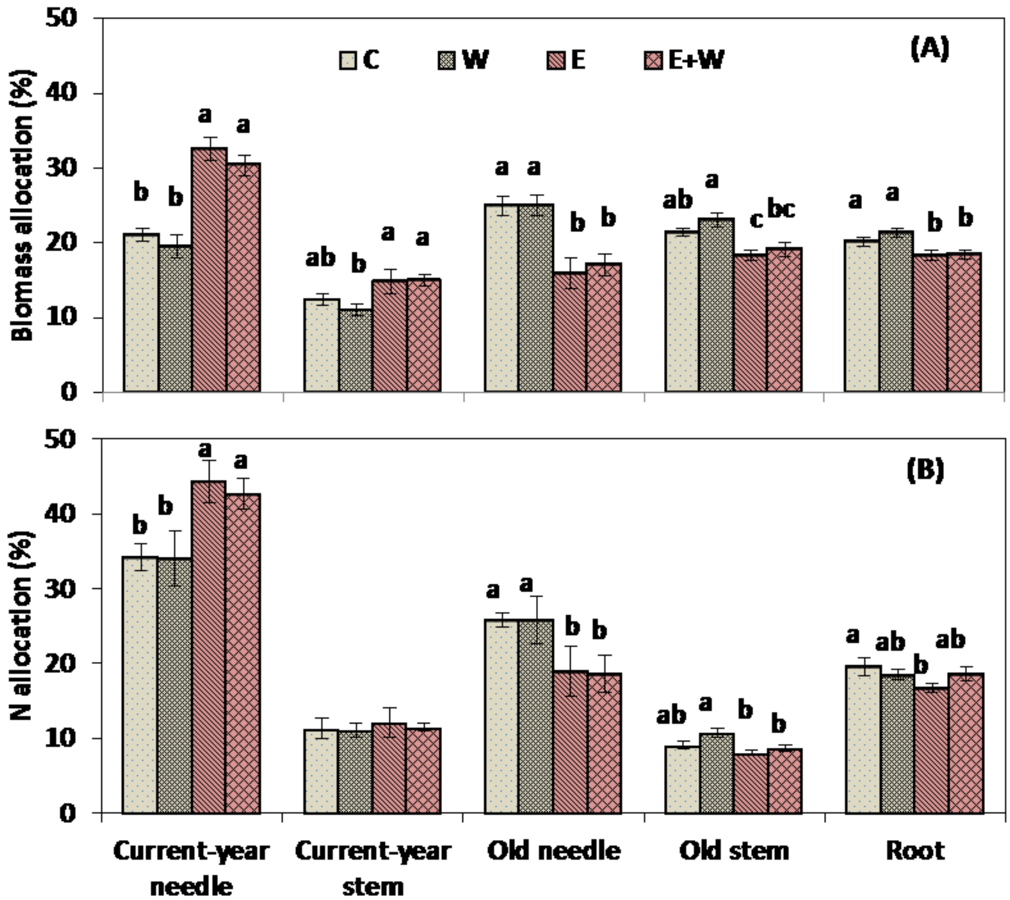

Figure 3-2 A) Percent biomass allocation to components of seedlings and B) percent nitrogen (N) allocation to components of seedlings at the end of the growing season. (C: control, W: weed removal, E: exponential fertilization and E+W: exponential fertilization + weed removal). Different letters represent significant differences among treatments within each seedling component at $p < 0.05$. Vertical bars are standard errors of means ($n = 5$).

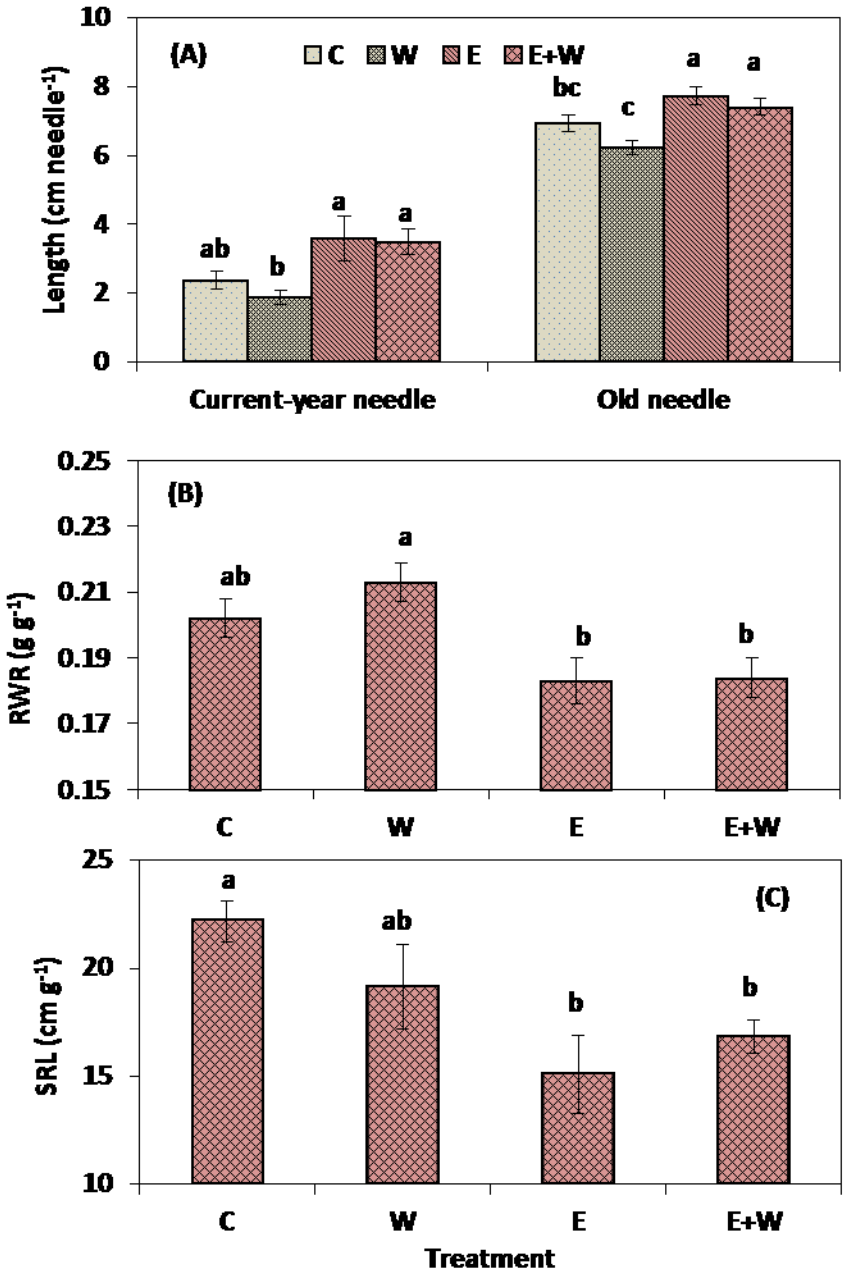

Figure 3-3 Effects of control (C) weed removal (W), exponential fertilization (E), and exponential fertilization and weed removal (E+W) treatments on A) needle length, B) root weight ratio (RWR), and C) specific root length (SRL) in seedling. Different letters represent significant differences between the treatments at $p < 0.05$. Vertical bars are standard errors of means ($n = 5$).

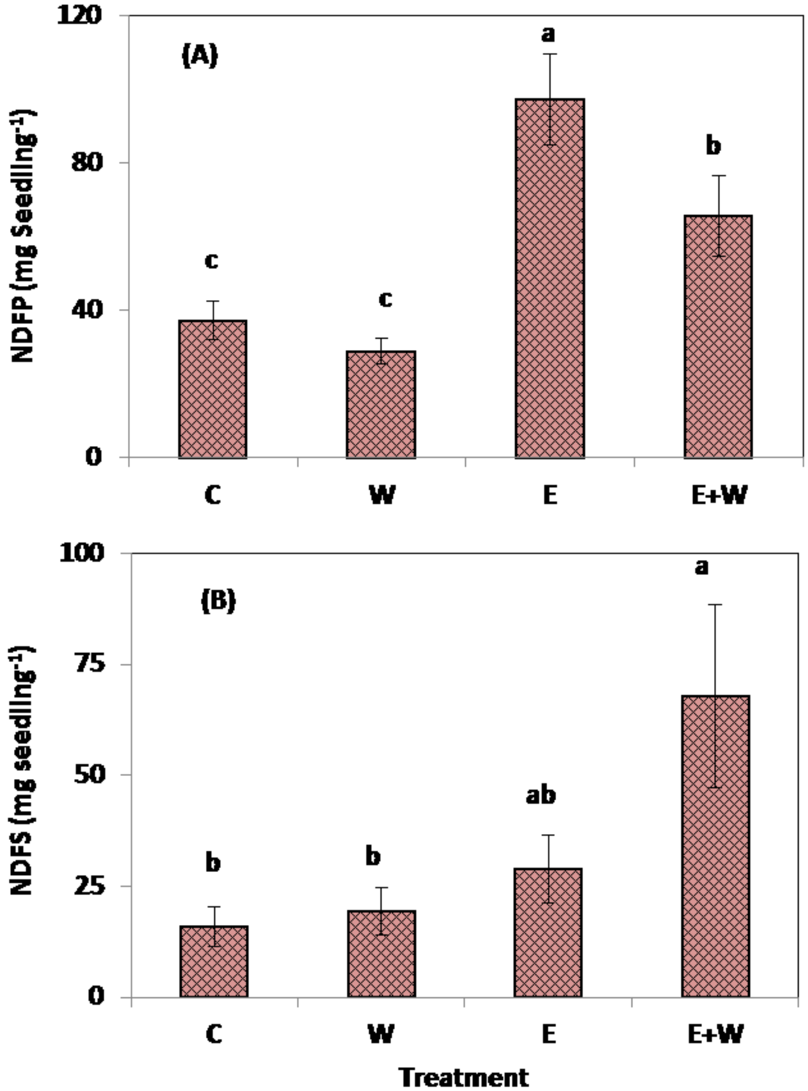

Figure 3-4 Effects of control (C), weed removal (W), exponential fertilization (E), exponential fertilization and weed removal (E+W) treatments on A) nitrogen derived from plant (NDFP) and B) nitrogen derived from the soil (NDFS) in seedling. Different letters represent significant differences between the treatments at $p < 0.05$. Vertical bars are standard errors of means ($n = 5$).

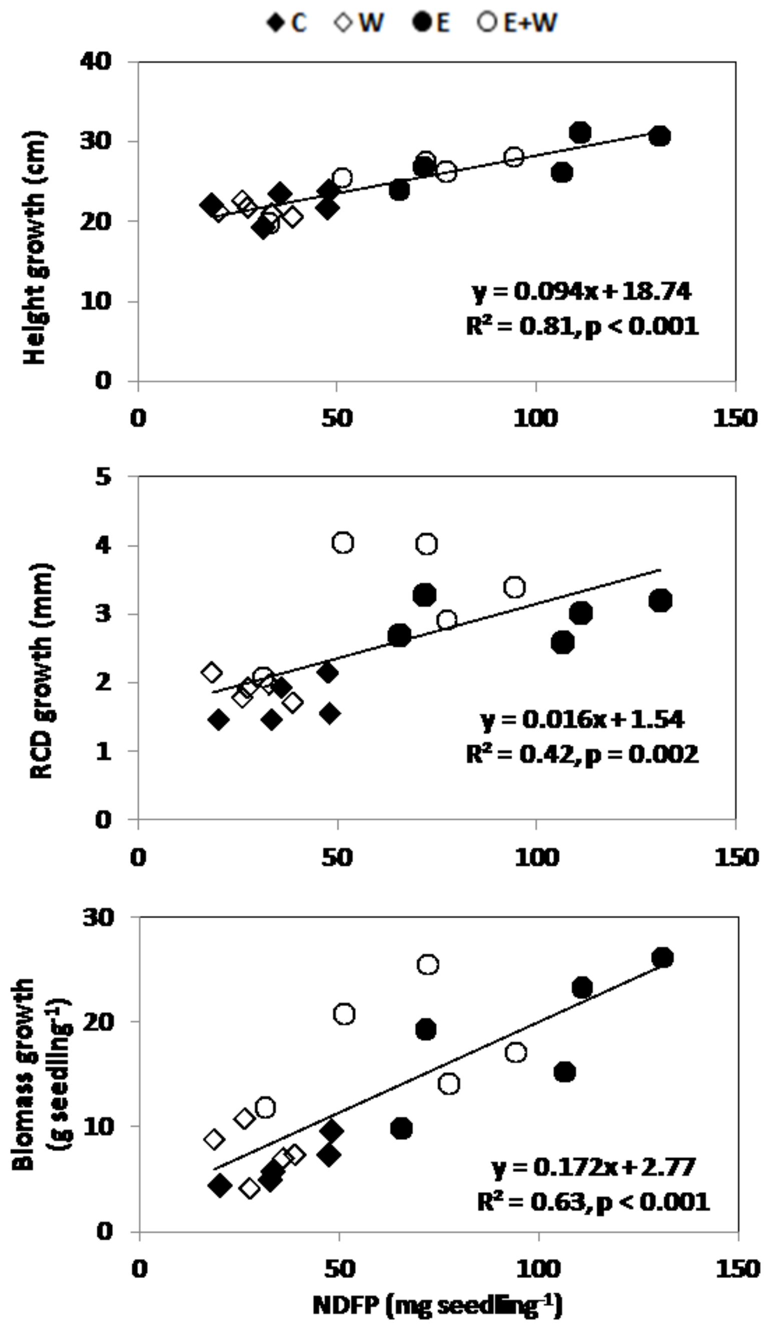

Figure 3-5 The relationship between height, root collar diameter (RCD), and biomass growth and NDFP in seedlings at the end of the first growing season. (C: control, W: weed removal, E: exponential fertilization and E+W: exponential fertilization + weed removal).

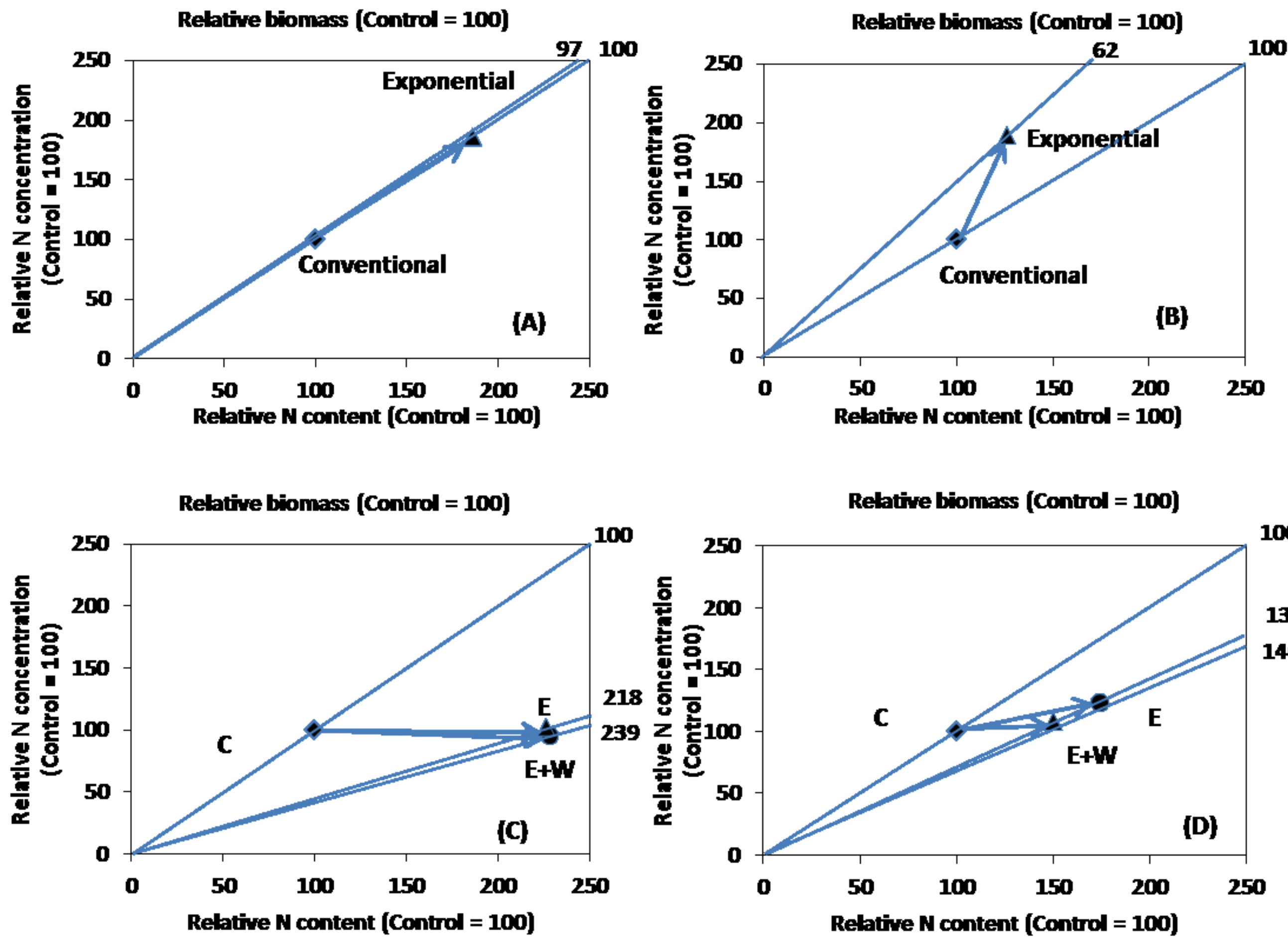

Figure 3-6 Vector monograms of relative changes in biomass, nitrogen (N) concentration, and N content before transplanting and at the end of the growing season for, A) needle before transplanting, B) root before transplanting, C) needle at the end of the growing season, and D) root at the end of the growing season. Conventional fertilization and control (C) were normalized to 100. The treatment codes are: E: exponential fertilization and E + W: exponential fertilization + weed removal.

CHAPTER 4 WEED CONTROL BUT NOT EXPONENTIAL FERTILIZATION INCREASES NITROGEN RETRANSLOCATION AND GROWTH OF WHITE SPRUCE SEEDLINGS ON A RECLAIMED OIL SANDS SOIL

1. Introduction

Reclaimed oil sands areas in northern Alberta, Canada are often revegetated with white spruce (*Picea glauca* [Moench] Voss), one of the most commonly distributed species in the mixedwood boreal region (Hardy BBT Limited, 1989; Fung and Macyk, 2000). However, survival and growth of nursery grown coniferous seedlings are often limited by transplanting stress (Rietveld, 1989; Grossnickle, 2000), which has been noted as a major factor contributing to the failure after planting (Burdett et al., 1984). Low soil nutrient availability caused by understory competition for resources such as nutrients and water can limit the early growth of seedlings (Matsushima and Chang, 2006; Sloan and Jacobs, 2013). In this context, building up a nutrient reserve in seedlings during nursery production (Timmer and Munson, 1991) and understory vegetation management (Rietveld, 1989; Matsushima and Chang, 2007; Man et al. 2008) to increase soil nutrient availability can reduce stress and thus enhance seedling establishment after outplanting.

The importance of internal nutrient cycling in supplying nutrients for new tissues of coniferous trees has been extensively reported (Nambiar and Fife, 1991; Millard, 1996). The internal cycling of nutrients is more important for initial growth and survival of seedlings due to their slow early growth and limited root systems, which limit their ability to take up nutrient and water (Burdett et al., 1984; Van den Driessche, 1985; Burdett, 1990).

The rationale of nutrient loading in nursery seedling production is to store nutrients in the seedling that can consequently be used during early growth through retranslocation before the root system of the seedling is established (Timmer, 1997). Exponential fertilization is a method for loading nutrients into seedlings, designed to supply nutrients to the seedlings at an exponential rate in excess of the demand for the current growth during nursery production, thereby inducing luxury uptake, resulting in an increased nutrient reserve in seedlings without increasing biomass (Timmer and Aidelbaum, 1996; Salifu and Timmer, 2003 b). One of the advantages of nursery nutrient loading over field fertilization is that it prevents the inadvertent supply of nutrients to understory vegetation that competes with seedlings (Chang et al., 1996).

Nursery nutrient loading has been used in a number of coniferous (Imo and Timmer, 2001; Jonsdottir et al. 2013) and broadleaved species (Close et al., 2004; Birge et al., 2007) in forest restoration. For white spruce, however, relevant studies are limited to nursery production of seedlings (Hu, 2012), green house experiments (Hu et al., 2015) and for planting in abandoned farm fields (McAlister and Timmer, 1998). Although disturbed oil sands sites are reclaimed by bringing in foreign soil materials such as peat mineral soil mix or LFH mineral soil mix as sources of organic matter and nutrients, the growth of seedlings may still be limited by low nutrient (particularly N) availability (Sloan and Jacobs, 2013; Duan et al., 2015). Available N (NH_4^+ and NO_3^-) in reclaimed soils is lower than that in adjoining natural forest soils (Jung and Chang, 2012; Jamro et al., 2014). It implies that seedling growth in reclaimed soils might benefit from improved conditions of nutrients by nutrient loading. However, research on the effect of nutrient loading on white spruce growth on reclaimed soils is lacking.

Control of understory vegetation, which either colonizes the site after disturbance or is grown purposefully in sloping areas for soil erosion control, soil stabilization and organic matter supply, can further help seedling growth by alleviating competition for nutrients, water and light (Brand, 1991; Munson et al., 1993; Matsushima and Chang, 2006). Understory vegetation can be a strong competitor with planted white spruce seedlings resulting in decreased soil water and nutrient availabilities for the seedlings (Man et al. 2008). However, we still lack experimental data on the effect of understory vegetation control on soil water and N availabilities that influence the growth of planted white spruce seedlings on reclaimed oil sands soils. Therefore, preliminary studies on the effect of two common silvicultural treatments (nursery nutrient loading and understory vegetation control) on seedling growth in reclaimed oil sands areas may provide insight into the implementation of the treatments at a larger scale.

Removal of understory vegetation can affect soil water and N availability to the planted seedlings. The available soil water and N can also be changed greatly over the growing season. During photosynthesis, carboxylation efficiency is controlled by soil N availability that affects photosynthetic enzyme activities, and CO_2 diffusion rates are controlled by available soil water affecting stomatal conductance (Hogberg et al., 1995; Korol et al., 1999). Both these activities can have significant effects on C isotope discrimination resulting into the change in carbon isotope composition ($\delta^{13}C$) of plant tissues. Therefore, the $\delta^{13}C$ isotope abundance in plant organic tissues can be a good indicator of water and N availability in the soil (Livingston et al., 1999). In this experiment, we used the foliar $\delta^{13}C$ technique to study the effects of nursery fertilization and understory competition on N and water stress on the seedlings caused by the change on N and water availability in the soil.

In the present study, I investigated the growth and nitrogen (N) uptake response of white spruce seedlings to nursery nutrient loading and understory vegetation control over two growing seasons after outplanting. Nitrogen was the main focus of this study because low N availability has been reported as a major factor to restrict the reestablishment of plant communities in reconstructed oil sands mining sites (Farnden et al., 2013; Duan et al., 2015). I hypothesized that exponential fertilization would increase seedling growth after outplanting through internal recycling of N in the reserve and understory vegetation control would also help seedling growth by increasing the soil N availability and N uptake from the soil.

2. Materials and methods

2.1 Nursery production of seedlings

Containerized white spruce seedlings were produced in styroblock containers (615 A with 45 cavities per block, 340 mL per cavity) filled with a mixture of peat moss and perlite (9:1, v:v) in a greenhouse at the Smoky Lake Forestry Nursery (54° 05ʹ N, 112° 14ʹ W) in Alberta following conventional and exponential fertilization regimes (Pokharel and Chang 2016). Following Hu et al. (2015), a commercial water-soluble fertilizer (20:20:20 N:P_2O_5:K_2O plus micronutrients, Plant products Co. Ltd., Brampton, Ontario) was applied at a total rate of 300 mg N per seedling, an optimum rate used by the Smoky Lake Forestry Nursery, for conventional fertilization and 450 mg N per seedling for a modified exponential fertilization regime for 22 weeks as suggested by Hu (2012). For conventional fertilization, an equal amount of fertilizer was applied each week, and for the modified exponential fertilization regime, the weekly fertilization rate decreased from 24 mg in the first week to 0.77 mg in the 9th week and then increased to 101.09 mg per seedling in the 22nd week; the rates were calculated following Birge et al. (2006) and Hu et al. (2015). In week 16, the seedlings were fed with a 20% replacement of the fertilizer with ^{15}N-urea (60.0 ^{15}N atom%) to label the seedlings with ^{15}N that allowed the quantification of N from old tissues to newly grown tissues of seedlings via remobilization of N.

During the 22 weeks, the seedlings were grown at 65−85% humidity and 18−30 °C temperature under a 20 h daily photoperiod using sodium vapor lamps at a light intensity 250 µmol m^{-2} s^{-1}. Seedlings were hardened from week 22 for two weeks in the greenhouse by lowering the photoperiod to 8 hours before the seedlings were harvested (Blgras and Daoust, 1992). The harvested seedlings were then kept in cold storage at −2 °C during winter time until they were taken out for transplanting to the field in June next year.

2.2 Site description and soil properties

The study was conducted at a reclaimed site (56° 58' N and 111° 19' W) in the Athabasca Oil Sands region in northern Alberta where lands disturbed for oil sands extraction have been reclaimed after open-pit mining activities. This area has a continental boreal climate with long cold winters and short warm summers; the mean annual temperature in the last 30 years was 1°C and the mean annual precipitation was 418.6 mm (316.3 mm as rainfall and 102.3 mm as snowfall) (Environment Canada 2015). The mean temperature and total precipitation in the growing season (May to September) were 13.8 °C and 297.7 mm, respectively, in 2014 and 13.8 °C and 194.9 mm, respectively, in 2015 (Environment Canada 2016). The area lies in the boreal forest consisting of coniferous trees such as black spruce (*Picea mariana* [Mill.] BSP), white spruce, jack pine (*Pinus banksiana* Lamb) and tamarack (*Larix lariciana* [Du Roi] K. Koch) and deciduous species including trembling aspen (*Populus tremuloides* Michx.), white birch (*Betula papyrifera* Marshall), and balsam poplar (*Populus balsamifera* L.) in pure as well as in mixed-wood stands (Fung and Macyk 2000).

During open pit mining, the forest was clear-cut; surface soil, overburden and oil sands were removed from the area. After oil sands extraction, overburden substrates were brought back to the area as part of the reclamation process, peat mineral soil mix was then placed on these substrates to complete land reclamation in 2008. The site was seeded with barley (*Hordeum vulgare* L.) in 2009 to control erosion and to stabilize the soil in some sloping areas. The percent coverage by understory vegetation (excluding moss and lichen) was assessed using a 50 × 50 cm quadrat and was 63 and 68% in August of the first (2014) and the second (2015) growing seasons, respectively, with dominant plant species consisting of *Calamogrostis canadensis*

[Michx.] Beauv., *Agropyron trachycaulum* [Link] Malte, *Hordeum vulgare* L., *Carex spp*., *Epilobium angustifolium* L., *Melilotus alba* Desr, *Taraxacum officinale* Weber, *Lathyrus venosus* Muhl., *Sonchus arvensis* L., and *Erigeron canadensis* L.

The soils of the natural ecosystems nearby the study site are xeric to sub-hydric Brunisols and Luvisols in uplands and Gleysols and Organic soils in lowlands (Oil Sands Vegetation Reclamation Committee 1998). The soil of our study site was further characterized by analyzing physical and chemical properties after collecting soil samples from plots (see below for the establishment of the plots) (Table 4-1). Soil samples were collected in June 2014 using an auger at two depths (0−10 and 10−30 cm) at five randomly selected points in each plot and composited for each depth. The samples were analyzed for total carbon (TC) and total N (TN) by the dry combustion method using an automated elemental analyzer (NA-1500 series, Carlo Erba, Milan, Italy), NH_4^+ with the indophenol blue method (Keeney and Nelson 1982) and NO_3^- with the vanadium oxidation method (Miranda et al. 2001), respectively, pH at 1:2 (w/v) of soil:$CaCl_2$ solution (0.01 molL^{-1}) using a pH meter (Orion, Thermo Fisher Scientific Inc., Beverly, MA, USA), electrical conductivity at 1:1 (w/v) of soil:water using an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA, USA). Bulk density of the soils was measured using a steel corer (98.12 cm^3).

2.3 Experimental design and plot establishment

The experimental design was a completely randomized block design with two treatments: nursery fertilization regime (conventional vs. exponential fertilization) and understory vegetation (i.e., weed) control (weed-intact vs. weed-removed) in four replicated blocks to block out possible environmental gradients such as slope and weed density. In June 2014 (six years after reclamation), 16 plots (each plot size was 6 × 6 m) were established with a 2 m buffer zone between the adjacent plots. In each plot, 36 seedlings were transplanted with 1 m spacing on June 12 three days after being taken out of cold storage. In total, 576 seedlings (2 fertilization regimes × 2 weed control treatments × 36 seedlings per plot × 4 replications) were planted in the plots. Measurements of growth and N uptake were conducted with 25 seedlings located at the center of each plot to avoid the edge effect. In the weed removal treatment, weeds were controlled manually by cutting the aboveground parts every three weeks during the active growing season from May to September. Seedlings were grown until the end of active growing season of 2015 under natural conditions except for the weed control.

2.4 Measurement of seedling growth and N uptake

Seedling survival rate was measured at the end of the growing season in 2014 and 2015. Seedling height and root collar diameter (RCD) at ground level were measured at the time of transplanting in June and at the end of the active growing season in September 2014 and 2015. Shoot height was measured with a meter stick (1 mm accuracy) and RCD was measured with a Vernier caliper (0.05 mm accuracy) in two directions perpendicular to each other and the average value was used to represent the RCD of seedlings. Absolute growth was calculated as the difference between final and initial size and percentage growth was calculated as the percentage change in size at the end of the first and second growing seasons relative to the initial size.

Biomass, total N and ^{15}N in seedling components were also determined at transplanting and at the end of the first and second growing seasons. At transplanting, five seedlings of each nursery fertilization regime were randomly selected from each of three storage boxes representing different replications in the cold storage. Seedlings were composited per replication separately for conventional and exponential fertilization treatments and were used for measurement of biomass and total N (concentration and % ^{15}N). At the end of the growing season, two (the first season) or four (the second season) seedlings were randomly selected and destructively harvested by cutting the seedling at ground level and roots were excavated intact with a shovel. A smaller number of seedlings were harvested at the first sampling to minimize site disturbance and to leave seedlings for the second harvest. The roots were washed onto a sieve (0.5 mm mesh) to remove soil and peat materials. The shoots and roots were then rinsed three times with deionized water. For seedlings sampled from the plots, shoots were separated into new (current year) and old (previous year) shoots which were further separated into stems and needles. Seedling components were composited per plot and dried at 70 °C for 72 h to determine biomass. The plant samples were mixed thoroughly and a portion of the samples were ground to fine powder with a Mixer Mill MM 200 ball mill (Thomas Scientific, Swedesboro NJ) for N analysis. The N concentration, ^{15}N, and ^{13}C isotope compositions were analyzed using a stable isotope ratio mass spectrometer (Thermo Delta Plus XP IRMS, Waltham, MA, USA) linked to a CN analyzer (Costech 4010, Valencia, CA, USA) at the Stable Isotope Facility of the

University of Wyoming, Laramie, WY, USA. Nitrogen and C isotope compositions were calculated as,

$$\delta\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000,$$

Where R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$, and the standards are the Pee Dee Belemnite (PDB) standard for C and atmospheric N_2 for N.

The N in the newly formed seedling components (stem and needle) was separated into N derived from old plant tissue ($NDFP_{old}$) and that from soil (NDFS). The $NDFP_{old}$ was calculated using the following equation (Hauck and Bremner 1976):

$$NDFP_{old} = TN_{new} \times (A_{new} / A_{old})$$

where TN_{new} is the total N content in the new tissue calculated as N concentration (mg g^{-1}) × dry weight of biomass (g), A_{new} is ^{15}N atom % excess of N (^{15}N atom % − 0.3663) in the new tissue, and A_{old} is ^{15}N atom % excess of N in the old tissue. The A_{old} was calculated as the biomass-weighted mean ^{15}N atom % of needles, stems and roots of seedlings at transplanting for the first growing season and as that of current and 1-year old needle and stem and root of the seedlings measured at the end of the first growing season for the second growing season (see Table 4-2 for ^{15}N atom % of seedling components). The NDFS was calculated as,

$TN_{new} - NDFP_{old}$.

2.5 Statistical analyses

The effect of fertilization regime during nursery production on seedling size, biomass, total N and ^{15}N was analyzed by a one-way analysis of variance (ANOVA) using the Proc MIXED model (SAS 9.2, SAS Institute, Cary NC):

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

where Y_{ij} is a dependent variable, μ is the overall mean, α_i is the fixed effect of the i^{th} fertilization regime and ε_{ij} is the random error within the experiment. For field-grown seedlings, the treatment effects (nursery fertilization and weed control) on the growth and N uptake of seedlings were analyzed by a two-way ANOVA:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijk}$$

where Y_{ij} is a dependent variable, μ is the overall mean, α_i is the random effect of i^{th} block, β_j and γ_k are the fixed effects of the j^{th} and k^{th} fertilization and weed control treatments, respectively, $(\beta\gamma)_{jk}$ is the fixed effect of interaction of the two treatments, and ε_{ijk} is the random error within the experiment.

Before implementing the ANOVA, the data were assessed for normality of distribution (Shapiro-Wilk test) in residuals using PROC UNIVARIATE. A log-transformation (base 10) was applied for NDFS of new needle and new stem of the first growing season data and $NDFP_{old}$ of new needle and NDFS of new needle and stem of the second growing season data to meet the assumption of normality, and back-transformed data are reported. Tukey's test at $P < 0.05$ was used to determine statistical significance of means separation of variables in all analyses.

3. Results

3.1 Seedling growth and N uptake at nursery

Total seedling biomass did not differ between conventional and exponential fertilization treatments; but exponential fertilization produced greater ($P = 0.045$) root biomass (Table 4-3) resulting in greater root:shoot ratio, with a ratio of 0.34 ± 0.02 (SE) in conventional and 0.52 ± 0.01 in exponential fertilization. Height and RCD were greater ($P < 0.001$ for both) in conventionally than in exponentially fertilized seedlings (Table 4-4). Exponential fertilization increased ($P = 0.003$) N concentration of stem by 36 % as compared to conventional fertilization but did not affect that of needle and root (Table 4-3). The N content in the seedling did not differ between fertilization regimes regardless of the seedling component (Table 4-3). Exponential fertilization in the nursery didn't increase nutrient content in the seedlings produced.

3.2 Seedling growth and N status after outplanting

Survival rates of planted seedlings were 92 and 89% in the first and second growing seasons, respectively, and the rate was similar across the treatments (data not shown). The growth and N uptake of the seedlings were independently affected by nursery fertilization and field weed control treatments without interactions between them (Tables 4-5 and 4-6). The effects of nursery fertilization and field weed control on seedling biomass production were only significant in the second but not in the first growing season (Table 4-5). In the second growing season, seedling with conventional fertilization had greater old shoot ($P = 0.014$) and root biomass ($P = 0.034$) than those growing with exponential fertilization in the second season. Weed control increased biomass of new shoot (by 63%, $P = 0.001$), root (by 46%, $P = 0.005$) and total biomass (by 29%, $P = 0.010$) over the weed-intact treatment.

The absolute height and percent height growth were affected by nursery fertilization treatment in both growing seasons (Figur 4-1 and Table 4-4). The absolute height was greater in conventional fertilization in both growing seasons (Figure 4-1A). While the percent height increment of seedlings during two growing seasons was greater ($P = 0.001$ in the first and $P < 0.001$ in the second growing seasons) for exponential fertilization (87 and 135%, respectively) than for conventional fertilization (57 and 89%, respectively) (Figure 4-1B).

Weed removal had significant effects on absolute as well as percent RCD growth in the second but not in the first growing season (Figure 4-1C and 4-1D). The weed removal treatment had 25% ($P < 0.001$) greater absolute RCD growth compared to the weed-intact treatment in the second growing season. The percent RCD increment in weed removed plots (152%) was significantly greater ($P < 0.001$) than that in the weed-intact plots (104%) in the second growing season.

The N concentration in seedling components decreased over the two growing seasons regardless of the nursery fertilization and field weed control treatment (Table 4-6). In the first growing season, N concentration in seedling components was affected neither by the nursery fertilization nor by field weed control except in old needles in which exponentially fertilized seedlings had greater ($P = 0.033$) N concentrations than conventional ones (Table 4-6). In the second growing season, weed removal increased N concentration by 58% ($P = 0.007$) in new needles and by 34% ($P = 0.011$) in new stems (Table 4-6). The pattern of N content followed that of N concentration (Figure 4-2 and Table 4-6). The nursery fertilization treatment did not affect N content in both growing seasons. Although not significant, weed removal increased N content of new needles by 26% and the total N in the seedling by 25% in the first growing season, while the increase was by 152% ($P = 0.005$) in new needles and by 175% ($P = 0.010$) in new stems in the second growing season (Table 4-6).

3.3 Foliar $\delta^{13}C$, $NDFP_{old}$ and NDFS

There were no interactions between the two treatments (nursery fertilization and field weed control) on foliar $\delta^{13}C$, $NDFP_{old}$ and NDFS. Nursery fertilization significantly affected foliar $\delta^{13}C$ in new needles in the first growing season ($P = 0.048$) but not in the second growing season ($P = 0.794$) but foliar $\delta^{13}C$ in new needles (both growing seasons) and old needles (the first growing season) were affected by weed control (Figure 4-3). Foliar $\delta^{13}C$ was lower in new needles in the first ($P < 0.001$) and second growing season ($P = 0.014$) and old needle in the first growing season ($P = 0.039$) in weed-removed plots than in weed-intact plots. The amount of $NDFP_{old}$ and thus the internal recycling of N were not affected by the nursery fertilization treatment in both growing seasons but were affected by weed removal in the second growing season regardless of the nursery fertilization regime (Figure 4-4A and 4-4B). In the second growing season, weed removal increased $NDFP_{old}$ by 40% ($P = 0.040$) for needles and by 50% ($P = 0.005$) for stems. On the other hand, NDFS was affected by fertilization in the first growing season and weed removal treatment in both seasons (Figure 4-4C and 4-4D). Exponential fertilization increased NDFS in needles by 134% ($P = 0.042$) and weed removal increased NDFS in needles in both the first ($P = 0.043$) and second ($P = 0.006$) growing seasons, while NDFS in stems was greater ($P = 0.008$) only in the first growing season as compared to weed-intact plots. The contributions of $NDFP_{old}$ and NDFS to N in the new tissues changed over the growing seasons (Figure 4-4 and Table 4-7). In the first growing season, the value of $NDFP_{old}$ was greater than that of NDFS but reversed in the second growing season in the weed-removed plots. However in the weed-intact plots, the value of $NDFP_{old}$ was greater than that of NDFS in both growing seasons. The % contribution of $NDFP_{old}$ to total N of new tissues was affected only by weed control but not by fertilization (Table 4-7).

3.4 Relationships of foliar $\delta^{13}C$ and $NDFP_{old}$ with seedling parameters

Regression analyses of the relationship between foliar $\delta^{13}C$ and seedling growth parameters in the second growing season showed that $\delta^{13}C$ was positively correlated with seedling biomass (r^2 = 0.34, P = 0.010), RCD (r^2 = 0.55, P = 0.001) and foliar N concentration (r^2 = 0.79, P < 0.001) (Figure 4-5). Similarly, $NDFP_{old}$ in the second growing season was positively correlated with RCD (r^2 = 0.50, P = 0.022) and biomass growth (r^2 = 0.58, P = 0.009). Although the relationship of $NDFP_{old}$ with height growth was relatively weak (r^2 = 0.40, P = 0.070), the trend was similar to that of RCD and biomass growth (Figure 4-5).

4. Discussion

4.1 Exponential fertilization increased root:shoot ratio but not nutrient reserve in seedlings

Exponential and conventional fertilization in the nursery produced similar seedling biomass but seedlings growing with exponential fertilization allocated greater biomass to roots resulting into greater root: shoot ratio; seedlings with greater ratios of root: shoot biomass are better able to handle drought and nutrient deficient conditions such as in oil sands reclamation sites where water and N availability are the major limiting factors for the planted trees (Farden et al., 2013; Duan et al., 2015). Although, the height of conventionally fertilized seedlings was consistently greater in nursery production and following both seasons of field growth, the percent of height growth was greater in exponentially fertilized seedlings, this may be linked to the greater ratio of root: shoot biomass of these seedlings in the nursery stage. Burdett et al. (1984) observed greater percent height growth in seedlings with greater root biomass in white spruce and concluded that early growth of planted spruce could be maximized by using seedling stocks with a high root growth capacity.

Exponential fertilization of seedlings during nursery production aims to increase nutrient reserves in seedling components taking advantage of the luxury uptake of nutrients (Timmer, 1997; Salifu and Timmer, 2003 b). Many studies report an increase in the nutrient reserve in exponentially fertilized seedlings of coniferous species such as black spruce (Salifu and Timmer, 2003 b) and Chinese-fir (*Cunninghamia lanceolata* [Lamb] Hook) (Xu and Timmer, 1999). In our previous studies, we found that the nutrient reserve of the seedling was significantly increased in trembling aspen (Pokharel and Chang, 2016) and jack pine (unpublished data) by a similar method of modified exponential fertilization used during nursery production. But in this experiment, the modified exponential fertilization technique did not increase the nutrient reserve in white spruce seedlings. The main reason for the lack of increase in the nutrient reserve in white spruce seedlings could be that the rate of fertilizer we used in the modified exponential fertilization regime was inappropriate and did not synchronize adequetly with the exponential growth and nutrient status of the seedlings of this species. Other possible reasons for this include a greater depletion of nutrient levels in exponentially fertilized seedlings during hardening. The seedlings were hardened for two weeks without supplying nutrients; but there is considerable growth in seedlings during the hardening phase which could lead to nutrient dilution (Miller and Timmer, 1994). A greater depletion in N concentration of exponentially fertilized seedlings has been observed in black spruce (Imo and Timmer, 1999) and Lutz spruce (*Picea x lutzii*) (Jonsdottir et al., 2013). It is also possible that the smaller size of vacuoles in the cells of white spruce needles could be a factor making them unsuitable to store excess N in the form of most common storage proteins such as vegetative storage proteins (VSPs) in conifers (Binnie et al., 1994). In white spruce, VSPs were observed in stems and roots but not in needles suggesting that white spruce needles may not be capable of storing excess N in the form of VSPs (Binnie et al., 1994). Therefore, further research is needed to optimize the nursery exponential fertilization regime to increase the nutrient reserve in white spruce seedlings during nursery production.

Although, in a separate experiment, I observed a 2.5- fold increase in N retranslocation by exponential fertilization in jack pine seedlings planted on reclaimed soils (unpublished data), $NDFP_{old}$ in white spruce planted in a similar environment (in this experiment) was not affected by nursery fertilization. The lack of exponential fertilization effect on N retranslocation in the first and second year field growth is linked to similar nutrient reserves in the needles of seedlings at the end of nursery production. Greater N reserve in the stem of exponentially fertilized seedlings did not affect $NDFP_{old}$, indicating that the other parts of the seedling such as needles may be the main site for nutrient storage and retranslocation in white spruce, similar to other spruce (Grossnickle, 2000). Our study also suggests that N retranslocation in white spruce is not affected by morphological features such as height, RCD and root:shoot biomass. Nutrient reserve in the seedling did not affect N retranslocation in white spruce. After outplanting, N concentration of seedling components decreased continuously over the two growing seasons because of growth dilution, and the decrease was uniform across the fertilization treatments. Current-year foliar N concentrations in field growth in both fertilization regimes were less than the critical level of 1.3% foliar N concentrations of white spruce (Ballard and Carter 1986), indicating that there was low N availability in the soil for the growth of planted seedlings.

4.2 Seedling growth, foliar $\delta^{13}C$ and soil N availability

Weed competition has been widely reported to affect the growth of coniferous as well as broadleaved seedlings in various environments. Munson et al., (1995) observed significant increase in growth of white pine (*Pinus strobus* L.) and white spruce seedlings planted in a clear-cut area subject to vegetation control. Aspen seedlings planted in oil sands reclaimed soils were found to increase biomass, height and RCD in weed removal treatment on sites with LFH cover soil (Pokharel and Chang, 2016). Removing competitive weeds can increase the availability of resources such as water, nutrients and light by decreasing competition for these resources with the increased availability of those resources will benefit the growth of crop plants. In a highly competitive site, the growth of newly planted seedlings can be limited by low soil nutrient, water and light availabilities. In our study, understory vegetation was cut short around the seedlings in the weed-intact plots to eliminate potential light limitation for seedling growth.

Although available nutrients and water in the soil could be major limiting factors for the growth of planted seedlings on reclaimed oil sands sites (Farden et al., 2013; Sloan and Jacobs, 2013; Duan et al., 2015), increased soil N availability by weed removal was found to be a major factor to improve the growth of planted white spruce seedlings in our study. The difference in foliar $\delta^{13}C$ (in the second year growth) between weed control treatments indicates that there was a significant influence of weed removal either on water or nutrient availability in the soil. The variation in foliar $\delta^{13}C$ is primarily due to changes in CO_2 diffusion rate linked with changes in available water or nutrients (Livingston et al., 1999). Less negative foliar $\delta^{13}C$ in weed removal plots could indicate the possible decrease in soil water availability caused by the potential increase in evaporation on the soil surface. However, we didn't find differences in soil moisture content (data not shown) between weed-intact and weed-removed plots in this study. This suggests that variation in foliar $\delta^{13}C$ in white spruce seedlings was more likely attributed to the increase in soil nutrient availability caused by weed removal. In both irrigated and dryland conditions, Livingston et al. (1999) found that N stressed seedlings of white spruce had the lowest foliar $\delta^{13}C$. A positive correlation between foliar $\delta^{13}C$ and seedling growth also supports that the growth of this species was affected by limitation of CO_2 diffusion linked to the limitation on carboxylation efficiency resulting from nutrient deficiency, but not by water limitation. Foliar $\delta^{13}C$ is usually positively correlated with growth when the variation in foliar $\delta^{13}C$ is related to the changes in carboxylation efficiency (Flagan and Johnsen, 1995; Choi et al., 2005). Moreover, available water is less likely a limiting factor on sites reclaimed with overburden substrate and PMM cover soil because of its high water holding capacity. The positive correlation between foliar $\delta^{13}C$ and the corresponding N concentration observed in this study also indicates that photosynthesis was limited by low N availability.

4.3 Weed removal increased $NDFP_{old}$ and NDFS

The increase in $NDFP_{old}$ and NDFS as well as biomass of new tissues by weed removal supports our second hypothesis. However, such positive effects were evident in the second, but not in the first growing season and this suggests that seedling growth was primarily dependent on the internal nutrient reserve rather than the resource available in the soil immediately after outplanting (Burdette et al., 1984). Spruce seedlings are known to have slow initial root development, and thus the interaction between roots and rhizosphere soils are most likely to develop in subsequent years after transplanting (Burdett et al., 1984). In the second growing season, roots were in better contact with the soil increasing their ability to exploit a greater volume of soil to absorb soil water and nutrients (Nambiar and Sands, 1993).

On average, N retranslocation contributed about 78% of the total N demand of new needles in the first year growth, indicating that the new growth soon after outplanting was sustained mainly by internal N remobilization. But in the second year growth, the contribution of $NDFP_{old}$ to new growth demand dropped to less than 50% in weed- removed plots. This suggests that N retranslocation in white spruce seedling is very important to sustain new growth immediately after outplanting; however, its contribution decreases sharply in subsequent years. In a white spruce stand established with bare-root seedlings, McAlister and Timmer (1997) also observed very small or negative net nutrient uptake from the soil by the plants in the first growing season after outplanting and concluded that nutrient accumulation in new growth was met mostly by internal rather than external nutrient sources. The restricted N uptake from the soil as a result of poor root-soil contact caused by slow root regeneration and extension is usually limited to the first growing season after outplanting. In subsequent years, the roots of transplanted seedlings establish firm contact with the soil, N uptake from the soil rapidly increases; hence the dependence on N retranslocation will be reduced (McAlister and Timmer, 1997). In the first year growth, weed removal didn't affect growth as well as $NDFP_{old}$; but in the second year growth, weed removal increased new tissue growth, resulting in an increase in $NDFP_{old}$. When the N demand of new tissue growth cannot be met by N uptake from the soil, plants require increasing N remobilization to meet the demand. The result suggests that the N retranslocation in white spruce is related to the growth rate of new tissue (Nambiar and Fife, 1987). In a pot study of N retranslocation in Sitka spruce (*Picea sitchensis* (Bong.) Carr.) Millard and Proe (1993) also showed that N retranslocation was controlled by the sink strength of current-year growth. However, positively significant relationships between $NDFP_{old}$ and growth parameters such as biomass and RCD growth in our study are opposite to Munson et al. (1995), suggesting that some ecological processes other than sink strength of current year growth controlled N retranslocation in white spruce. Weed removal did not only increase the N uptake from the soil, but also increased the percent contribution of NDFS in new tissue growth. As a result of increased N availability in the soil by weed removal, the seedlings took up more N from the soil in weed-removed plots.

5. Conclusions

Nursery exponential fertilization in white spruce did not increase the nutrient reserve in the seedlings but increased the root:shoot ratio of the seedlings obtained at the end of nursery production. In the field experiment, nursery exponential fertilization increased the percent height growth but not seedlings component biomass and N retranslocation in the seedlings. Weed removal in the field experiment increased soil N availability to the planted seedlings as demonstrated by foliar $\delta^{13}C$ analysis. Nitrogen uptake from the soil, N retranslocation within the seedlings, and growth of the seedlings were also increased by weed removal. New tissue growth after outplanting of white spruce seedlings was mainly sustained by $NDFP_{old}$, contributing more than 78% of the new tissue demand in the first year while the contribution decreased to less than 50 % in the second year in weed-removed plots. Internal nutrient cycling in white spruce seedlings planted on reclaimed oil sands soils appears to be important during early establishment (i.e. the first year) but soil nutrient availability plays a more significant role in seedling growth in subsequent years. Vegetation management in the form of weed control was effective to improve white spruce seedling growth by increasing soil N availability. We recommend further research on the optimization of exponential fertilization to build up nutrient reserve in white spruce seedlings during nursery production. Long term study of weed control at various levels of weed competition to improve growth of white spruce seedlings on reclaimed soils is required to implement weed control in vegetation management practices for land reclamation in the oil sands.

Table 4-1 Selected properties of the soil in the study site.

Depth (cm)	pH	Electrical conductivity (ds m^{-1})	Bulk density (g cm^{-3})	Total C (g kg^{-1})	Total N (g kg^{-1})	C:N	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
0-10	6.71 (0.06)	1.22 (0.15)	0.59 (0.12)	93.12 (19.22)	3.71 (0.9)	26.77 (2.22)	7.20 (0.9)	6.05 (1.38)
10-30	6.85 (0.07)	1.17 (0.14)	0.69 (0.15)	88.70 (28.65)	3.45 (1.35)	29.45 (2.72)	5.30 (1.16)	3.62 (1.24)

Values are means with standard errors in the parentheses (n=16).

Table 4-2 Atom % ^{15}N (mean ± SE, n = 4) in seedling components after outplanting over two growing seasons.

Treatments	First growing season					Second growing season	
	New needle	Old needle	New stem	Old stem	Root	New needle	New stem
C	0.92 (0.05)	0.84 (0.02)	0.92 (0.05)	0.85 (0.03)	0.85 (0.05)	0.73 (0.05)	0.77 (0.04)
W	0.89 (0.08)	0.85 (0.09)	0.93 (0.03)	0.89 (0.11)	0.84 (0.08)	0.57 (0.05)	0.58 (0.06)
E	0.99 (0.02)	1.19 (0.02)	1.01 (0.01)	1.09 (0.02)	1.03 (0.02)	0.90 (0.03)	0.86 (0.04)
E+W	0.84 (0.07)	1.03 (0.08)	0.95 (0.08)	0.90 (0.06)	0.79 (0.09)	0.63 (0.04)	0.65 (0.06)

Abbreviations: C = conventional + weed-intact, W = conventional + weed-removed, E = exponential + weed-intact, E+W = exponential + weed removed.

Table 4-3 Biomass and concentration, content and ^{15}N atom % of nitrogen (N) of seedlings with different fertilization regimes (conventional, CF; exponential, EF) before transplanting.

Seedling components	Dry mass (g seedling^{-1})		N concentration (mg N g^{-1})		N content (mg N seedling^{-1})		Atom % ^{15}N	
	CF	EF	CF	EF	CF	EF	CF	EF
Needle	2.61 (0.35)	2.09 (0.08)	30.15 (0.91)	34.07 (3.55)	79.32 (12.41)	71.30 (7.77)	0.98 b (0.01)	1.19 a (0.04)
Stem	1.41 (0.24)	1.35 (0.12)	16.61 b (0.38)	22.53 a (0.86)	23.54 (4.33)	30.45 (3.26)	0.91 b (0.03)	1.15 a (0.02)
Root	1.36 b (0.15)	1.79 a (0.10)	22.25 (1.10)	23.02 (0.96)	30.65 (4.90)	41.31 (2.71)	0.96 (0.01)	1.02 (0.07)
Needle + Stem + root	5.38 (0.68)	5.24 (0.27)	24.59 (0.78)	27.43 (1.98)	133.51 (20.40)	143.07 (7.60)	0.95 b (0.02)	1.14 a (0.04)

Values are means with standard errors in the parentheses (n=3).

Different letters indicate significant differences in the values between fertilization regimes.

Table 4-4 Effects of fertilization (in the nursery) and weed control (in the field) on height and root collar diameter of seedlings over two growing seasons.

Treatments	Height					Root collar diameter (mm)				
	Initial	First growing season		Second growing season		Initial	First growing season		Second growing season	
		Final	Growth	Final	Growth		Final	Growth	Final	Growth
	(cm)	(cm)	(%)	(cm)	(%)	(mm)	(mm)	(%)	(mm)	(%)
Fertilization										
Conventional	25.76a (1.32)	39.88a (1.07)	57.26b (8.12)	48.05a (0.86)	89.21b (7.79)	4.11a (0.07)	6.01 (0.17)	46.11 (3.97)	9.09 (0.43)	121.67 (11.86)
Exponential	18.68b (0.18)	35.05b (0.85)	87.62a (4.32)	43.94b (0.86)	135.30a (4.55)	3.63b (0.02)	5.30 (0.30)	46.56 (8.02)	8.53 (0.44)	135.67 (11.18)
Weed control										
Weed-intact	22.31 (1.74)	38.90 (1.25)	78.68 (8.62)	45.90 (0.77)	112.17 (11.91)	3.86 (0.13)	5.49 (0.32)	42.28 (6.98)	7.85b (0.21)	104.48b (7.84)
Weed-removed	22.13 (1.53)	36.02 (1.16)	66.21 (8.06)	46.08 (1.45)	112.33 (9.55)	3.87 (0.08)	5.82 (0.21)	50.39 (5.17)	9.77a (0.30)	152.86a (7.15)
Effects	ANOVA (P > F)[a]									
Fertilization (F)	**<0.001**	**0.001**	**0.006**	**0.008**	**<0.001**	**<0.001**	0.694	0.961	0.154	0.214
Weed control (W)	0.898	0.029	0.203	0.895	0.986	0.906	0.381	0.399	**<0.001**	**<0.001**
F×W	0.716	0.324	0.753	0.762	0.549	0.496	0.466	0.599	0.736	0.598

Different letters indicate significant differences in the values within fertilization regime and weed control treatments.

Values are means with standard errors in the parentheses (n = 4).

[a]Values in bold indicate significance at $P < 0.05$.

Table 4-5 Effects of fertilization regime (in seedling nursery production) and weed control (in the field) on biomass of seedling components over two growing seasons after outplanting.

Treatments	First growing season				Second growing season			
	New shoot	Old shoot	Root	Total	New shoot	Old shoot	Root	Total
	(g seedling^{-1})							
Fertilizer regime								
Conventional	6.51 (0.55)	7.65 (0.56)	3.36 (0.33)	17.52 (1.36)	7.74 (0.89)	15.65a (0.57)	6.06a (0.42)	29.46 (1.55)
Exponential	6.54 (0.62)	6.02 (0.61)	3.31 (0.25)	15.88 (1.44)	7.64 (0.94)	12.71b (0.83)	4.65b (0.65)	25.01 (2.32)
Weed control								
Weed-intact	6.69 (0.71)	6.81 (0.81)	3.12 (0.26)	16.62 (1.68)	5.84b (0.21)	13.60 (0.81)	4.35b (0.42)	23.79b (1.09)
Weed-removed	6.36 (0.41)	6.86 (0.48)	3.55 (0.31)	16.78 (1.14)	9.55a (0.82)	14.76 (0.94)	6.36a (0.52)	30.67a (2.15)
Effects	ANOVA ($P > F$)[a]							
Fertilizer regime (F)	0.963	0.076	0.919	0.445	0.920	**0.014**	**0.034**	0.074
Weed control (W)	0.715	0.953	0.332	0.941	**0.001**	0.285	**0.005**	**0.010**
F×W	0.524	0.208	0.465	0.345	0.384	0.883	0.514	0.914

Values are means with standard errors in the parentheses (n=4).

[a]Values in bold indicate significance at $P < 0.05$.

Different letters indicate significant differences in the values within fertilization regime and weed control treatments

Table 4-6 Effects of fertilization (in nursery) and weed control (in field) on nitrogen concentration (TN) and N content in seedling components after outplanting over two growing seasons.

Treatments	First growing season						Second growing season			
	New needle		New stem		Total		New needle		New stem	
	TN	N content	TN	N content	TN	N content	TN	N content	TN	N content
	%	mg seedling^{-1}	%	mg seedling^{-1}	%	mg seedling^{-1}	%	mg seedling^{-1}	%	mg seedling^{-1}
Fertilization										
Conventional	1.04 (0.11)	48.79 (5.49)	0.92 (0.12)	15.69 (3.06)	0.70 (0.06)	147.35 (11.72)	1.03 (0.13)	64.78 (13.26)	0.69 (0.06)	13.89 (3.74)
Exponential	1.28 (0.13)	57.86 (7.50)	1.02 (0.11)	18.57 (2.76)	1.00 (0.13)	163.40 (20.49)	0.96 (0.12)	60.32 (15.99)	0.65 (0.05)	13.51 (3.58)
Weed control										
Weed-intact	1.03 (0.10)	47.27 (2.62)	1.02 (0.12)	16.67 (2.89)	0.75 (0.08)	138.26 (5.03)	0.78 b (0.05)	35.51 b (2.79)	0.58 b (0.02)	7.30 b (0.51)
Weed-removed	1.29 (0.14)	59.39 (8.66)	0.91 (0.11)	17.43 (3.07)	0.95 (0.13)	172.49 (21.60)	1.22 a (0.12)	89.58 a (14.71)	0.77 a (0.06)	20.10 a (3.85)
Effects	ANOVA (P > F)[a]									
Fertilization (F)	0.176	0.353	0.568	0.515	0.051	0.493	0.609	0.786	0.520	0.931
Weed control (W)	0.146	0.221	0.557	0.848	0.178	0.157	**0.007**	**0.005**	**0.011**	**0.010**
F×W	0.640	0.810	0.864	0.265	0.509	0.374	0.745	0.837	0.813	0.813

Values are means with standard errors in the parentheses (n=4).

[a]Values in bold indicate significance at $P < 0.05$.

Different letters indicate significant differences in the values within fertilization regime and weed control treatments.

Table 4-7

Effects of fertilization (in nursery) and weed control (in field) on contribution of $NDFP_{old}$ (%) to total nitrogen of new tissues over two growing seasons.

Treatment	First growing season		Second growing season	
	New needle	New stem	New needle	New stem
Fertilization				
Conventional	85.6 (5.9)	89.1 (4.5)	54.5 (7.8)	61.0 (10.8)
Exponential	72.1 (5.1)	79.0 (8.4)	65.9 (7.9)	65.1 (7.5)
Weed control				
Weed-intact	87.2 (4.7)a	88.0 (4.21)	74.6 (4.4)a	77.2 (7.6)a
Weed-removed	71.4 (5.9)b	80.2 (8.9)	45.8 (7.3)b	48.9 (7.6)b
Effects	ANOVA (P > F)[a]			
Fertilization (F)	0.061	0.369	0.204	0.710
Weed control (W)	**0.044**	0.483	**0.005**	**0.023**
F×W	0.815	0.955	0.582	0.217

Values are means with standard errors in the parentheses (n=4).

[a] Values in bold indicate significance at $P < 0.05$.

Different letters indicate significant differences in the values within fertilization regime and weed control treatments.

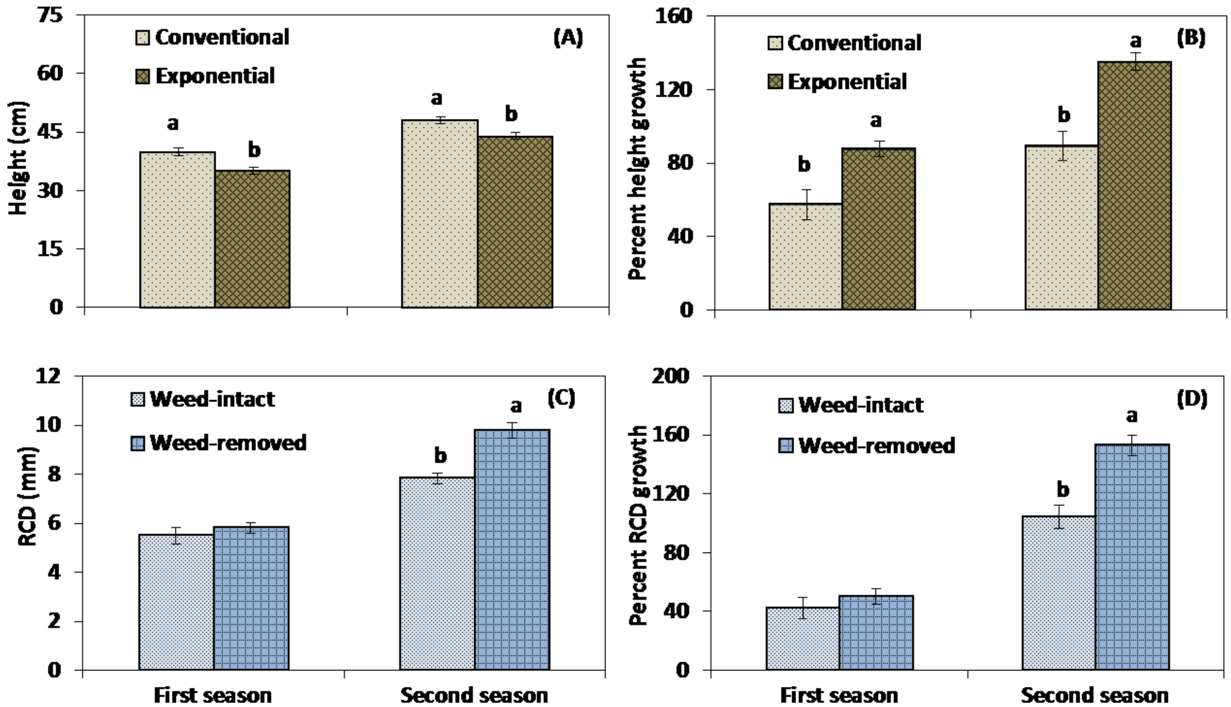

Figure 4-1 Seedling height and RCD (root collar diameter) over two growing seasons after outplanting: A) final height, B) relative height growth, C) final RCD, and D) relative RCD growth. Different letters represent significant differences among treatments at $P < 0.05$. Vertical bars are standard errors of means (n = 4).

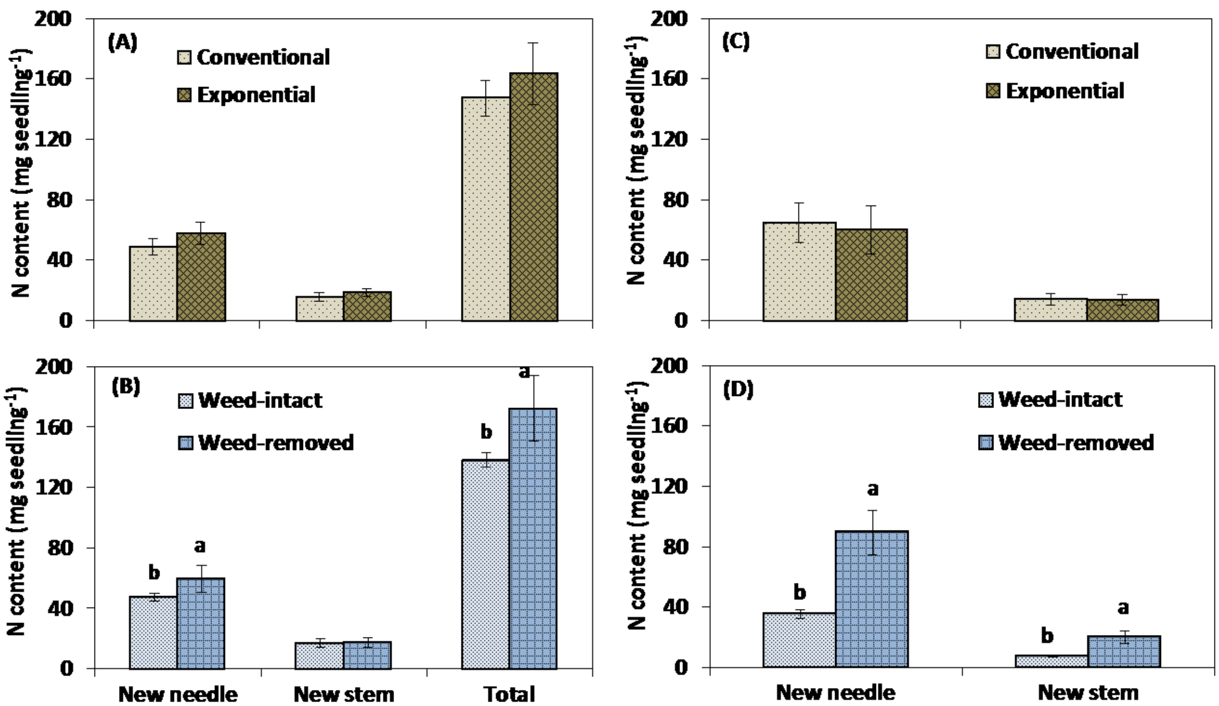

Figure 4-2 Effects of fertilization and weed removal treatments on total nitrogen (N) in seedling components over two growing seasons: A) and B) the first growing season and C) and D) the second growing season. Different letters represent significant differences between the treatments at $P < 0.05$. Vertical bars are standard errors of means (n = 4).

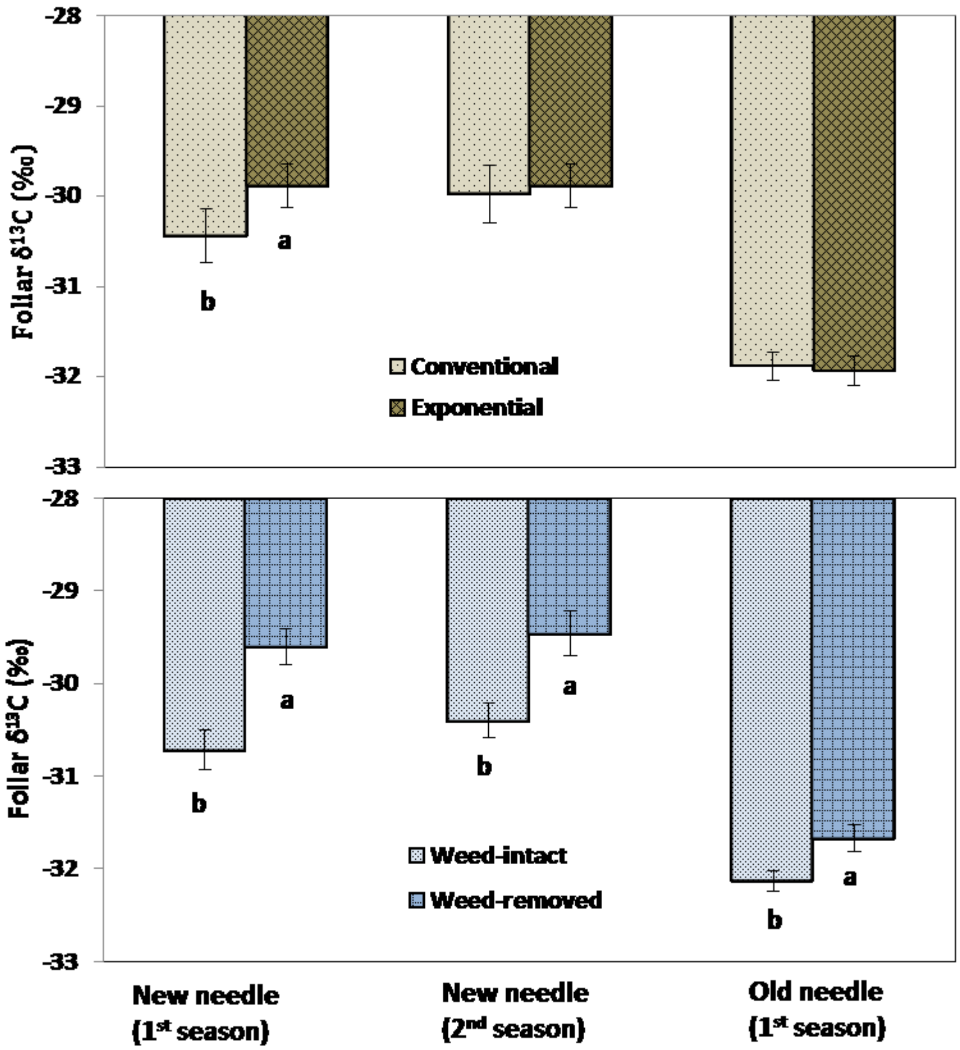

Figure 4-3 Foliar $\delta^{13}C$ of seedlings in the first and second growing seasons. Different letters represent significant differences between the treatments at $P < 0.05$. Vertical bars are standard errors of means (n = 4).

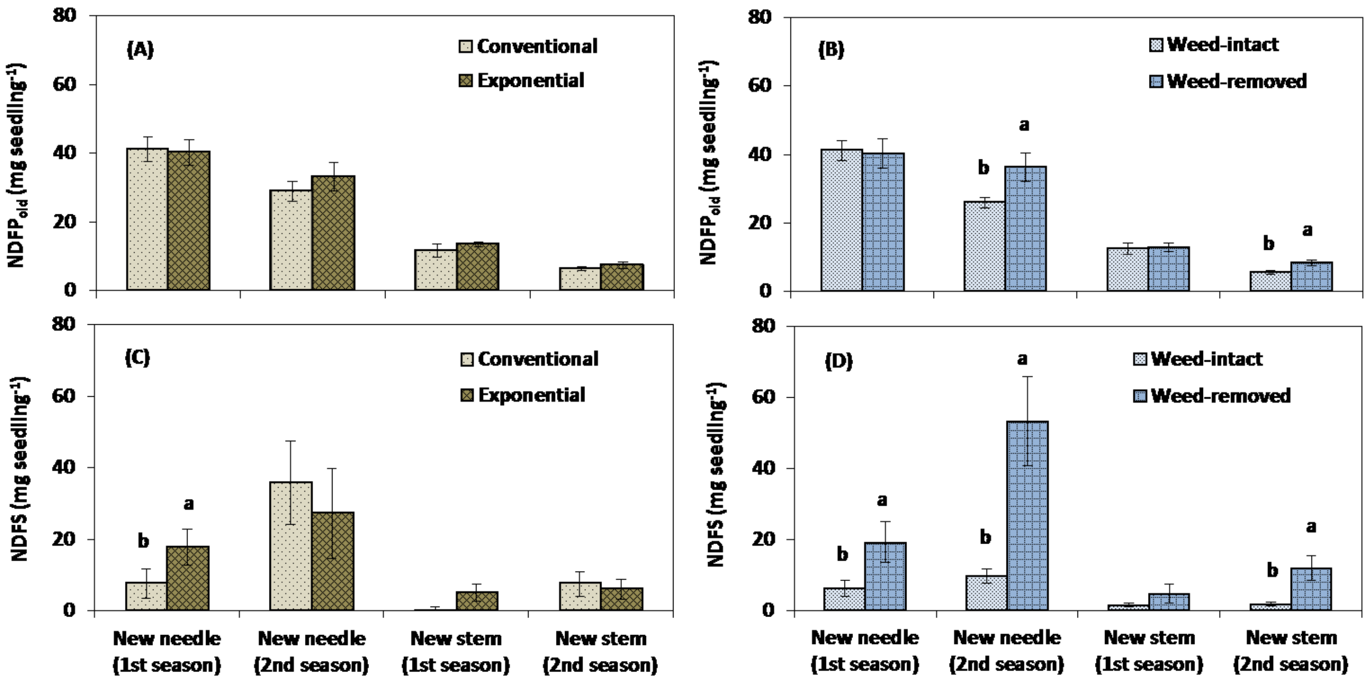

Figure 4-4 Effects of fertilization and weed removal treatments on nitrogen (N) derived from older components of the plant ($NDFP_{old}$) and N derived from the soil (NDFS) by new tissues in the first and second growing seasons: A) and B) $NDFP_{old}$, and C) and D) NDFS. Different letters represent significant differences between the treatments at $P < 0.05$. Vertical bars are standard errors of means (n = 4).

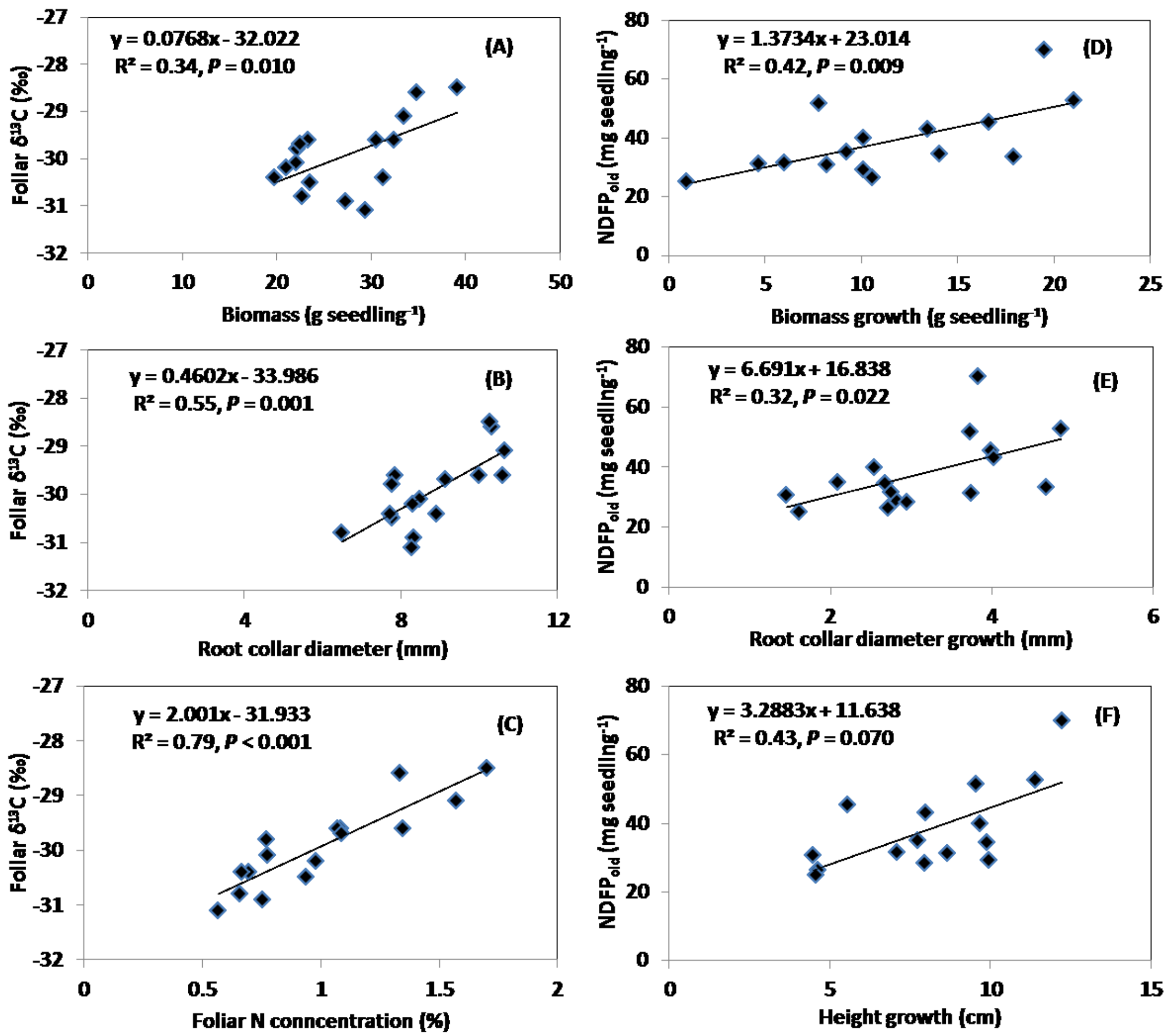

Figure 4-5 The relationship of foliar $\delta^{13}C$ and nitrogen derived from older components of the plant ($NDFP_{old}$) with the growth parameters of seedlings in the second growing season (n =16).

CHAPTER 5 SUMMARY, CONCLUSIONS AND FUTURE RESEARCH RECOMMENDATION

1. Research Overview

The overall objective of this research was to examine the effects of nutrient loading in seedlings during nursery fertilization and weed control in the field on vegetation re-establishment on reclaimed soil in oil sands region. Seedlings of trembling aspen, jack pine and white spruce were produced in nursery with exponential and conventional fertilization regimes and planted on weed competitive reclaimed sites amended with peat mineral soil mix (PMM) and LFH mineral soil mix (LFH). The field experiments were established on an overburden dump at the Suncor Energy Inc mine, approximately 25 km north of Fort McMurray. Field study was conducted from June 2014 to October 2015 to assess the survival rate of seedlings, nitrogen retranslocation and uptake from the soil and growth of these planted seedlings. To assess the growth of the seedlings, height, root collar diameter, seedlings component biomass, foliar and root size were measured for two years in field experiments. Nutrient status of the seedlings was evaluated based on N concentration and total N content in seedling components. Nitrogen retranslocation and N uptake from the soil were determined by using ^{15}N isotope labeling in the seedlings during nursery production. Vector analysis was also used in jack pine to assess the growth and nutrient status of the seedlings at nursery production and in field growth.

2. Summary of research results

2.1 Growth and N retranslocation in aspen seedlings

At the end of nursery production, exponentially fertilized seedlings had greater nutrient reserves than that of conventionally fertilized ones but had similar size and biomass. In field growth, exponential fertilization and weed removal significantly increased seedling size on both PMM and LFH sites. The effects of nursery fertilization on seedlings height and RCD growth were similar on PMM and LFH sites but the effects of weed removal were more pronounced on LFH than on PMM site. In terms of biomass growth, the effect of exponential fertilization was consistent in first and second growing season but the effect of weed removal was greater on second growing season than in the first growing season. Percent root biomass allocation was not affected by nursery fertilization or weed competition while new stem biomass allocation was significantly increased by both exponential fertilization and weed removal on the PMM site and by weed removal on the LFH site. The N concentration was not significantly affected by both treatments at the end of first growing season of field growth; however there was a greater reduction of N concentration in field growth compared with nursery stage in exponentially fertilized seedlings than in conventionally fertilized seedlings. Exponential fertilization increased N content in the seedlings on both PMM and LFH site while weed competition reduced N content only on LFH site.

The N retranslocation from the old to new tissue was much greater than N uptake from the soil in both treatments on both soils. On average, 80% of total N demand of new tissue growth was met by N retranslocation on PMM site and 73% on the LFH site during first year growth of seedlings after outplanting. There was no significant difference in percent N retranslocation among the treatments but the total N derived from plant (NDFP) was significantly increased by exponential fertilization and weed control on PMM as well as on LFH site. The N derived from soil (NDFS) was increased by weed removal treatment on LFH site while the effect of exponential fertilization was not significant on both PMM and LFH sites.

2.2 Growth, biomass allocation and N retranslocation in jack pine seedlings

Exponential fertilization increased N concentration and total N in seedling components but did not affect on biomass of seedling during nursery production. Height and RCD of exponentially and conventionally fertilized seedlings were also not significantly different. Vector analysis of biomass, N concentration and N content in the seedlings showed that the exponentially fertilized seedlings had luxury consumption of N during nursery production that induced nutrient reserves in these seedlings. In the field growth, seedling size and biomass were significantly increased by exponential fertilization but not by weed removal treatment. Exponentially fertilized seedlings had greater N concentration in old tissues at the end of first growing season but the N concentration in new tissues were not affected by exponential fertilization. Weed removal treatment did not change the N concentration of new as well as old tissues. Exponential fertilization increased N content of new and old tissues but weed removal treatment was ineffective to change N content of the seedlings. In terms of biomass allocation, greater percent of biomass was allocated to new tissues in exponentially fertilized seedlings. But the percent root biomass allocation was greater in conventionally fertilized than in exponentially fertilized seedlings. Exponential fertilization also increased needle length and decreased root weight ratio and specific root length of the seedlings. Exponential fertilization increased N retranslocation but weed removal treatment decreased. The N uptake from the soil was increased by both exponential fertilization and weed removal treatments. In first year growth, N retranslocation was more important than N uptake from the soil as N retranslocation contributed about 82% in exponentially fertilized and 70 % in conventionally fertilized seedlings to meet the total N demand of new tissues.

2.3 Growth, N uptake from the soil and N retranslocation in white spruce seedlings

Exponential fertilization in white spruce seedlings increased root:shoot biomass but not nutrient content in the seedlings compared to conventional fertilization during nursery production. Biomass production was affected by both nursery fertilization and field weed control only on second but not on first growing season. Exponential fertilization increased percent height growth and weed control increased percent RCD growth in seedlings on second growing season. Foliar N concentration of the seedlings was decreased in field growth regardless of the treatments compared to the seedlings during nursery production. Within the weed control treatment, foliar N concentration and the total N content were significantly increased by weed removal treatment. Seedlings on weed-removed plots had lower foliar $\delta^{13}C$ than on weed-intact plots indicating that weed removal increased soil available N to the planted seedlings. In second growing season, foliar $\delta^{13}C$ was positively correlated with seedling biomass, RCD and foliar N concentration suggesting that the growth of planted white spruce seedling was limited by N availability in the soil. Exponential fertilization didn't affect on N retranslocation within the seedling but increased N uptake from the soil. Weed removal increased both N retranslocation as well as N uptake from the soil. Increase in N uptake by weed removal can be attributed to the increase in soil N availability.

3. Conclusions

Plantation of nursery grown seedling is a common practice in oil sand reclamation in northern Alberta to speed up the vegetation re-establishment process because natural recovery in the reclaimed soil is very slow. But the survival and growth of the planted seedlings on the reclaimed soils are often limited by soil nutrient deficiencies. Several studies have shown that total N, organic and inorganic N in the reclaimed soils are much less than adjacent natural forest soils. High competition of understory vegetation decreases soil N availability to the planted seedlings. In this context, we examined the effects of nursery nutrient loading of seedlings by exponential fertilization on growth, N retranslocation and N uptake from the soil by the seedlings after outplanting on oil sands reclaimed soils. Although similar, the three experiments (for trembling aspen, jack pine and white spruce) were different in terms of fertilization regimes used to produce seedlings in nursery, reclamation sites (PMM and LFH), growth and nutritional parameters analysed and the time of seedling components harvest for nutrition analyses.

Nutrient loading in trembling aspen and jack pine seedlings through exponential fertilization during nursery production increased nutrient reserves in the seedlings without significant change in the biomass. In the field growth, exponentially fertilized seedlings increased seedling size and biomass. N retranslocation was increased by exponential fertilization in both species; the study demonstrated that the increase in nutrient reserve by exponential fertilization could help in improving growth of seedlings after outplanting. The studies concluded that N remobilization in both species is more important than N uptake soon after ouplanting to the field and also demonstrated that building up of nutrient reserves in these seedlings during nursery production is crucial to improve field growth. In aspen, N uptake from the soil was not affected by nutrient pool size in the seedling prior transplantation. The effect of understory vegetation competition in aspen growth was site specific. Understory removal was more effective on LFH site than on PMM site to improve growth of planted seedlings, N retranslocation and uptake from the soil in these seedlings. In field growth of jack pine seedlings, exponential fertilization increased N retranslocation as well as N uptake from the soil that led to the improvement of seedling growth. Exponentially fertilized seedlings of both species allocated greater biomass into metabolically active new tissues than conventionally fertilized ones, validating their improved competitiveness in growth when planted in weed-prone sites. Vector analysis of biomass and nutrient status of jack pine seedlings at nursery production and field growth also demonstrated that the exponential fertilization in these seedlings was useful to improve their growth and nutrient status.

In white spruce, exponential fertilization did not increase nutrient reserve in the seedlings prior outplanting to the field. Although percent growth was improved by exponential fertilization, absolute growth, N retranslocation and N uptake from the soil was not affected by exponential fertilization. Weed control treatment increased soil N availability, N uptake from the soil and growth of seedlings. Nitrogen retranslocation appears more important for the new tissue growth in first year of outplanting but soil N availability and N uptake from the soil appear more important in second year growth.

These studies demonstrated the potential benefits of applying nutrient loading technique through exponential fertilization to enhance vegetation re-establishment on reclaimed soils by improving growth and nutrient status of planted seedlings of aspen and jack pine. In white spruce, further study is required to appropriately optimize exponential fertilization regime to successfully load nutrients into the seedlings during nursery production. Vegetation management by weed control could be used to help seedling growth for aspen on LFH site and white spruce on PMM site. However, the studies examined the field growth performance of exponentially fertilized seedlings of aspen and white spruce for two and jack pine for one growing season; the utility of the nutrient loading technique needs to be operationally tested for the longer term and applied in future land reclamation practices in oil sands region.

4. Future research recommendation

- Long term monitoring: This study was limited for two years growth for aspen and white spruce and one year growth for jack pine. To examine the benefit of exponential fertilization on vegetation re-establishment and productivity of the forest, growth and N retranslocation of these seedlings should be monitored for long term.
- Optimization of exponential fertilization and field testing of other boreal species: This study demonstrated the benefit of exponential fertilization in only two species. Since the optimization of exponential fertilization varies with the time period of nursery culture, growth pattern and nutritional requirement of species, it seems to be important to optimize the exponential fertilization regime for other species which are common in boreal forest.
- Examine the growth of exponentially fertilized seedling on reclaimed soils at the different stages of reclamation. In this study, nursery-grown seedlings were planted on site six years of reclamation. Physical, chemical and biological properties of the reclaimed soil

change significantly with time of reclamation. The effects of exponential fertilization should be tested in reclaimed soils of different ages.

- Examine the growth of exponentially fertilized seedling in tailing sands. The current study was done on reclaimed site with overburden as substrate material for reclamation. In oil sands reclamation in northern Alberta, tailings sand is another common reclamation substrate which has different properties than overburden, so the effect of exponential fertilization on growth response of seedling may be different than that in overburden substrate.
- Fertilizer and moisture effects on growth of exponentially fertilized seedlings: There are few literatures recommending the use of fertilizer to increase the nutrient availability in reclaimed soil and productivity of the planted seedlings. However, the question whether the nutrient loaded seedlings are benefitted with fertilizer application remains unanswered. Fertilizer could increase understory vegetation competition that may adversely affect the growth of planted seedlings. So, it is important to elucidate how these seedlings respond to fertilizer application in competition with understory vegetation. Drought has also been reported a factor to limit the growth of planted seedlings on some reclaimed soil. Evaluating the growth response of exponentially fertilized seedlings against various levels of moisture and nutrient availability in the reclaimed soil would give better understanding of improving vegetation re-establishment on reclaimed soils.
- Moisture and salinity effects on growth of exponentially fertilized seedlings: As shown in the previous study (Duan et al., 2015), the growth of planted seedlings on oil sands reclaimed soil is affected by drought (*Pinus contorta*) and high salinity (*Picea glauca*). Soil moisture also decreases shoot/root ratio and increases N accumulation in roots in exponentially fertilized red pine seedling (Timmer and Miller, 1991). The effects of drought and salinity in growth of nutrient loaded seedlings of boreal forest have not been elucidated.

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