

**Effects of Non-segregated Tailings, Nitrogen, Phosphorus and Elemental Sulphur
on Growth of Plants in Oil Sands Reclamation Soils**

by

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Abstract

Oil sands mining in northeastern Alberta, Canada, has disturbed large areas of the northern boreal forest. These disturbed areas must be reclaimed to forest ecosystems with equivalent land capability after mining closure. The extraction of bitumen is carried out with recycled hot water containing NaOH and, as a result, the oil sands tailings have high pH and elevated levels of Na⁺, which are harmful to plants. To accelerate tailings consolidation, other chemicals may be added, which further affect tailings chemistry and can potentially contribute to their phytotoxicity. To alleviate this concern, Canadian Natural Resources Limited (CNRL) has developed novel tailings technologies to consolidate fine tailings and produce non-segregated tailings (NST) using thickeners in combination with CO₂. However, NST may still have negative effects on plants. During oil sands reclamation, a layer of forest mineral soil mix (FMM), that is salvaged from upland boreal forest sites, or peat mineral mix (PMM), that is stripped from the peatlands, are placed on the top of overburden materials and coarse tailings sands before revegetation. The pH of the topsoil may increase due to the high pH of the underlying layers and affect the revegetation efforts. In the present thesis project, two studies have been conducted to address some of the above concerns.

In the first study, I examined the effects of soil pH and elemental sulphur on growth and physiological parameters in Saskatoon (*Amelanchier alnifolia*) and beaked hazelnut (*Corylus cornuta*) seedlings. I found that elemental sulphur was effective in lowering soil pH. However, the addition of elemental sulphur to the pH 5.7 lowered the soil pH to very low levels and impaired growth and physiological performance of

Saskatoon and beaked hazelnut plants. Saskatoon and beaked hazelnut seedlings growing in the soil of pH 8.5 did not substantially benefit from the addition of 5 and 25 g kg⁻¹ elemental sulphur to the soil. The results demonstrated that 5 g kg⁻¹ was not sufficient to lower soil pH to the desirable neutral to slightly acidic level, and 25 g kg⁻¹ was too high and resulted in excessive soil acidity.

In the second study, I examined the effects of nitrogen and phosphorus levels in the NST-affected soil on growth and physiological parameters of trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) seedlings. I found that the growth and physiological responses of seedlings were increased in the mixture of NST and FMM compared with NST and PMM. Trembling aspen was more affected by NST than white spruce and benefitted more from the higher N and P soil levels. The results of both studies may be helpful in addressing the concerns of high pH and improve oil sands reclamation efforts.

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List of Abbreviations

FMM	forest mineral soil mix
PMM	peat mineral mix
S	sulphur
ANOVA	analysis of variance
LSD	least square difference
SE	standard error
cm	centimeter
PPFD	photosynthetic photon flux density
H	hours
nm	nanometer
Mg	milligram
pH	power of hydrogen
Pn	photosynthesis rate
E	transpiration rate
DMSO	dimethyl sulfoxide
LDW	leaf dry weight
SDW	shoot dry weight
RDW	root dry weight
St: Rt	shoot: root
TDW	total dry weight
Chl	chlorophyll
K	potassium
Mn	manganese
Fe	iron
Zn	zinc
B	boron
Mg	magnesium
Ca	calcium

P	phosphorus
N	nitrogen
NST	non segregated tailings
L	litter
ml	milliliter
CO ₂	carbon dioxide
HNO ₃	nitric acid

Chapter 1 Introduction and literature review

1.1 Background

Oil sands surface mining activities in northern Alberta, Canada have disturbed large areas of northern boreal forest and produced large volume of tailings (Government of Alberta, 2017). Tailings are produced as a result of bitumen extraction with hot water that contains NaOH (Masliyah et al., 2004). They consist of water with suspended clays, residual bitumen, and various dissolved chemicals as the by-products of extraction (Fung and Macyk, 2000), and are further processed and deposited in mining pits prior to mine reclamation. Since all oil sands mining areas must be returned to equivalent land capability as per the pre-disturbance state following mine closures (Province of Alberta, 2014), these oil sands areas must be reclaimed and revegetated. A new tailings processing technology, referred to as non-segregated tailings (NST), was developed by the Canadian Natural Resources Limited (CNRL) to accelerate the process of consolidation using thickeners in combination with carbon dioxide (CO₂) (CNRL, 2019; COSIA, 2020).

During the soil placement process of site preparation, suitable subsoil or overburden are placed (Dietrich and MacKenzie, 2018). The final stages of site preparation include adding a layer of forest mineral mix (FMM) salvaged from upland boreal forest sites, or peat mineral soil mix (PMM), salvaged from lowlands within the mining footprint as cover soils with thickness ranging from 15 cm to 100 cm (Tuttle and Powter, 1991; Fung and Macyk, 2000; Keshta et al., 2010). However, the thickness of FMM and PMM is usually 20 cm and 30 cm, respectively (Dietrich and MacKenzie, 2018). In addition to providing organic matter and nutrients, these soils contain microorganisms that are essential to the establishment and growth of plants in the reclaimed areas (Sydnor and Redente, 2002; Mackenzie and Naeth, 2010).

Numerous studies reveal that elemental sulphur can lower soil pH, reduce availability of heavy metal in soil and provide elemental sulphur for plant growth (Tichý et al., 1996; Wu et al., 2007; Zhang et al., 2007; Zhao et al., 2008). Therefore, application of

elemental sulphur to the soil could be useful for improving revegetation success of these oil sands reclamation areas, because the optimum pH for boreal forest is typically lower than 6 (Zhang et al., 2020).

Nitrogen and phosphorus are important factors limiting plant growth, since they are required for many important processes in plants, for example, the synthesis of nucleic acids, amino acids and other metabolites (Mengel et al., 2001; Taiz and Zeiger, 2002; Epstein and Bloom, 2005; Jing et al., 2010).

My thesis research project focused on the soil pH, elemental S levels, soil substrates and N&P levels that may affect the survival and growth of plants in oil sands reclamation areas. In the first research study, I examined the effects of soil supplementation with elemental sulphur on the growth and physiological parameters of Saskatoon (*Amelanchier alnifolia*) and beaked hazelnut (*Corylus cornuta*) seedlings. In the second study, I focused on the effects of phosphorus and nitrogen nutrition on trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) growing in reclamation soil containing NST. The plant species that were used in the two studies are native to the boreal forest. Saskatoon and beaked hazelnut used in first study are not commonly selected but could provide another option for oil sands reclamation. In addition, aspen and white spruce are the dominant tree species in the boreal forest of Central Boreal Mixedwood (Fung and Macyk, 2000; Downing and Pettapiece, 2006) and are commonly planted for oil sands reclamation (TSCEMA, 2009).

The objectives of the present study were to:

- 1) Examine the effectiveness of elemental sulphur in lowering the pH of reclamation soil.
- 2) Determine the effects of soil pH and sulphur supplementation on subsequent growth and physiological responses of Saskatoon and beaked hazelnut.
- 3) Investigate how the amendment of two types of reclamation soil with NST affects growth and physiological parameters in aspen and white spruce.
- 4) How growth and physiological parameters in aspen and white spruce are affected by soil N and P levels.

The following hypotheses were tested:

- 1) Soil supplementation with sulphur is effective in lowering the pH of reclamation soil.
- 2) Sulphur supplementation is beneficial for growth and physiology of Saskatoon and beaked hazelnut.
- 3) NST presence in the soil has detrimental effects on plant growth and the effects vary depending on the type of soil.
- 4) Soil supplementation with N and P is more effective in enhancing growth and physiological responses in aspen compared with white spruce.

1.2 Literature review

1.2.1 Oil sands mining and reclamation

Athabasca, Cold Lake and Peace River oil sands deposits cover over 140,000 km² of largely forested land in northern Alberta, Canada (Natural Resources Canada, 2017). The bitumen is mixed with sand at the depths that are commonly from 50 to 200 m below the surface and is recovered one of the two main mining methods depending on their depth. Bitumen (Natural Resource Canada, 2017). Bitumen that is less than 75 meters below the surface is usually recovered through open-pit mining (Government of Alberta, 2014; Alberta Chamber of Resources, 2015). This accounts for about 20% of the oil deposit, while the other 80% is deeper than 75 m below the surface and must be exploited by *in-situ* mining (Alberta Chamber of Resources, 2015).

In preparation for open-pit mining activities, all vegetation, muskeg, topsoil, subsoil and overburden (including rock, clay and non-bituminous sands) are removed (MacKenzie, 2012; Forsch, 2014). In the extraction plant, bitumen is extracted by adding hot water with NaOH (Schramm and Smith, 1989; Masliyah et al., 2004) and the slurry is transferred to big vessels to separate bitumen on the surface. During the extraction process, large volumes of tailings consisting of water with clays, tailings sand, residual bitumen, and chemicals are produced after adding hot water and alkaline chemicals (Howat, 2000; Fung and Macyk, 2000). These tailings are settled in tailings

pond and ready for land reclamation (CNRL, 2019).

During open-pit mining activities, more than 1000 km² of boreal forest were disturbed up to 2017 (Government of Alberta, 2017). The disturbed area must be restored to the equivalent land capability, which refers to the ability of reclaimed land to support similar land uses, although not necessarily the same as before (Province of Alberta, 2014; Government of Alberta, 2015).

Oil sands reclamation includes multiple processes, that start with a survey of pre-disturbance conditions followed by salvage and storage of reclamation material, landscape planning, site construction, revegetation, monitoring, and finally, certification of the reclaimed land (Fung and Macyk, 2000; Naeth et al., 2013). Following the placement of suitable subsoil or overburden (Dietrich and MacKenzie, 2018), the final stages of site preparation include the placement of cover soil, such as forest floor mineral mix (FMM) salvaged from upland boreal forest sites and peat mineral soil mix (PMM) stripped from lowlands (Naeth et al., 2013). Both soil types add organic matter, improve water holding capacity and fertility as well as plant propagules and microorganisms that are essential for site revegetation (Deput, 1984; Sydnor and Redente, 2002; Mackenzie and Naeth, 2010).

Mine tailings that are produced as by-product during the extracting process, consist of water with suspended sand, silt, clay, residual bitumen, and chemical agent like NaOH that may be harmful to plants (Fung and Macyk, 2000). The pH of these tailings is high due to a large amount of NaOH added during bitumen extraction (Masliyah et al., 2004). High pH and elevated Na levels can affect the soil in reclamation sites and affect vegetation establishment and growth (Renault et al., 2003; Zhang et al., 2015).

1.2.2 Biology of the studied plant species

In the two studies encompassed by this thesis research project, four species of woody plants were investigated including Saskatoon (*Amelanchier alnifolia*), beaked hazelnut (*Corylus cornuta*), trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*). The biology of these four species is summarized below.

1.2.2.1 Biology of Saskatoon (*Amelanchier alnifolia*)

Saskatoon is a deciduous shrub native to North America, that usually grows from sea level up to 2600 and 3400 meters in California and the Rocky Mountains, respectively. It can grow in different types of soil including brown, dark brown and chernozemic soils, with soil pH ranging from 5 to 8 (Wang, 2014). However, Saskatoon does not grow well in poorly drained soils, in heavy clay soils with limited organic matter, and in shallow soils with high or fluctuating water table (Schooley, 2008).

1.2.2.2 Biology of beaked hazelnut (*Corylus cornuta*)

Beaked hazelnut is a deciduous shrub distributed across most of the North America (Native Plants PNW, 2016; ACRRE, 2016). It grows in dry woodlands and may reach the heights of up to 8 meters (ACRRE, 2016). As the hardiest shrub of all hazelnut species, it can survive at temperatures below -50°C (ACRRE, 2016).

The seeds of beaked hazelnut are dispersed by birds such as jays and other animals such as American red squirrels. Beaked hazelnut has the ability to recover after fires that kills above-ground parts of the shrub (Fryer and Janet, 2007).

1.2.2.3 Biology of trembling aspen (*Populus tremuloides*)

Trembling aspen is a deciduous tree that is widely distributed in North America (Zasada and Phipps, 1990). The tree is relatively fast growing and usually reaches heights from 20 to 25 meters at maturity (Ministry of Natural Resources and Forestry, 2014). Trembling aspen can grow in a wide range of soils with pH varying from 5.3 to 8.4 (Renault et al., 1999). However, it grows poorly in sandy and heavy clay soils due to limiting moisture, nutrients, and poor aeration (Perala, 1977). Aspen trees grow better in soil that is well drained, loamy, and abundant in organic matter and mineral nutrients, such as calcium, magnesium, potassium and nitrogen, due to its fast growth rate and high nutrient demand (Boyle, 1973; Alban, 1982; Debyle, 1985).

Trembling aspen has the ability to reproduce through seeds and root suckers arising

along its long lateral roots (Brinkman and Roe, 1975), root sucker is important for the successful reestablishment of aspen after disturbance (Frey et al., 2003). Its seeds usually mature in 4 to 6 weeks after bloom and are spread by wind or water (Debyle and Winokur, 1985).

There are many uses for trembling aspen trees. Trembling aspen provides wildlife with good habitat (Patton and Jones, 1977; Ohmann et al., 1978) and it is often planted as a wind breaking tree, allows more water recharge and steam flow compared with conifer forests (Gifford et al., 1984), and is suitable as a restoration species due to its quick colonization of disturbed soils (Shepperd, 1986).

1.2.2.4 Biology of white spruce (*Picea glauca*)

White spruce is a large coniferous evergreen tree native to northern temperate and boreal forests in North America and may reach 15 to 30 m in height at maturity (Farjon, 1990). It is able to adapt to a wide range of soils with pH from 4.7 to 7.0 and have relatively high tolerance to high root zone pH (Sutton, 1969; Stiell, 1976; Brand et al., 1986; Nienstaedt and Zasada, 1990; Xu et al., 2020), it can tolerate temperatures below -50°C (Maini, 1966). In addition, white spruce can tolerate a wide range of soil fertility levels and moisture conditions (Nienstaedt and Zasada, 1990). Similar to other conifers, white spruce trees can acidify soil pH over time (Brand, 1986).

White spruce is a prolific seed producer at maturity, which is approximately 30 years of age (Nienstaedt and Telch, 1972). The seeds may be dispersed by wind as far as 300 m away from the tree (Sutton, 1969; Zasada, 1986).

White spruce has a variety of uses. It provides foliage as food for deer and rabbits during winter (Whitney, 1985) and its wood is used for pulp and paper as well as lumber (Wang et al., 2012).

1.2.3 Soil pH and plants

1.2.3.1 Soil pH

Soil pH plays an important role in affecting plant growth and geographic distribution. High pH soils account for more than 30% of terrestrial areas in the world (Chen and Barak, 1982; Marschner, 2012) and the soil pH in semi-arid and arid areas is usually higher than 7 (Marschner, 2012). The optimum pH for most plants ranges from 6 to 6.5 (Fageria and Baligar, 2003). However, the optimum pH for boreal forest is typically lower than 6 (Zhang et al., 2020). High soil pH can affect plant growth directly through high concentrations of OH^- in soil (Zieslin and Snir 1989; Kopittke and Menzies, 2005), and indirectly by affecting microorganism like bacteria and fungi activities (Baath et al., 1980; Wang et al., 1985; Nicol et al., 2008; Rousk et al., 2009) and associated nutrient availability (Brady and Weil, 1996).

1.2.3.2 Effects of high pH on plants growth

Different plant species may respond differently to the effects of high soil pH (Bertoni et al., 1992; Tang et al., 1992; Zhang et al., 2013). For example, previous studies showed that white spruce is more tolerant to high root zone pH when compared with paper birch and green alder (Zhang et al., 2013; Zhang and Zwiazek, 2016). In addition, some studies report that high root zone pH had negative effects on the formation of lateral roots and root hairs, with the optimum pH for root development ranges from 4 to 6 (Ewens and Leigh, 1985; Takahashi et al., 2003).

High soil pH inhibits water uptake by plants. Numerous studies indicate that high pH in the root zone area may reduce shoot and root growth, aggravate water deficit stress, inhibit stomatal conductance, and reduce shoot water potential (Atwell, 1991; Bertoni et al., 1992; Tang and Turner, 1999; Kamaluddin and Zwiazek, 2004). The hydraulic conductivity and water flow of roots in paper birch measured at pH 8 were lower than that occurring at pH of 6 (Kamaluddin and Zwiazek, 2004). This may be related to aquaporin activities or abundance (Voicu and Zwiazek, 2004; Aroca et al., 2006), which

regulate root water flux through cell-to-cell pathways (Steudle and Peterson, 1998; Voicu and Zwiazek, 2004; Aroca et al., 2006). Numerous studies reveal that aquaporin activities or abundance are affected by pH and water uptake might be indirectly affected by an inhibition of root growth under high pH (Tang et al., 1992; Felle and Hanstein, 2002; Kamaluddin and Zwiazek 2004; Vander et al., 2004; Törnroth-Horsefield et al., 2006). Ultimately, shoot growth, stomatal conductance and photosynthetic rates can be severely affected by a reduction in water uptake and root water flow rate (Tang and Turner, 1999).

1.2.3.3 Effects of high pH on nutrient availability

High soil pH reduces the availability of several nutrients, such as Fe, Mn, P and Zn, B and N (Lindsay, 1984; Yang et al., 1994; Iles, 2001; Valentine et al., 2006; Marschner, 2012) by affecting the solubility and mobility of nutrients in soil (Comerford, 2005).

Fe deficiency can result in leaf chlorosis and poor plant growth, due to low solubility of Fe at high pH (Mengel, 1994; Kosegarten et al., 2001; Boukhalfa and Crumbliss, 2002; Tang et al., 2006). However, some plants can grow well in high pH soils despite low concentrations of available Fe. For example, barley developed more lateral roots that released more phytosiderophores under alkaline and low Fe availability conditions (Yousfi et al., 2007).

Research indicates that Mn availability decrease with increasing soil pH (Stone, 1968; Parker and Walker, 1986). When soil pH ranges from 6 to 8, Mn is oxidized to MnO_2 and forms manganese calcite with $CaCO_3$ (Hewitt et al., 1974; Jauregui and Reisenauer, 1982).

Solubility of P decreases at both low and high soil pH, with the pH for maximum solubility at 6.5 (Valentine et al., 2006). Zhang et al (2013) found that phosphorus concentrations in young leaves of trembling aspen and white spruce decreased under high pH conditions (Zhang et al., 2013).

Zinc deficiency usually occurs in high pH soils, especially in calcareous soils (Graham et al., 1992; Graham, 2008). Some plants growing in high pH soil can improve

Zn uptake through different strategies, including increased root mass and releasing more Zn-mobilizing phytosiderophores from roots (Dong et al., 1995; Cakmak et al., 1996).

Boron also shows reduced availability in high pH and calcareous soils. Goldberg (1997) indicated that B adsorption increased strongly at a pH of 6.5 and was highest at pH 9 (Goldberg and Glaubig, 1986; Goldberg, 1997).

Nitrogen may be deficient in alkaline soils (Marschner, 1986) because alkaline-adapted and acid-adapted plants have different strategies to utilize N. Alkaline-adapted plants prefer absorbing nitrate while acid-adapted plants prefer utilizing ammonium (Merhaut, 1993; Claussen and Lenz, 1999; Marschner, 2012).

1.2.4 Soil treatments with elemental sulphur

Elemental sulphur can be used to reduce soil pH (Cai et al., 2008; Cao et al., 2010; Li et al., 1999; Liu et al., 2008; Sun et al., 2014; Tichý et al., 1996; Wu et al., 2007; Zhang et al., 2007; Zhao et al., 2008). Many studies show that elemental sulphur has an effect on the activities of soil microorganisms (Banerjee and Chapman, 1996; Rao et al., 2000), soil acid-base balance (Xing et al., 1997; Zhou and Lin, 1997), and redox balance (Slaton et al., 2001) as well as the availability of soil mineral nutrients (Modaihsh et al., 1989). Elemental sulphur was proven effective in improving the availability of phosphorus and trace elements (Nomik and Vahtras, 1982; Zhao et al., 1999; Eriksen and Mortensen, 2002). However, soil treatments with excessive elemental sulphur can significantly reduce the biomass of plants due to the much lower pH (Zhang et al., 2007).

1.2.5 Importance of nitrogen and phosphorus

Nitrogen availability is one of the most commonly limiting factors to plant growth, since N accounts for 80% of the total nutrients absorbed by plants and is required for many important processes in plants, including the synthesis of nucleic acids, amino acids, proteins and other metabolites (Mengel et al., 2001; Taiz and Zeiger, 2002;

Epstein and Bloom, 2005). Nitrogen deficiency can occur in alkaline soils (Marschner, 1986) and affect photosynthetic processes directly or indirectly by affecting chlorophyll synthesis, photosynthesis rates, and the activities of enzymes participating in carbon fixation (Oaks et al., 1977). Nitrogen availability affects plant growth, richness, composition of species, and forest productivity (Frelich et al., 2003; Rowe et al., 2006; Walker and del Moral, 2009; Gundersen et al., 2009; Yan et al., 2012; Duan et al., 2015).

Phosphorus is another common factor limiting plant growth (Jing et al., 2010), since it is a component of many essential molecules such as nucleic acids, phospholipids, and ATP (Marschner, 2012). Studies indicate that many forests growing on soils with considerable age, growth and nutrient cycling are more probably limited by phosphorus (Attiwill and Adams, 1993). Phosphorus deficiency that occurs in acidic and alkaline soil inhibits leaf initiation and expansion (Fredeen et al., 1989; Lynch et al., 1991), decreases shoot to root ratios due to greater limitations on shoot growth (Marschner, 2012), reduces root hydraulic conductivity due to a reduction of aquaporin gene expression (Clarkson et al., 2000), changes root architecture such as distribution patterns that related to spatial availability and acquisition of phosphorus (Shen et al., 2011), induces root cluster formation related to the exudation of organic acids (Marschner, 1995), and changes availability of other nutrients including Fe (Svistoonoff et al., 2007; Ward et al., 2008).

1.3 References

- ACRRE, 2016. *Corylus cornuta*. Alberta Centre for Reclamation and Restoration Ecology. University of Alberta [WWW Document]. URL http://acrr.ualberta.ca/Portals/14/ACRREDocuments/Corylus_cornuta.pdf.
- Alban, D.H., 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. *Soil Science Society of America Journal* 46, 853–861.
- Alberta Chamber of Resources, 2015. Caring for the land [WWW Document]. URL <https://www.acralberta.com/app/uploads/Reclamation-Brochure-Caring-For->

The Land.pdf.

- Aroca, R., Ferrante, A., Vernieri, P., Chrispeels, M.J., 2006. Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Annals of Botany* 98, 1301–1310.
- Attiwill, P.M., Adams, M.A., 1993. Nutrient cycling in forests. *New Phytologist* 124, 561–582.
- Atwell, B.J., 1991. Factors which affect the growth of grain legumes on a solonized brown soil. II. Genotypic responses to soil chemical factors. *Australian Journal of Agricultural Research* 42, 107–119.
- Baath, E., Berg, B., Lohm, U., Lundgren, B., Lundkvist, H., Rosswall, T., Soderstrom, B., Wirén, A., 1980. Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. *Pedobiologia* 20, 85–100.
- Banerjee, M.R., Chapman, S.J., 1996. The significance of microbial biomass sulphur in soil. *Biology and Fertility of Soils* 22, 116–125.
- Bertoni, G.M., Pissaloux, A., Morard, P., Sayag, D.R., 1992. Bicarbonate-pH relationship with iron chlorosis in white lupine. *Journal of Plant Nutrition* 15, 1509–1518.
- Boukhalfa, H., Crumbliss, A.L., 2002. Chemical aspects of siderophore mediated iron transport. *Biometals* 15, 325–339.
- Boyle, J.R., Phillips, J.J., Ek, A.R., 1973. “Whole tree” harvesting: nutrient budget evaluation. *Journal of Forestry* 71, 760–762.
- Brady N.C., Weil R.R., 1996. *The nature and properties of soils*. Prentice-Hall Inc., New Jersey, USA.
- Brand, D.G., Kehoe, P., Connors, M., 1986. Coniferous afforestation leads to soil acidification in central Ontario. *Canadian Journal of Forest Research* 16, 1389–1391.
- Brinkman, K.A., Roe, E.I., 1975. *Quaking aspen: silvics and management in the Lake States*. Agriculture Handbook, US Department of Agriculture. US Government Printing Office, Washington D.C., USA.

- Cai, X., Ma, Y., Wang, W., Wei, C., Wang, X., Dun, S., 2008. Effects of applying sulphur on alkali soil pH and its consequence on yield and quality of flue-cured Tobacco. *Acta Tabacaria Sinica* 14, 19–22.
- Cakmak, I., Sari, N., Marschner, H., Ekiz, H., Kalayci, M., Yilmaz, A., Braun, H.J., 1996. Phytosiderophore release in bread and durum wheat genotypes differing in zinc efficiency. *Plant and Soil* 180, 183–189.
- Cao, Y., Zhao, Y., Zhao, W., Lv, J., 2010. Research on effect of sulfur application on saline-alkaline soil pH and availability of trace elements in Yellow River irrigation district in Ningxia. *Agricultural Research in the Arid Areas* 28, 199–201.
- Chen, Y., Barak, P., 1982. Iron nutrition of plants in calcareous soils. *Advances in Agronomy* 35, 217–240.
- Clarkson, D.T., Carvajal, M., Henzler, T., Waterhouse, R.N., Smyth, A.J., Cooke, D.T., Steudle, E., 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* 51, 61–70.
- Claussen, W., Lenz, F., 1999. Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant and Soil* 208, 95–102.
- CNRL, 2019. Managing Tailings. <https://www.cnrl.com/corporate-responsibility/advancements-in-technology/managing-tailings>.
- Comerford, N.B., 2005. Soil factors affecting nutrient bioavailability. *Nutrient Acquisition by Plants* 1–14. Springer, Berlin, Germany.
- COSIA, 2020. Carbon Dioxide Amended Tailings. <https://cosia.ca/initiatives/tailings/projects/carbon-dioxide-amended-tailings#:~:text=In%202015%2C%20Canadian%20Natural%20began,use%20of%20cyclones%20and%20thickeners>.
- DeByle, N.V., Winokur, R.P., 1985. *Aspen: ecology and management in the western United States*. US Department of Agriculture, Forest Service, Rocky Mountain

- Forest and Range Experiment Station. Fort Collins, Colorado, USA.
- DePuit, E.J., 1984. Potential topsoiling strategies for enhancement of vegetation diversity on mined lands. *Minerals and the Environment* 6, 115–120.
- Dietrich, S.T., MacKenzie, M.D., 2018. Comparing spatial heterogeneity of bioavailable nutrients and soil respiration in boreal sites recovering from natural and anthropogenic disturbance. *Frontiers in Environmental Science* 6, 126.
- Dong, B., Rengel, Z., Graham, R.D., 1995. Root morphology of wheat genotypes differing in zinc efficiency. *Journal of Plant Nutrition* 18, 2761–2773.
- Downing, D.J., Pettapiece, W.W., 2006. Natural regions and subregions of Alberta. Natural Regions Committee, Edmonton.
- Duan, M., House, J., Chang, S.X., 2015. Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region. *Ecological Engineering* 75, 323–331.
- Eriksen, J., Mortensen, J.V., 2002. Effects of timing of sulphur application on yield, S-uptake and quality of barley. *Plant and Soil* 242, 283–289.
- Epstein, E., Bloom, A.J., 2005. Mineral nutrition of plants: principles and perspectives, 2nd Edition. Sinauer, Sunderland, UK.
- Ewens M., Leigh, R.A., 1985. The effect of nutrient solution composition on the length of root hairs of wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* 36, 713–724.
- Fageria, N.K., Baligar, V.C., 2003. Fertility management of tropical acid soils for sustainable crop production. *Handbook of Soil Acidity*, 359–385. Marcel Dekker Inc., New York, USA.
- Farjon, A., 1990. Pinaceae. Drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Koeltz Scientific Books, Königstein, Germany.
- Felle, H.H., Hanstein, S., 2002. The apoplastic pH of the substomatal cavity of *Vicia faba* leaves and its regulation responding to different stress factors. *Journal of Experimental Botany* 53, 73–82.

- Forsch, K.B.C., 2014. Oil sands reclamation using woody debris with LFH mineral soil mix and peat mineral soil mix cover soils: impacts on select soil and vegetation properties. M.Sc. Thesis. Department of Renewable Resources, University of Alberta. Edmonton, AB, Canada.
- Fredeen, A.L., Rao, I.M., Terry, N., 1989. Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. *Plant Physiology* 89, 225–230.
- Frelich, L.E., Machado, J.-L., Reich, P.B., 2003. Fine-scale environmental variation and structure of understorey plant communities in two old-growth pine forests. *Journal of Ecology* 91, 283–293.
- Frey, B.R., Lieffers, V.J., Landhäusser, S.M., Comeau, P.G., Greenway, K.J., 2003. An analysis of sucker regeneration of trembling aspen. *Canadian Journal of Forest Research* 33, 1169–1179.
- Fryer, Janet, L., 2007. *Corylus cornuta*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). [WWW Document]. URL <https://www.fs.fed.us/database/feis/plants/shrub/corcor/all.html>.
- Fung, M.Y., Macyk, T.M., 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands* 41, 755–774.
- Gifford, G.F., Humphries, W., Jaynes, R.A., 1984. A preliminary quantification of the impacts of aspen to conifer succession on water yield. *Journal of the American Water Resources Association* 20, 181–186.
- Goldberg, S., 1997. Reactions of boron with soils. *Plant and Soil* 193, 35–48.
- Goldberg, S., Glaubig, R.A., 1986. Boron adsorption and silicon release by the clay minerals kaolinite, montmorillonite, and illite. *Soil Science Society of America Journal* 50, 1442–1448.
- Government of Alberta, 2017. Oil sands – Overview [WWW Document]. URL <https://www.alberta.ca/oil-sands-overview.aspx>.
- Government of Alberta, 2015. Alberta’s oil sands: reclamation [WWW Document]. URL <https://www.oilsands.alberta.ca/reclamation.html>.

- Government of Alberta, 2014. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region (2nd edition). Fort McMurray, AB, Canada.
- Graham, M.J., Stephens, P.A., Widholm, J.M., Nickell, C.D., 1992. Soybean genotype evaluation for iron deficiency chlorosis using sodium bicarbonate and tissue culture. *Journal of Plant Nutrition* 15, 1215–1225.
- Graham, R.D., 2008. Micronutrient deficiencies in crops and their global significance. *Micronutrient Deficiencies in Global Crop Production* 41-61. Springer, Dordrecht, Netherlands.
- Gundersen, P., Sevel, L., Christiansen, J.R., Vesterdal, L., Hansen, K., Bastrup-Birk, A., 2009. Do indicators of nitrogen retention and leaching differ between coniferous and broadleaved forests in Denmark? *Forest Ecology and Management* 258, 1137–1146.
- Hewitt, E. J., Smith, T. A., et al. 1974. *Plant mineral nutrition*. English Universities Press Ltd., London, UK.
- Howat, D., 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Alberta Environment. Environmental Sciences Division, Edmonton, AB, Canada.
- Iles, J., 2001. *Community Trees: Community tree steward program. Requirements for plant growth*. Department of Horticulture, Iowa State University. Ames, IA, USA.
- Jauregui, M.A., Reisenauer, H.M., 1982. Dissolution of oxides of manganese and iron by root exudate components 1. *Soil Science Society of America Journal* 46, 314–317.
- Jing, J., Rui, Y., Zhang, F., Rengel, Z., Shen, J., 2010. Localized application of phosphorus and ammonium improves growth of maize seedlings by stimulating root proliferation and rhizosphere acidification. *Field Crops Research* 119, 355–364.
- Kamaluddin, M., Zwiazek, J.J., 2004. Effects of root medium pH on water transport in paper birch (*Betula papyrifera*) seedlings in relation to root temperature and

- abscisic acid treatments. *Tree Physiology* 24, 1173–1180.
- Keshta, N., Elshorbagy, A., Barbour, S.L., 2010. Comparative probabilistic assessment of the hydrological performance of reconstructed and natural watersheds. *Hydrological Processes* 24, 1333–1342.
- Kopittke, P.M., Menzies, N.W., 2005. Control of nutrient solutions for studies at high pH. *Plant and Soil* 266, 343–354.
- Kosegarten, H., Hoffmann, B., Mengel, K., 2001. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the re-greening effect brought about by acidic foliar sprays. *Journal of Plant Nutrition and Soil Science* 164, 155–163.
- Li, C., Liu, Y., He, T., Yang, H., Huang, L., Teng, Y., 1999. Relation between soil pH and potassium nutrition of flue-cured tobacco. *Acta Pedologica Sinica* 2, 276–282.
- Lindsay, W.L., 1984. Soil and plant relationships associated with iron deficiency with emphasis on nutrient interactions. *Journal of Plant Nutrition* 7, 489–500.
- Liu, G., Li, X., Zhang, Y., Lv, J., Wei, Y., 2008. Primary study on function of sulphur on saline-alkali soil in Yinbei Area. *Agricultural Research in the Arid Areas* 4, 79–82.
- Lynch, J., Lauchli, A., Epstein, E., 1991. Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Science* 31, 380–387.
- MacKenzie, D., Alberta, Alberta Environment and Water, 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Alberta Environment and Water, Edmonton, Alberta.
- Mackenzie, D.D., Naeth, M.A., 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. *Restoration Ecology* 18, 418–427.
- Maini, J.S., 1966. Phytoecological study of sylvotundra at Small Tree Lake, NWT. *Arctic* 19, 220–243.
- Marschner, P., 2012. *Marschner's mineral nutrition of higher plants*. Elsevier Ltd., 225

- Wyman Street, Waltham, MA 02451, USA.
- Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press, London, UK.
- Marschner, H., Römheld, V., Kissel, M., 1986. Different strategies in higher plants in mobilization and uptake of iron. *Journal of Plant Nutrition* 9, 695–713.
- Masliyah, J., Zhou, Z.J., Xu, Z., Czarnecki, J., Hamza, H., 2004. Understanding water-based bitumen extraction from Athabasca oil sands. *The Canadian Journal of Chemical Engineering* 82, 628–654.
- Mengel, K., 1994. Iron availability in plant tissues-iron chlorosis on calcareous soils. *Plant and Soil* 165, 275–283.
- Mengel, K., Kosegarten, H., Kirkby, E.A., Appel, T., 2001. Principles of plant nutrition. Springer, New York, USA.
- Merhaut, D.J., 1993. Effects of nitrogen form on vegetative growth, and carbon/nitrogen assimilation, metabolism, and partitioning in blueberry. Ph.D. Dissertation, University of Florida, USA.
- Ministry of Natural Resources and Forestry, 2014. Trembling aspen [WWW Document]. URL <https://www.ontario.ca/page/trembling-aspen>.
- Modaihsh, A.S., Al-Mustafa, W.A., Metwally, A.I., 1989. Effect of elemental sulphur on chemical changes and nutrient availability in calcareous soils. *Plant and Soil* 116, 95–101.
- Naeth, M.A., Wilkinson, S.R., Mackenzie, D.D., Archibald, H.A., Powter, C.B., 2013. Potential of LFH mineral soil mixes for reclamation of forested lands in Alberta. OSRIN Report No. TR-35. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, AB, Canada.
- Native Plants PNW, 2016. Beaked Hazelnut, *Corylus cornuta*. Native Plants PNW. URL <http://nativeplantspnw.com/beaked-hazelnut-corylus-cornuta/>.
- Natural Resources Canada, N.R., 2017. Oil Sands Processes [WWW Document]. URL <https://www.nrcan.gc.ca/energy/energy-sources-distribution/crude-oil/technical-overview/oil-sands-processes/5853>.

- Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology* 10, 2966–2978.
- Nienstaedt, H., Teich, A., 1972. The genetics of white spruce. Forest Service Research Paper, (WO-15). US Department of Agriculture, USA.
- Nienstaedt, H., Zasada, J.C., 1990. *Picea glauca* (Moench) Voss. Pages: 204–226. In: Burns, R.M. and Honkala, B.H. (eds.). *Silvics of North America: 1. Conifers*. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington D.C., USA.
- Nommik, H., Vahtras, K., 1982. Retention and fixation of ammonium and ammonia in soils. *Nitrogen in Agricultural Soils* 22, 123–171.
- Oaks, A., Aslam, M., Boesel, I., 1977. Ammonium and amino acids as regulators of nitrate reductase in corn roots. *Plant Physiology* 59, 391–394.
- Ohmann, L.F., Batzer, H.O., Buech, R.R., Lothner, D.C., Perala, D.A., Schipper Jr, A.L., Verry, E.S., 1978. Some harvest options and their consequences for the aspen, birch and associated conifer forest types of the Lake States. North Central Forest Experiment Station, St. Paul, Minnesota, USA.
- Parker, M.B., Walker, M.E., 1986. Soil pH and manganese effects on manganese nutrition of peanut 1. *Agronomy Journal* 78, 614–620.
- Patton, D.R., Jones, J.R., 1977. Managing aspen for wildlife in the Southwest. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.
- Perala, D.A., 1977. Manager's handbook for aspen in the north-central states. General Technical Report NC-36. US Department of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota, USA.
- Province of Alberta, 2014. Conservation and reclamation regulation of environmental protection and enhancement act. Alberta Queen's Printer, Edmonton, Canada.
- Rao, P.S., Karunasagar, Iddya, Otta, S.K., Karunasagar, Indrani, 2000. Incidence of bacteria involved in nitrogen and sulphur cycles in tropical shrimp culture ponds.

- Aquaculture International 8, 463–472.
- Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D., 1999. Responses of boreal plants to high salinity oil sands tailings water. *Journal of Environmental Quality* 28, 1957–1962.
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* 75, 1589–1596.
- Rowe, E.C., Healey, J.R., Edwards-Jones, G., Hills, J., Howells, M., Jones, D.L., 2006. Fertilizer application during primary succession changes the structure of plant and herbivore communities. *Biological Conservation* 131, 510–522.
- Schooley, K., 2008. Introduction to Saskatoons [WWW Document]. URL https://web.archive.org/web/20081212072602/http://www.omafra.gov.on.ca/english/crops/facts/info_introduction_saskatoons.htm.
- Schramm, L.L., Smith, R.G., 1989. Some parametric studies of oil sand conditioning in the hot water flotation process. *AOSTRA Journal of Research* 5, 87–107.
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011. Phosphorus dynamics: from soil to plant. *Plant Physiology* 156, 997–1005.
- Shepperd, W.D., 1986. *Silviculture of Aspen Forests in the Rocky Mountains and in the Southwest*. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.
- Slaton, N.A., Norman, R.J., Gilmour, J.T., 2001. Oxidation rates of commercial elemental sulfur products applied to an alkaline silt loam from Arkansas. *Soil Science Society of America Journal* 65, 239–243.
- Steudle, E., Peterson, C.A., 1998. How does water get through roots? *Journal of Experimental Botany* 49, 775–788.
- Stiell, W.M., 1976. *White spruce: artificial regeneration in Canada*. Canadian Forestry Service, Department of the Environment, Canada.
- Stone, E.L., 1968. Microelement nutrition of forest trees: a review. *Forest Fertilization, Theory and Practice*, 132–175.

- Sun, L., Duan, D., Peng, C., He, J., Shi, J., 2014. Influence of sulfur on the speciation transformation and phyto-availability of heavy metals in soil: A review. *Chinese Journal of Applied Ecology* 25, 2141–2148.
- Sutton, R.F., 1969. Silvics of white spruce (*Picea glauca* (Moench) Voss). *Canadian Forestry Services* 1250, 57. Ottawa, ON, Canada.
- Svistoonoff, S., Creff, A., Reymond, M., Sigoillot-Claude, C., Ricaud, L., Blanchet, A., Nussaume, L., Desnos, T., 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genetics* 39, 792.
- Sydnor, M.E., Redente, E.F., 2002. Reclamation of high-elevation, acidic mine waste with organic amendments and topsoil. *Journal of Environmental Quality* 31, 1528–1537.
- Taiz, L., Zeiger, E., 2002. *Plant Physiology*, 3rd Edition. Sinauer, Sunderland, UK.
- Takahashi, H., Kawahara, A., Inoue, Y., 2003. Ethylene promotes the induction by auxin of the cortical microtubule randomization required for low-pH-induced root hair initiation in lettuce (*Lactuca sativa* L.) seedlings. *Plant and Cell Physiology* 44, 932–940.
- Tang, C., Longnecker, N.E., Thomson, C.J., Greenway, H., Robson, A.D., 1992. Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in their sensitivity to pH above 6.0. *Journal of Plant Physiology* 140, 715–719.
- Tang, C., Turner, N.C., 1999. The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus angustifolius* L. and *Lupinus pilosus* Murr. *Australian Journal of Experimental Agriculture* 39, 457–464.
- Tang, C., Zheng, S.J., Qiao, Y.F., Wang, G.H., Han, X.Z., 2006. Interactions between high pH and iron supply on nodulation and iron nutrition of *Lupinus albus* L. genotypes differing in sensitivity to iron deficiency. *Plant and Soil* 279, 153–162.
- Tichý, R., Fajtl, J., Kužel, S., Kolář, L., 1996. Use of elemental sulphur to enhance a cadmium solubilization and its vegetative removal from contaminated soil.

- Nutrient Cycling in Agroecosystems 46, 249–255.
- Törnroth-Horsefield, S., Wang, Y., Hedfalk, K., Johanson, U., Karlsson, M., Tajkhorshid, E., Neutze, R., Kjellbom, P., 2006. Structural mechanism of plant aquaporin gating. *Nature* 439, 688–694.
- TSCEMA, 2009. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. Second edition - Open Government [WWW Document]. URL <https://open.alberta.ca/publications/9780778588252>.
- Tuttle, S., Powter, C.B., 1991. An overview of land reclamation at Suncor. *Land Reclamation of Oil Sands and Heavy Oil Developments*, 1–23.
- Valentine, D.W., Kielland, K., Chapin III, F.S., McGuire, A.D., Van Cleve, K., 2006. Patterns of biogeochemistry in Alaskan boreal forests. *Alaska's Changing Boreal Forest* 241–266. Oxford University Press, New York, USA.
- Vander, W. C., Verdoucq, L., Boursiac, Y. and Maurel, C. 2004. Aquaporins in plants. In: Blatt MR, ed. *Membrane transport in plants*. Annual Plant Reviews 8, Sheffield: Blackwell Publishing 221–250.
- Voicu, M.C., Zwiazek, J.J., 2004. Cycloheximide inhibits root water flow and stomatal conductance in aspen (*Populus tremuloides*) seedlings. *Plant, Cell & Environment* 27, 199–208.
- Walker, L.R., del Moral, R., 2009. Lessons from primary succession for restoration of severely damaged habitats. *Applied Vegetation Science* 12, 55–67.
- Wang G.M., Stribley D., Tinker P., 1985. Soil pH and vesicular-arbuscular mycorrhizas. *Special Publications Series of the British Ecological Society* 4, 219–224.
- Wang, L., 2014. On the current research situation of *Amelanchier alnifolia*. *Journal of Agriculture* 4, 82–84.
- Wang, W., Lu, A., Hou, D., Cao, Y., 2012. Cultivation technique of white spruce seedling. *Journal of Liaoning Forestry Science and Technology*, 60–62.
- Ward, J.T., Lahner, B., Yakubova, E., Salt, D.E., Raghothama, K.G., 2008. The effect of iron on the primary root elongation of *Arabidopsis* during phosphate deficiency. *Plant Physiology* 147, 1181–1191.

- Whitney, S., 1985. Western forests. New York: Knopf: Distributed by Random House.
- Wu, X., Chen, M.C., Yang, Z.P., 2007. Effects of sulfur application on the growth of cole, soil pH and available P in alkaline soil. *Plant Nutrition and Fertilizer Science* 13, 671–677.
- Xing, Z., Guo, J., Yan, Z., 1997. Behaviors of coated-urea in calcareous soil. *Journal of Plant Nutrition and Fertilizers* 1, 16–23.
- Xu, F., Vaziriyeganeh, M., Zwiazek, J.J., 2020. Effects of pH and mineral nutrition on growth and physiological responses of trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and white spruce (*Picea glauca*) seedlings in sand culture. *Plants* 9, 682.
- Yan, E.-R., Hu, Y.-L., Salifu, F., Tan, X., Chen, Z.C., Chang, S.X., 2012. Effectiveness of soil N availability indices in predicting site productivity in the oil sands region of Alberta. *Plant and Soil* 359, 215–231.
- Yang, X., Römheld, V., Marschner, H., 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). *Plant and Soil* 164, 1–7.
- Yousfi, S., Mahmoudi, H., Abdelly, C., Gharsalli, M., 2007. Effect of salt on physiological responses of barley to iron deficiency. *Plant Physiology and Biochemistry* 45, 309–314.
- Zasada, J., 1986. Natural regeneration of trees and tall shrubs on forest sites in interior Alaska. *Forest Ecosystems in the Alaskan Taiga* 44–73. Springer, New York, USA.
- Zasada, J.C., Phipps, H.M., 1990. *Populus balsamifera* L. Balsam poplar. *Silvics of North America* 2, 518–529.
- Zhang, C., Zhang, M., Zeng, Y., 2007. Effects of sulphur on chemical properties of calcareous soil. *Chinese Journal of Applied Ecology* 7, 1453–1458.
- Zhang, W., Calvo-Polanco, M., Chen, Z.C., Zwiazek, J.J., 2013. Growth and physiological responses of trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*) and tamarack (*Larix laricina*) seedlings to root zone pH. *Plant*

- and Soil 373, 775–786.
- Zhang, W., Fleurial, K., Sherr, I., Vassov, R., Zwiazek, J.J., 2020. Growth and physiological responses of tree seedlings to oil sands non-segregated tailings. *Environmental Pollution* 259, 113945.
- Zhang, W., Xu, F., Zwiazek, J.J., 2015. Responses of jack pine (*Pinus banksiana*) seedlings to root zone pH and calcium. *Environmental and Experimental Botany* 111, 32–41.
- Zhang, W., Zwiazek, J.J., 2016. Responses of reclamation plants to high root zone pH: effects of phosphorus and calcium availability. *Journal of Environmental Quality* 45, 1652–1662.
- Zhao, F.J., Wood, A.P., McGrath, S.P., 1999. Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). *Plant and Soil* 212, 207–217.
- Zhao, X., Li, Y., Mai, W., Tian, X., Lai, H., Lv, J., 2008. A preliminary study on amendment of saline soil through addition of sulphur. *Agricultural Research in the Arid Areas* 4, 74–78.
- Zhou, W., Lin, B., 1997. The progress in study on the behavior of sulphur in soil and plants. *Soils and Fertilizers* 5, 8–11.
- Zieslin, N., Snir, P., 1989. Responses of rose plants cultivar ‘Sonia’ and *Rosa indica major* to changes in pH and aeration of the root environment in hydroponic culture. *Scientia Horticulturae* 37, 339–349.

Chapter 2 Effects of elemental sulphur on growth of Saskatoon (*Amelanchier alnifolia*) and beaked hazelnut (*Corylus cornuta*) in oil sands reclamation soils

2.1 Introduction

Soil pH is among the major environmental factors affecting plant growth and survival. It has a profound effect on soil chemistry and affects the uptake of essential nutrients as well as the presence of phytotoxic compounds exposed to plants within the soil (Brady and Weil, 1999; Rengel, 2002). The optimum range of soil pH varies among different plant species, but the processes contributing to pH tolerance outside of this range remains poorly understood (Rengel, 2002).

Oil sands mining in northeastern Alberta, Canada results in a disturbance of large areas of the northern boreal forests (Government of Alberta, 2017). Revegetation of these areas is challenging due to the presence of saline-sodic overburden and mine tailings, which affect the soil pH (Howat, 2000). During oil sands reclamation, a layer of forest mineral soil mix (FMM), that is salvaged from upland boreal forest sites, or peat mineral mix (PMM), that is stripped from the peatlands, are placed on the top of suitable subsoil or overburden materials and coarse tailings sands before revegetation (Fung and Macyk, 2000; Dietrich and MacKenzie, 2018). The pH of the topsoil increases due to high pH of the underlying layers (Howat, 2000; Fung and Macyk, 2000). As a result, soil pH in oil sands mining reclamation sites commonly ranges from 7.0 to 8.5, while the soil pH in the surrounding boreal forests is lower than 6 (Howat, 2000; Dietrich and MacKenzie, 2018). Since native plant species of the boreal forest are planted in the reclaimed mining areas, they may not survive or grow well in these sites (Alberta Environment, 2010).

The effects of high pH on plants are complex. High pH reduces the availability of Mg, Ca, Fe, Mn, P and Zn to plants (Yang et al., 1994; Brady and Wei, 1999; Valentine et al., 2006; Marschner, 2011; Zhang et al., 2015; Xu et al., 2019). It can also reduce root water flux (Tang et al. 1993; Kamaluddin and Zwiazek, 2004; Voicu and Zwiazek,

2004; Siemens and Zwiazek, 2011), likely due to negative pH effects on the function of aquaporins (Tournaire-Roux et al., 2003; Törnroth-Housefield et al., 2006; Aroca et al., 2006; Siemens and Zwiazek, 2011). The effects of high pH on nutrient and water uptake may result in stomatal closure, a reduction of shoot water potentials, and growth inhibition (Bertoni et al., 1992; Tang et al., 1992; Tang and Turner, 1999; Kamaluddin and Zwiazek, 2004).

Several studies have indicated that elemental sulfur can be added to the soil to lower the pH (Wu et al., 2007; Cai et al., 2008; Cao et al., 2010; Sun et al., 2014) and restore the acid-base balance of the soil (Xing et al., 1997; Zhou and Lin, 1997). Elemental S can affect redox balance in the soil (Slaton et al., 2001) and alter the availability of mineral nutrients (Modaihsh et al., 1989; Cui et al., 2004). Supplementing the soil with elemental S can also affect the activities of soil microorganisms (Banerjee and Chapman, 1996; Rao et al., 2000).

In the present study, I examined the effects of elemental S addition to soil on growth parameters, nutrient uptake, leaf chlorophyll concentrations and gas exchange in both Saskatoon (*Amelanchier alnifolia*) and beaked hazelnut (*Corylus cornuta*). These native boreal plants are among the species recommended for the revegetation of oil sands due to their ecological importance (Alberta Environment, 2010; Wildflower Center, 2013; Wildflower Center, 2017). These two species could provide another option for oil sands reclamation if they grow well in this study. The main objectives of this research were to examine the effectiveness of elemental S in lowering soil pH and improving plant growth. I hypothesized that soil supplementation with S would be beneficial for growth and physiology of Saskatoon and beaked hazelnut in soil with elevated pH, but would be harmful to plants when applied to soil with inherently lower pH.

2.2 Materials and Methods

2.2.1 Soil preparation

Approximately 30-cm of the top layer of soil (forest mineral mix, FMM) was collected from the boreal forest site near the oil sands mining areas of the Canadian Natural Resources Limited (CNRL) Horizon lease, north of Fort McMurray, Alberta, Canada. The soil was sealed in pails and delivered to the University of Alberta. Large aggregates, stones, grass and tree branches were removed and the soil was air dried for 3-4 days. The initial pH of the soil was 5.7 and measured in deionized water with a soil to water ratio of 1:2 (w/v) using an Orion STAR A111 pH meter (Thermo Fisher Scientific Inc., Waltham, MA). The soil was mixed with 50 g kg⁻¹ Ca(OH)₂ (Wang et al., 2011) to raise the initial soil pH to 8.5. After adjusting the pH, elemental S (99.5%, Thermo Fisher Scientific Chemicals, Inc., Ottawa, ON, Canada) was mixed into the soil at the concentrations of 0 (control), 5 and 25 g kg⁻¹ soil. These concentrations were selected based on the results of a previous study, which used 0, 2.5, 5, 10 and 20 g S kg⁻¹ soil concentrations (Dede and Ozdemir, 2016).

2.2.2 Plant material and experimental set-up

One-year-old greenhouse-grown Saskatoon (*Amelanchier alnifolia*) and beaked hazelnut (*Corylus cornuta*) dormant seedlings were obtained from the Tree Time Services Inc., Edmonton, Alberta, Canada. Seedlings were stored for two weeks at 4°C prior to the experiment. After removing from cold storage, the seedlings were planted in 2 L pots. The plants were grown for two months in the growth room at 22/18°C (day/night) temperature, 65±10% relative humidity and 16-h photoperiod with 300 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPDF) at the top of the seedlings provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

The experiment was a 2 x 3 completely randomized factorial design (fully randomized factorial design) with two initial pH levels (5.7 and 8.5) and three elemental

S concentrations (0, 5 and 25 g kg⁻¹ soil). There were ten replicates for each treatment combination. All plants were watered with tap water in growth chamber every two days and provided with 500 ml 100% Hoagland's mineral solution (Epstein, 1972) to each pot once a week. Soil pH during the experiment was monitored weekly with the pH meter (IQ160G, IQ Scientific Instruments, USA). Six plants were randomly selected for measurements from ten replicates of each treatment combination.

2.2.3 Growth parameters

Seedling height measurements were taken from the base of the stem to the shoot tip of the terminal leader in six randomly-selected seedlings from ten replicates per treatment combination per species. Stem diameter measurements were taken with calipers at the base of the stem. Shoot heights and stem diameters were measured twice, at the time of planting and at harvest after two months of growth.

To determine leaf, shoot and root dry weights, six seedlings were randomly selected from each pH and S treatment combination for each species. Leaves were separated from stems and placed in an ultra-low temperature freezer at -80°C before freeze-drying for 72 h. Roots and stems were dried in an oven at 70°C for 72 h. Shoot dry weights were determined by adding dry weights of stems and leaves. For the total dry weights, stem, shoot and root dry weights from each plant were combined.

2.2.4 Leaf chlorophyll concentrations

Leaf chlorophyll-a, chlorophyll-b and total chlorophyll concentrations were determined in fully-expanded leaves removed from the mid-parts of the shoots in six randomly-selected seedlings per treatment combination per species. The leaves were dried in a freeze-drier (Freeze Dry Lyph-Lock, Labconco, Kansas, USA) for 72 h and ground in a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from the leaf samples (10 mg dry weight) with 8 ml dimethyl sulfoxide (DMSO) at 65°C for 22 h. After filtering, chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher

Scientific, Massachusetts, USA), at 648 nm and 665 nm for chlorophyll-a and chlorophyll-b concentrations, respectively. Total chlorophyll concentration was calculated using the Arnon's equation (Sestak et al., 1971).

2.2.5 Net photosynthesis (P_n) and transpiration (E) rates

Six seedlings per species were randomly taken from each treatment combination for the measurements of gas exchange after two months of treatments. The P_n and E rates were measured in the uppermost fully-developed leaves using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, Nebraska USA) at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPDF) at approximately 4 - 9 hours after the onset of photoperiod.

2.2.6 Leaf elemental concentrations

Leaf elemental concentrations were determined in six plants of each plant species from each treatment combination. For K, Mn, Fe, Zn, B, Mg, Ca, P and S analysis, ground dry leaf samples (0.2 g dry weight) were digested with 10 ml 70% HNO₃ and diluted with Milli-Q water to 40 ml. The extracts were then filtered and analyzed by Thermo iCAP6300 Duo (N. America) inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo Fisher Corp., Cambridge, United Kingdom). For total N analysis, ground dry leaf samples (3 mg dry weight) were packed into a tin or silver capsule, and then analyzed by the Thermo FLASH 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., Bremen, Germany 2016). All elemental analyses were conducted in the Natural Resources Analytical Laboratory of the University of Alberta, Edmonton, Canada.

2.2.7 Statistical analysis

All data were analyzed by two-way ANOVA using the R software (Version 3.5.2, R Core Team, R Foundation for Statistical Computing, Vienna, Austria) to determine statistically significant ($p \leq 0.05$) differences between the treatments. Soil pH and elemental S levels were the main factors. Comparisons between the treatment means

were conducted using the Fisher's LSD test.

2.3 Results

2.3.1 Soil pH

The soil pH fluctuated around the original pH values when no S was added at both pH levels (Fig. 1). In the control soil with no $\text{Ca}(\text{OH})_2$ added, the addition of 5 g kg^{-1} S lowered the pH over time by up to about two units and the addition of 25 g kg^{-1} S lowered the pH by up to about four units. In the pH 8.5 soil with $\text{Ca}(\text{OH})_2$ added, the pH declined by up to about one unit with the addition of 5 g kg^{-1} S, and by up to about five units with the addition of 25 g kg^{-1} S (Fig. 2.1).

2.3.2 Plant heights and stem diameters

There were no significant interactions between pH and S on height increments and diameter increments of both plants (Table 2.1, Table 2.2).

Diameter increments of both plants were significantly decreased with the addition of elemental S in pH 5.7 soil (Fig. 2.2b, d).

The addition of elemental S to pH 8.5 soil didn't significantly reduce height increment (Fig. 2.2a, c) and diameter increment (Fig. 2.2b, d) of both plants.

2.3.3 Dry weights

There were significant interactions between pH and S on leaf dry weights of Saskatoon (Table 2.1, Table 2.2).

At pH 5.7, the addition of elemental S significantly reduced leaf (Fig. 2.3a) and shoot dry weights (Fig. 2.3b) in Saskatoon, leaf dry weights (Fig. 2.3d) were significantly decreased in beaked hazelnut.

At pH 8.5, root dry weights (Fig. 2.3f) were significantly decreased with addition of elemental S in Saskatoon.

2.3.4 Chlorophyll concentrations

There were significant interactions between pH and S on chlorophyll-a: chlorophyll-b ratios of Saskatoon and beaked hazelnut (Table 2.1, Table 2.2).

At pH 5.7, elemental S significantly decreased chlorophyll-a: chlorophyll b ratios (Fig. 2.6a) in Saskatoon, chlorophyll a concentrations (Fig. 2.5c), chlorophyll b concentrations (Fig. 2.5d), chlorophyll-a: chlorophyll-b ratio (Fig. 2.6c) and total chlorophyll concentrations (Fig. 2.6d) were significantly reduced with addition of elemental S in beaked hazelnut.

At pH 8.5, elemental S significantly reduced chlorophyll a concentrations (Fig. 2.5a) and chlorophyll-a: chlorophyll-b ratio (Fig. 2.6a) in Saskatoon, chlorophyll a (Fig. 2.5c), chlorophyll b concentrations (Fig. 2.5d) and total chlorophyll concentrations (Fig. 2.6d) were significantly decreased with the addition of elemental S in beaked hazelnut.

2.3.5 Net photosynthesis (Pn) and transpiration (E) rates

There were significant interactions between pH and S on Pn of Saskatoon and E of beaked hazelnut (Table 2.1, Table 2.2).

At pH 5.7, the addition of elemental S significantly reduced Pn (Fig. 2.7a) and E (Fig. 2.7b) in Saskatoon, only E (Fig. 2.7d) was significantly decreased with addition of elemental S in beaked hazelnut.

At pH 8.5, elemental S significantly reduced Pn (Fig. 2.7a) and E (Fig.2.7b) in Saskatoon.

2.3.6 Leaf elemental concentrations

There were significant interactions between pH and S on leaf Ca, P, S and total N concentrations of Saskatoon, leaf Mn concentrations of beaked hazelnut (Table 2.1, Table 2.2).

At pH 5.7, elemental S significantly increased leaf K (Fig. 2.8a), Mn (Fig. 2.8b), S (Fig. 2.12a) concentrations in Saskatoon and leaf Mn (Fig. 2.8d), Fe (Fig. 2.9c), Zn (Fig.

2.9d), Mg (Fig. 2.10d), Ca (Fig. 2.11c), S (Fig. 2.12c) concentrations in beaked hazelnut. However, leaf total N (Fig. 2.12d) concentrations were significantly reduced in beaked hazelnut with elemental S added.

At pH 8.5, the addition of elemental S significantly increased leaf K (Fig. 2.8a), B (Fig. 2.10a), S (Fig. 2.12a) concentrations in Saskatoon and leaf Mn (Fig. 2.8d), B (Fig. 2.10c), Mg (Fig. 2.10d), Ca (Fig. 2.11c), S (Fig. 2.12c) concentrations in beaked hazelnut. However, elemental S significantly decreased leaf Mg (Fig. 2.10b), Ca (Fig. 2.11a), total N (Fig. 2.12b) concentrations in Saskatoon. In addition, leaf P (Fig. 2.11b) concentrations in Saskatoon significantly increased with the addition of 5 g kg⁻¹ S, but significantly decreased with 25 g kg⁻¹ S added.

2.4 Discussion

In the present study, growth and physiological responses to different levels of elemental S added to the soil of 5.7 and 8.5 pH were examined in Saskatoon and beaked hazelnut. The soil pH values in the boreal forest are typically below 6.0 with wet peatland sites having the lowest pH (Natural Regions Committee, 2006; Calvo-Polanco et al., 2017). The soil pH in oil sands reclamation areas located in Northeastern Alberta, Canada is commonly higher than 8.0 (Zhang et al., 2020). In the reclamation sites affected by oil sands tailings, part of the reason for high soil pH in addition to the effect of overburden may also be NaOH, which is added during the processes of oil extraction and increases the pH of tailing sands deposited in the reclamation sites (Fung and Macyk, 2000; Chalaturnyk et al., 2002; Xu et al., 2019).

In the present study, I added Ca(OH)₂ to the soil to increase its pH to 8.5, which may be expected in many reclamation sites. Ca(OH)₂ is usually used to amend acid soil since it has no significant negative impact on plants (Wang et al., 2011). Calcium is also an abundant element that is present in some reclamation soils (Ramos-Padrón et al., 2010). Since bitumen refining produces large amounts of elemental S (Engelhardt and Todirescu, 2005), it could be potentially useful as an additive to lower soil pH. Previous

studies demonstrated that soil bacteria oxidize elemental S and, during this process, H^+ is released into the soil (Zhang et al., 2007; Zhao et al., 2008; Cao et al., 2010; Sun et al., 2014). The results of soil pH changes in my study also demonstrated that elemental S was effective in reducing soil pH, which declined by one and five units, respectively, when 5 and 25 g of S was added per kg of pH 8.5 soil. Therefore, the amount of S that would be required to lower the pH of this soil to the optimum pH value of 5 to 7 for most plants (Perry, 2003), falls somewhere within the range that was used in my study. However, the exact amount would depend on the soil chemical and physical properties (Soaud et al., 2011). In the present study, adding 5 and 25 g kg^{-1} S to the 8.5 pH soil did not bring pH to the desirable level. The addition of 5 g kg^{-1} S decreased the pH only to 7.7, while 25 g kg^{-1} S lowered the pH to 3.3. The optimum soil pH is considered to be 6.0 - 7.0 for Saskatoon (CNPS, 2020) and 5.3 – 6.1 for beaked hazelnut (Gill et al., 1974). In addition, tap water was used to water plants, the pH of tap water is usually higher than 8 (EPCOR, 2020), it may affect soil pH change. However, tap water had less effects on soil pH change, because soil pH just fluctuated around the original pH value.

The addition of 5 and 25 g of S per kg of soil at pH 5.7 resulted in high soil acidity (pH 3.7 and pH 2.7, respectively), which was likely to negatively affect the plants (Tang et al., 2013). The plants growing in low pH soil may face a variety of stresses including ion toxicity, nutrient deficiencies, altered cell wall formation and enzyme activities, which can affect plant growth and increase mortality (Rout et al., 2001; Brady and Weil, 2002; Kopittke et al., 2016). My results clearly demonstrate that excessive soil acidity can be of concern when elemental S is added to the slightly acidic soil. Therefore, careful measurements of soil pH across the reclamation site must be carried out before S is added.

In the pH 5.7 soil, a significant decrease of Pn, E and chlorophyll a:b ratios were observed in Saskatoon when 5 g kg^{-1} elemental S was added to the soil at pH 5.7 and in beaked hazelnut, these decreases were also accompanied by the reduced total chlorophyll concentrations. The decreases of leaf dry weights were additionally

observed at the pH 5.7 level in both plant species after the addition of 25 g kg⁻¹ elemental S compared with no addition of S to the soil. The decrease of chlorophyll a:b ratios in plants indicates that chlorophyll a was more affected by the S treatment compared with chlorophyll b. This agrees with other studies, which demonstrated that chlorophyll a is less stable than chlorophyll b under various environmental conditions including low and high pH soils (Koca et al., 2007; Zhang et al., 2015). The reduction of stomatal conductance is among the main factors that limiting photosynthesis and transpiration (Tang et al., 1993; Kamaluddin and Zwiazek, 2004; Xu et al., 2019). The decrease of Pn and E in Saskatoon was also likely due to the samples with some leaves in bad condition when collecting randomly. The decrease of leaf dry weight was likely due to the reduction of Pn. However, the non-linear decrease of diameter increment of beaked hazelnut was likely due to the lower pH caused by high oxidization rate of 5 g kg⁻¹ S in some pots that limiting plants growth.

In the pH 8.5 soil, the decreases of root dry weights, chlorophyll a:b ratios, Pn and E were observed in Saskatoon when 25 g kg⁻¹ elemental S was added, compared with 0 and 5 g kg⁻¹ elemental S treatments. However, contrary to beaked hazelnut, there was no significant decrease in the total leaf chlorophyll concentrations. The reduction of stomatal conductance may be still the main factor for lowering Pn and E in Saskatoon. The decrease of root dry weights in Saskatoon by the 25 g kg⁻¹ S treatment also points to the sensitivity of plants to low pH, which could directly or indirectly impact root growth through the effects on root elongation, formation of lateral roots and root hair development (Canmore-Neumann et al., 1996; Rout et al., 2001; Zhang et al., 2013). In beaked hazelnut, the decrease of total chlorophyll concentrations was likely mainly due to the inhibition of chlorophyll synthesis by low pH (Karimizarch et al., 2014). However, the Pn and E in Saskatoon as well as the total leaf chlorophyll concentrations in beaked hazelnut were significantly higher in plants subjected to the 5 g kg⁻¹ S treatment compared with the 25 g kg⁻¹ S treatment, but not compared with 0 g kg⁻¹ S treatment. Therefore, none of these two S soil amendments could be considered effective in improving plant performance when added to the pH 8.5 soil.

Since pH impacts availability of mineral nutrients. Plants may face nutrient deficiencies when grown under very low or high pH conditions (Zhang et al., 2015). In the pH 5.7 soil, significant increases of leaf K and S concentrations were observed in Saskatoon with the addition of S and significant increases of leaf Mn concentrations were observed when 5 g kg⁻¹ S was added to the soil. In beaked hazelnut, the significant increases of leaf Mn, Mg and S concentrations were observed with the addition of 5 g kg⁻¹ and 25 g kg⁻¹ S. Also leaf Fe, Zn and Ca concentrations significantly increased in the 5 g kg⁻¹ S treatment compared with control. These increases are likely due to the biomass dilution effect (Jarrell and Beverly, 1981; Zhang et al., 2020). However, the significant decrease of the total leaf N concentrations that was observed in beaked hazelnut after the addition of 5 g kg⁻¹ S to the soil was contrary to the reported increase in the leaf N concentrations in blueberry after soil amendment with S (Spiers and Braswell, 1992). The decrease in leaf N may be due to unbalanced ratio of available N and S in the soil that resulted in a reduced N uptake (Janzen and Bettany, 1984; Orman, 2012).

In the pH 8.5 soil, significant increases of leaf K and B concentrations were observed in Saskatoon after the addition of 5 g kg⁻¹ and 25 g kg⁻¹ S and leaf S concentrations significantly increased after the addition of 25 g kg⁻¹ S. In beaked hazelnut, the leaf Mn, B, Mg and Ca concentrations significantly increased with the addition of 25 g kg⁻¹ S and the leaf S concentrations significantly increased by both 5 g kg⁻¹ and 25 g kg⁻¹ S treatments. These results were likely due to the high concentrations of H⁺ that can facilitate nutrient uptake (Lambers et al., 2008; Viani et al., 2014). However, in Saskatoon, leaf Mg, Ca, and N concentrations significantly decreased with the addition of 25 g kg⁻¹ S and leaf P concentrations significantly increased by the 5 g kg⁻¹ S treatment, but decreased when 25 g kg⁻¹ S was added to the soil. These differences between the plant species likely reflect their different pH requirements for nutrient uptake (Zhang et al., 2015).

In beaked hazelnut, leaf Ca concentrations significantly increased with the addition of 25 g kg⁻¹ S, which is in agreement with the results reported for lowbush blueberry

(Starast, 2007) and demonstrate that S can improve Ca uptake from the soil. However, leaf Ca concentrations in Saskatoon in the 25 g kg⁻¹ S treatment of pH 8.5 soil showed an opposite trend, with a decrease in the leaf Ca concentrations suggesting that high S concentrations in the soil interfered with Ca uptake and (or) root to leaf transport at this soil pH level.

The results indicated that Saskatoon and beaked hazelnut responded differently to elemental S treatments, the results were likely due to the different range of suitable pH range of two species, which were 6.0 - 7.0 and 5.3 - 6.1 for Saskatoon and beaked hazelnut, respectively (Gill et al., 1974; CNPS, 2020). Saskatoon may be more sensitive than beaked hazelnut to much lower soil pH that caused by elemental S treatment. However, previous study had illustrated that lower pH can limit the nutrient uptake (Zhang et al., 2015), but the results above showed that some leaf element increased under much lower soil pH. The results were likely due to long process of S oxidation in soil (Modaihsh et al., 1989) and the short period for growing both plants, which affected plants slowly.

In conclusion, the results of this study demonstrated that elemental S was effective in lowering soil pH. The addition of S to the pH 5.7 soil lowered its pH to the levels that can be considered toxic to plants and resulted in impaired growth and physiological performance of Saskatoon and beaked hazelnut plants. However, Saskatoon and beaked hazelnut seedlings growing better in pH 8.5 soil with no addition of S than the addition of 5 and 25 g kg⁻¹ S to the soil. In addition to the initial soil pH, the exact S concentration required to lower soil pH to the desirable level may also likely vary depending on the soil type and chemistry and should be experimentally determined for the different sites. However, this study clearly demonstrated the effectiveness of using S to lower the pH in reclamation sites and potential beneficial effects of this treatment to plants.

2.5 References

Alberta Environment, 2010. Guidelines for reclamation to forest vegetation in the

- Athabasca Oil Sands Region, 2nd Edition. Prepared by the Terrestrial Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, Fort McMurray, AB, Canada.
- Aroca, R., Ferrante, A., Vernieri, P., Chrispeels, M.J., 2006. Drought, abscisic acid and E rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Annals of Botany* 98, 1301–1310.
- Banerjee, M.R., Chapman, S.J., 1996. The significance of microbial biomass sulphur in soil. *Biology and Fertility of Soils* 22, 116–125.
- Bertoni, G.M., Pissaloux, A., Morard, P., Sayag, D.R., 1992. Bicarbonate-pH relationship with iron chlorosis in white lupine. *Journal of Plant Nutrition* 15, 1509–1518.
- Brady, N.C., Weil, R.R., 1999. *The nature and properties of soils*. 12th Edition. Prentice-Hall Inc., New Jersey, USA.
- Brady, N.C., Weil, R.R., 2002. *The nature and properties of soils*. 13th Edition. Prentice-Hall Inc., New Jersey, USA.
- Cai, X., Ma, Y., Wang, W., Wei, C., Wang, X., Dun, S., 2008. Effects of applying sulphur on alkali soil pH and its consequence on yield and quality of flue-cured tobacco. *Acta Tabacaria Sinica* 14, 19–22.
- Calvo-Polanco, M., Zhang, W., Macdonald, S.E., Señorans, J., Zwiazek, J.J., 2017. Boreal forest plant species responses to pH: ecological interpretation and application to reclamation. *Plant and Soil* 420, 195–208.
- Canmore-Neumann, R., Silber, A., Mitchnick, B., Gilad, S., Ben-Jaacov, J., 1996. Effect of pH on the development of *Leucadendron* ‘Safari Sunset’. IV International Protea Working Group Symposium 453, 47–52.
- Cao, Y., Zhao, Y., Zhao, W., Lv, J., 2010. Research on effect of sulfur application on saline-alkaline soil pH and availability of trace elements in Huanghe River irrigation district in Ningxia. *Agricultural Research in the Arid Areas* 28, 199–201.
- Chalaturnyk, R.J., Don Scott, J., Özüm, B., 2002. Management of oil sands tailings. *Petroleum Science and Technology* 20, 1025–1046.

- CNPS, 2020. Saskatoon Serviceberry, *Amelanchier alnifolia* [WWW Document]. URL [https://calscape.org/Amelanchier-alnifolia-\(\)](https://calscape.org/Amelanchier-alnifolia-()).
- Cui, Y., Wang, Q., Dong, Y., Li, H., Christie, P., 2004. Enhanced uptake of soil Pb and Zn by Indian mustard and winter wheat following combined soil application of elemental sulphur and EDTA. *Plant and Soil* 261, 181–188.
- Dede, G., Ozdemir, S., 2016. Effects of elemental sulphur on heavy metal uptake by plants growing on municipal sewage sludge. *Journal of Environmental Management* 166, 103–108.
- Dietrich, S.T., MacKenzie, M.D., 2018. Comparing spatial heterogeneity of bioavailable nutrients and soil respiration in boreal sites recovering from natural and anthropogenic disturbance. *Frontiers in Environmental Science* 6, 126.
- Epstein, E. 1972. *Mineral nutrition of plants: principles and perspectives*. Wiley, New York, USA.
- Engelhardt, R., Todirescu, M., 2005. *An introduction to development in Alberta's oil sands*. University of Alberta. AB, Canada.
- EPCOR, 2020. Daily Water Quality [WWW Document]. URL <https://www.epcor.com/products-services/water/water-quality/Pages/daily-water-quality.aspx>.
- Fung, M.Y., Macyk, T.M., 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands* 41, 755–774.
- Gill, J.D., Healy, W.M., 1974. *Shrubs and vines for northeastern wildlife*. USDA Forest Service, Northeastern Forest Experiment Station, Upper Darby, Pennsylvania, USA.
- Government of Alberta, 2017. Oil sands – Overview [WWW Document]. URL <https://www.alberta.ca/oil-sands-overview.aspx>.
- Howat, D., 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Alberta Environment. Environmental Sciences Division, Edmonton AB, Canada.
- Janzen, H.H., Bettany, J.R., 1984. Sulfur nutrition of rapeseed: I. Influence of fertilizer

- nitrogen and sulfur rates. *Soil Science Society of America Journal* 48, 100–107.
- Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* 34, 197–224.
- Kamaluddin, M., Zwiazek, J.J., 2004. Effects of root medium pH on water transport in paper birch (*Betula papyrifera*) seedlings in relation to root temperature and abscisic acid treatments. *Tree Physiology* 24, 1173–1180.
- Karimizarchi, M., Aminuddin, H., Khanif, M.Y., Radziah, O., 2014. Elemental sulphur application effects on nutrient availability and sweet maize (*Zea mays* L.) response in a high pH soil of Malaysia. *Malaysian Journal of Soil Science* 18, 75–86.
- Koca, N., Karadeniz, F., Burdurlu, H.S., 2007. Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry* 100, 609–615.
- Kopittke, P.M., Menzies, N.W., Wang, P., Blamey, F.P.C., 2016. Kinetics and nature of aluminium rhizotoxic effects: a review. *Journal of Experimental Botany* 67, 4451–4467.
- Lambers, H., Chapin III, F.S., Pons, T.L., 2008. *Plant physiological ecology*. Springer Science & Business Media, New York, USA.
- Marschner, H., 2011. *Marschner's mineral nutrition of higher plants*. Academic Press, London, UK.
- Modaihsh, A.S., Al-Mustafa, W.A., Metwally, A.I., 1989. Effect of elemental sulphur on chemical changes and nutrient availability in calcareous soils. *Plant and Soil* 116, 95–101.
- Natural Regions Committee, 2006. *Natural regions and subregions of Alberta*. Compiled by DJ Downing and WW Pettapiece. Government of Alberta, AB, Canada.
- Orman, S., 2012. Effects of elemental sulphur and farmyard manure applications to calcareous saline clay loam soil on growth and some nutrient concentrations of tomato plants. *Journal of Food, Agriculture & Environment* 10, 720–725.
- Perry, L., 2003. pH for the Garden. URL <http://pss.uvm.edu/ppp/pubs/oh34.htm>.
- Ramos-Padrón, E., Bordenave, S., Lin, S., Bhaskar, I.M., Dong, X., Sensen, C.W.,

- Fournier, J., Voordouw, G., Gieg, L.M., 2011. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environmental Science & Technology* 45, 439–446.
- Rao, P.S., Karunasagar, Iddya, Otta, S.K., Karunasagar, Indrani, 2000. Incidence of bacteria involved in nitrogen and sulphur cycles in tropical shrimp culture ponds. *Agriculture International* 8, 463-472.
- Rengel, Z., 2002. *Handbook of Plant Growth pH as the Master Variable*. CRC Press.
- Rout, G., Samantaray, S., Das, P., 2001. Aluminium toxicity in plants: a review. *Agronomie* 21, 4–5.
- Sestak, Z., Catský, J., Jarvis, P.G., 1971. *Plant photosynthetic production. Manual of methods*. Dr. W. Junk Publishers, The Hague, Netherlands.
- Siemens, J.A., Zwiazek, J.J., 2011. *Hebeloma crustuliniforme* modifies root hydraulic responses of trembling aspen (*Populus tremuloides*) seedlings to changes in external pH. *Plant and Soil* 345, 247–256.
- Slaton, N.A., Norman, R.J., Gilmour, J.T., 2001. Oxidation rates of commercial elemental sulfur products applied to an alkaline silt loam from Arkansas. *Soil Science Society of America Journal* 65, 239–243.
- Soaud, A.A., Al Darwish, F.H., Saleh, M.E., El-Tarabily, K.A., Sofian-Azirun, M., Rahman, M.M., 2011. Effects of elemental sulfur, phosphorus, micronutrients and ‘*Paracoccus versutus*’ on nutrient availability of calcareous soils. *Australian Journal of Crop Science* 5, 554.
- Spiers, J.M., Braswell, J.H., 1992. Soil-applied sulfur affects elemental leaf content and growth of ‘*Tifblue*’ rabbiteye blueberry. *Journal of the American Society for Horticultural Science* 117, 230–233.
- Starast, M., Karp, K., Vool, E., 2007. Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry. *Agricultural and Food Science* 16, 34–45.
- Sun, L., Duan, D., Peng, C., He, J., Shi, J., 2014. Influence of sulfur on the speciation transformation and phyto-availability of heavy metals in soil: A review. *Chinese*

- Journal of Applied Ecology 25, 2141–2148.
- Tang, C., Cobley, B.T., Mokhtara, S., Wilson, C.E., Greenway, H., 1993. High pH in the nutrient solution impairs water uptake in *Lupinus angustifolius* L. Plant Nutrition—from Genetic Engineering to Field Practice 763–765. Springer, Dordrecht, Netherlands.
- Tang, C., Longnecker, N.E., Thomson, C.J., Greenway, H., Robson, A.D., 1992. Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in their sensitivity to pH above 6.0. Journal of Plant Physiology 140, 715–719.
- Tang, C., Turner, N.C., 1999. The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus angustifolius* L. and *Lupinus pilosus* Murr. Australian Journal of Experimental Agriculture 39, 457–464.
- Tang, K., Zhu, W., Zhou, W., Yi, Z., Tu, N., 2013. Research progress on effects of soil pH on plant growth and development. Crop Research 27, 207–212.
- Törnroth-Horsefield, S., Wang, Y., Hedfalk, K., Johanson, U., Karlsson, M., Tajkhorshid, E., Neutze, R., Kjellbom, P., 2006. Structural mechanism of plant aquaporin gating. Nature 439, 688–694.
- Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., Bligny, R., Maurel, C., 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature 425, 393–397.
- Valentine, D.W., Kielland, K., Chapin III, F.S., McGuire, A.D., Van Cleve, K., 2006. Patterns of biogeochemistry in Alaskan boreal forests. Alaska's Changing Boreal Forest 241–266. Oxford University Press, New York, USA.
- Viani, R.A., Rodrigues, R.R., Dawson, T.E., Lambers, H., Oliveira, R.S., 2014. Soil pH accounts for differences in species distribution and leaf nutrient concentrations of Brazilian woodland savannah and seasonally dry forest species. Perspectives in Plant Ecology, Evolution and Systematics 16, 64–74.
- Voicu, M.C., Zwiazek, J.J., 2004. Cycloheximide inhibits root water flow and stomatal conductance in aspen (*Populus tremuloides*) seedlings. Plant, Cell & Environment

27, 199–208.

Wang, S., Hu, C., Cheng, D., Liao, W., Li, N., Chen, L., Fang, J., 2011. Effects of soil pH regulation on indigenous microflora and physiological groups in tea plantations. *Soils* 43, 76–80.

Wildflower Center, 2013. *Amelanchier alnifolia* (Saskatoon serviceberry serviceberry). Native Plants of North America. URL https://www.wildflower.org/plants/result.php?id_plant=AMAL2.

Wildflower Center, 2017. *Corylus cornuta* (Beaked hazelnut). Native Plants of North America. URL https://www.wildflower.org/plants/result.php?id_plant=COCO6.

Wu, X., Chen, M., Yang, Z., 2007. Effects of sulphur application on the growth of cole, soil pH and available P in alkaline soil. *Plant Nutrition and Fertilizer Science* 4, 671–677.

Xing, Z., Guo, J., Yan, Z., 1997. Behaviors of coated-urea in calcareous soil. *Journal of Plant Nutrition and Fertilizers* 3, 16–23.

Xu, F., Tan, X., Zhang, W. Q., Zwiazek, J. J., 2019. Effects of iron and root zone pH on growth and physiological responses of paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*) and red-osier dogwood (*Cornus stolonifera*) seedlings in a split-root hydroponic system. *Acta Physiologiae Plantarum* 41, 142.

Yang, X., Römheld, V., Marschner, H., 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). *Plant and Soil* 164, 1–7.

Zhang, C., Zhang, M., Zeng, Y., 2007. Effects of sulphur on chemical properties of calcareous soil. *Chinese Journal of Applied Ecology* 7, 1453–1458.

Zhang, W., Fleurial, K., Sherr, I., Vassov, R., Zwiazek, J.J., 2020. Growth and physiological responses of tree seedlings to oil sands non-segregated tailings. *Environmental Pollution* 259, 113945.

Zhang, W., Calvo-Polanco, M., Chen, Z.C., Zwiazek, J.J., 2013. Growth and physiological responses of trembling aspen (*Populus tremuloides*), white spruce

- (*Picea glauca*) and tamarack (*Larix laricina*) seedlings to root zone pH. *Plant and Soil* 373, 775–786.
- Zhang, W., Xu, F., Zwiazek, J.J., 2015. Responses of jack pine (*Pinus banksiana*) seedlings to root zone pH and calcium. *Environmental and Experimental Botany* 111, 32–41.
- Zhao, X., Li, Y., Mai, W., Tian, X., Lai, H., Lv, J., 2008. A preliminary study on amendment of saline soil through addition of sulphur. *Agricultural Research in the Arid Areas* 4, 74–78.
- Zhou, W., Lin, B., 1997. The progress in study on the behavior of sulphur in soil and plants. *Soils and Fertilizers* 5, 8–11.

2.6 Figures

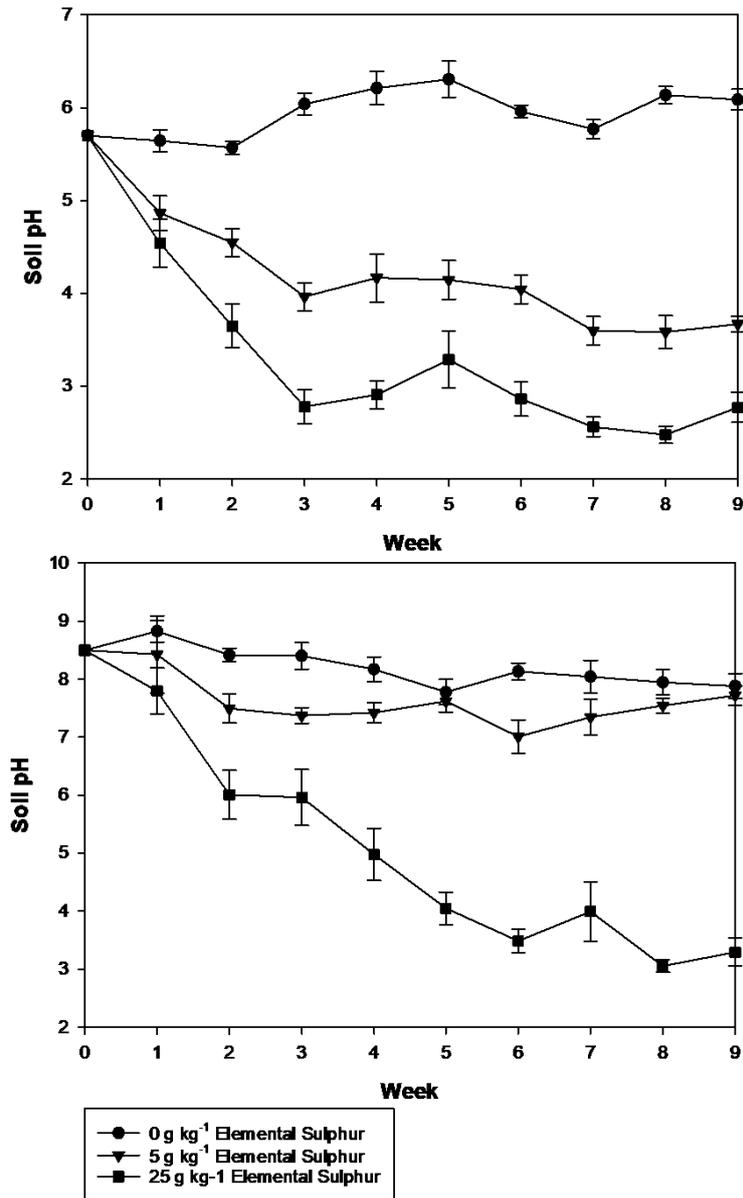


Figure 2.1 Changes of soil pH following the addition of 0, 5, and 25 g kg⁻¹ elemental S to soil with an initial pH of 5.7 (a) and 8.5 (b). Means (n = 6) ± SE are shown.

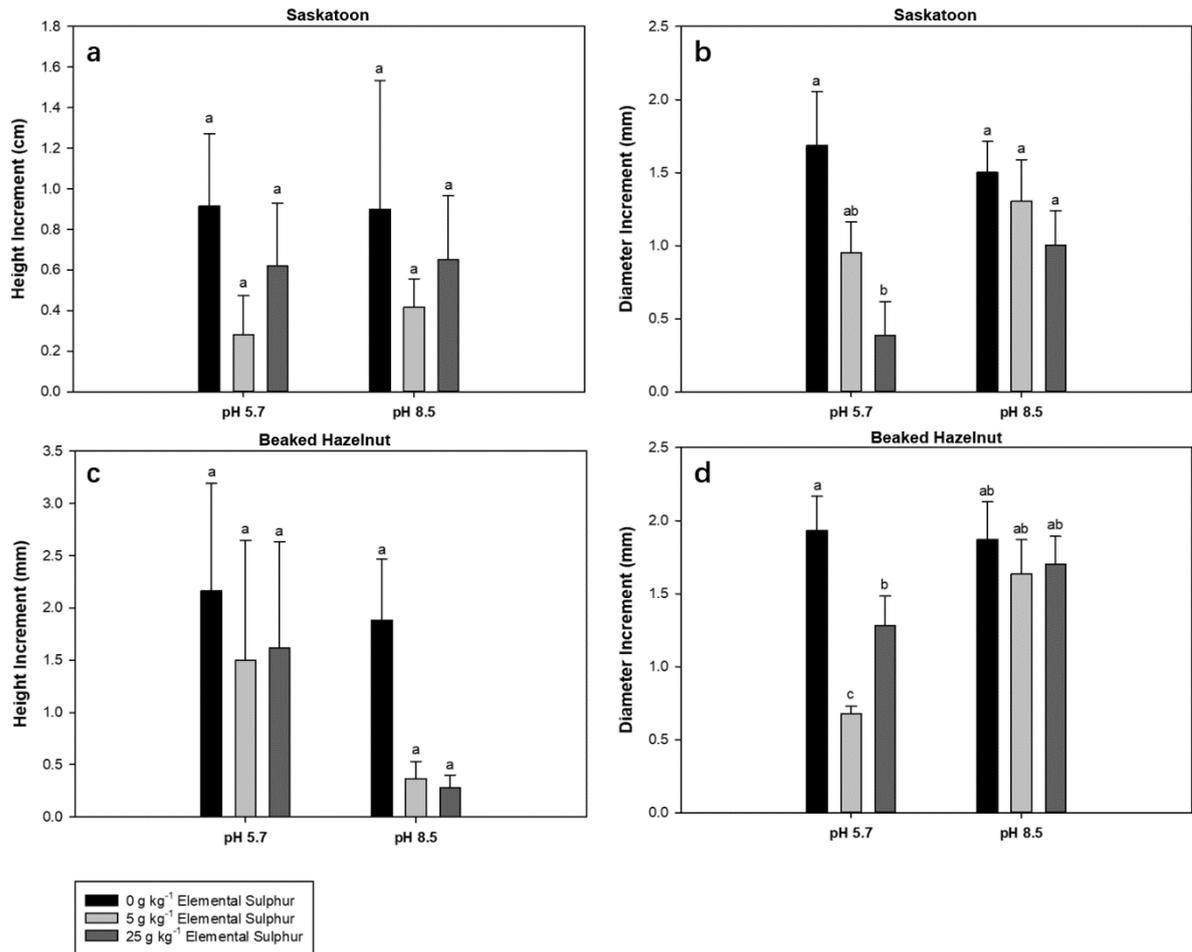


Figure 2.2 Height increment (cm) and diameter increment (mm) in Saskatoon (a, b) and beaked hazelnut (c, d) plants after two months of growth in soil with an initial pH of either 5.7 or 8.5 and supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments as determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

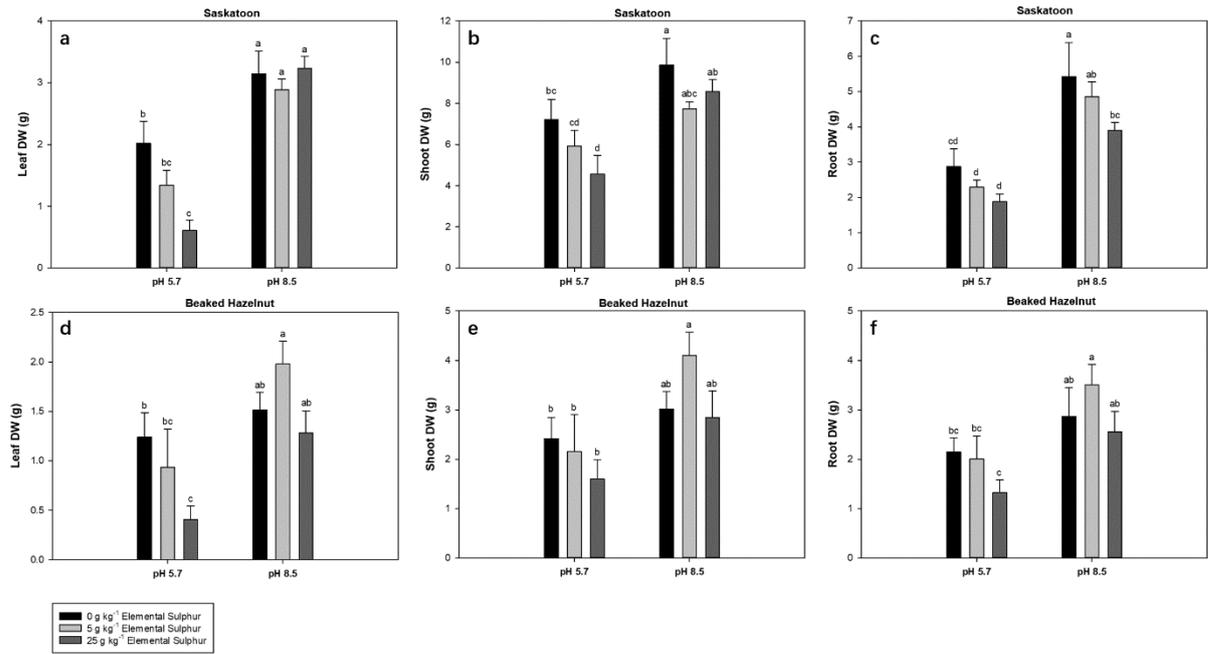


Figure 2.3 Leaf, shoot, and root dry weights in Saskatoon (a, b, c) and beaked hazelnut (d, e, f). Plants were grown for two months in soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

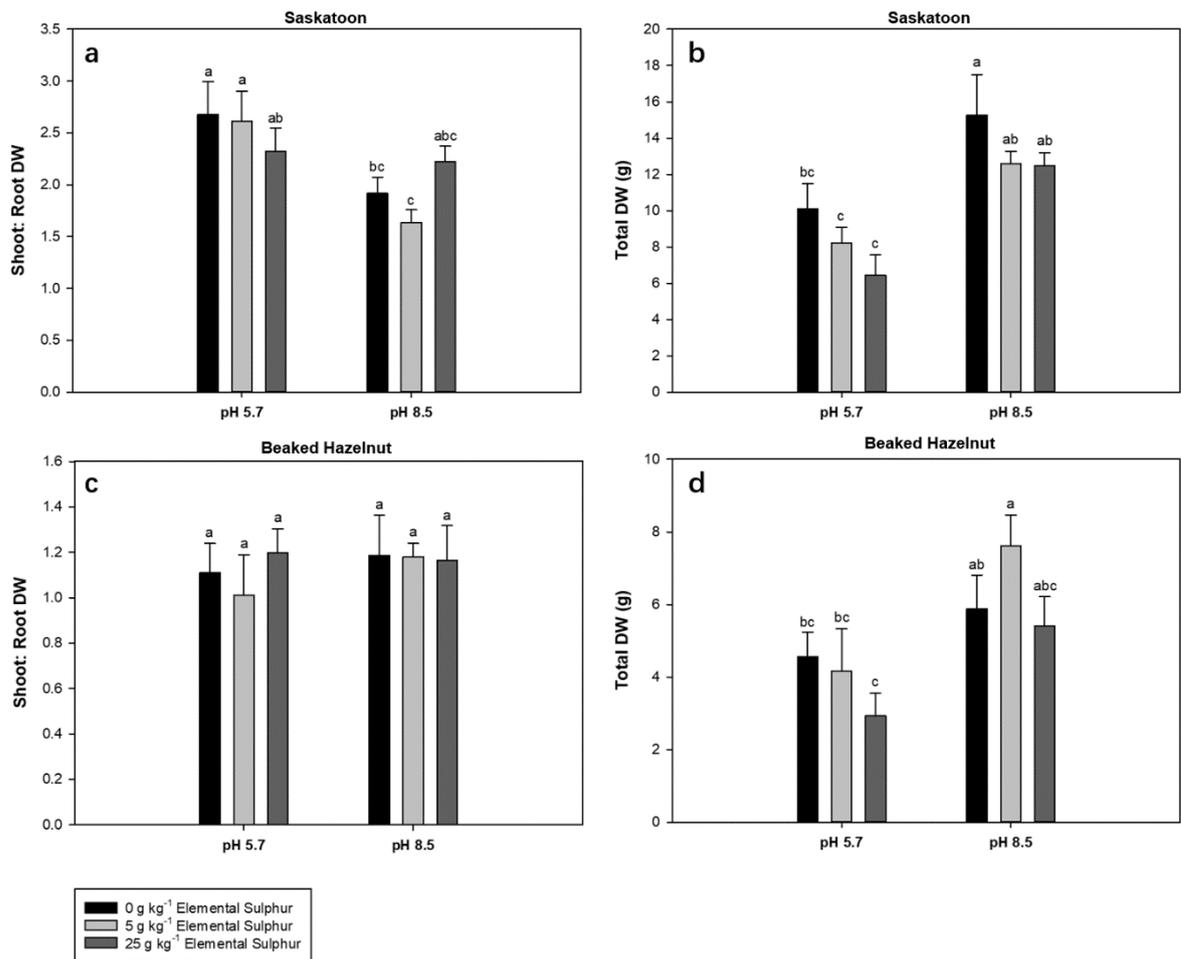


Figure 2.4 Shoot: Root dry weight ratios and total plant dry weights in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

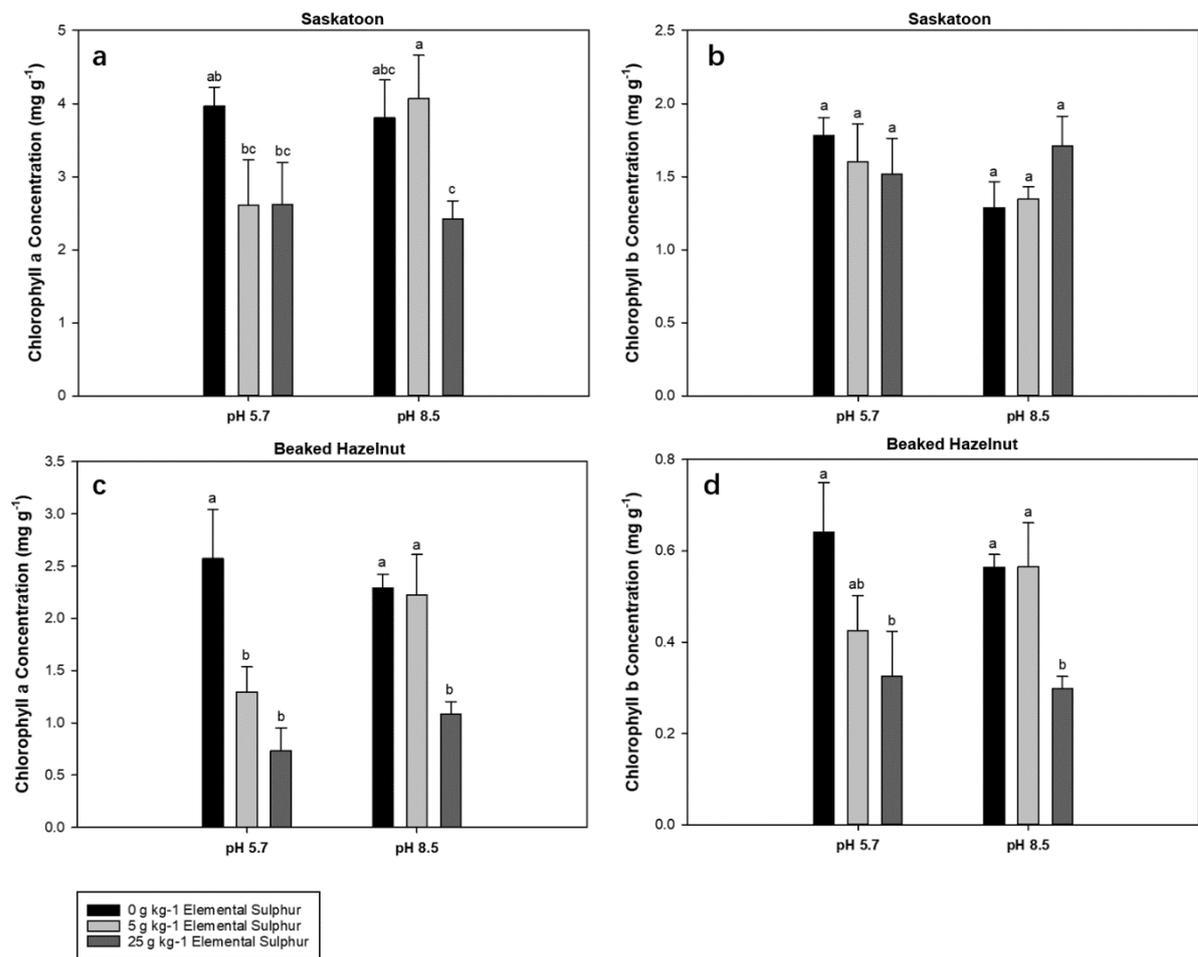


Figure 2.5 Leaf chlorophyll-a and chlorophyll-b concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

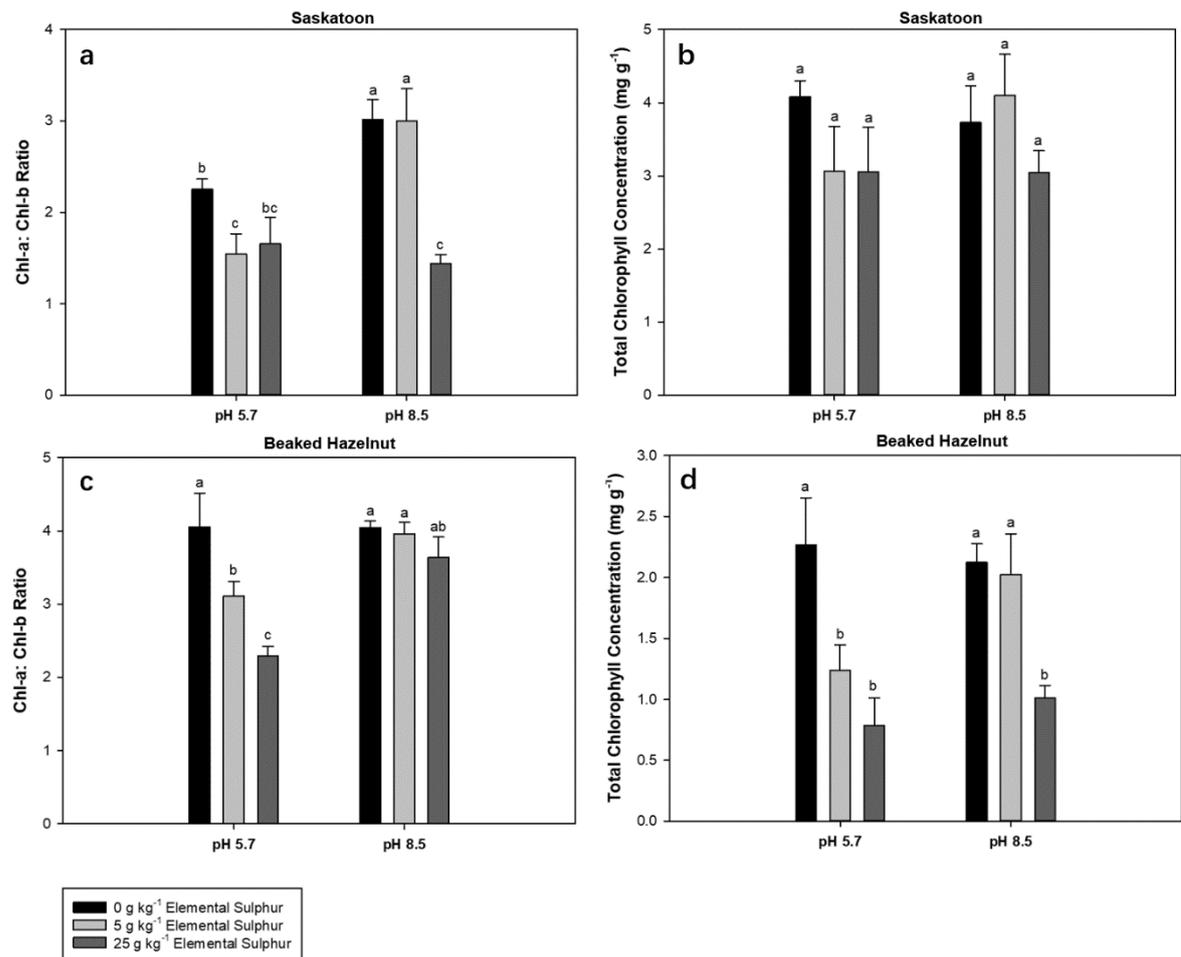


Figure 2.6 Leaf chlorophyll-a: chlorophyll-b ratios and total chlorophyll concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

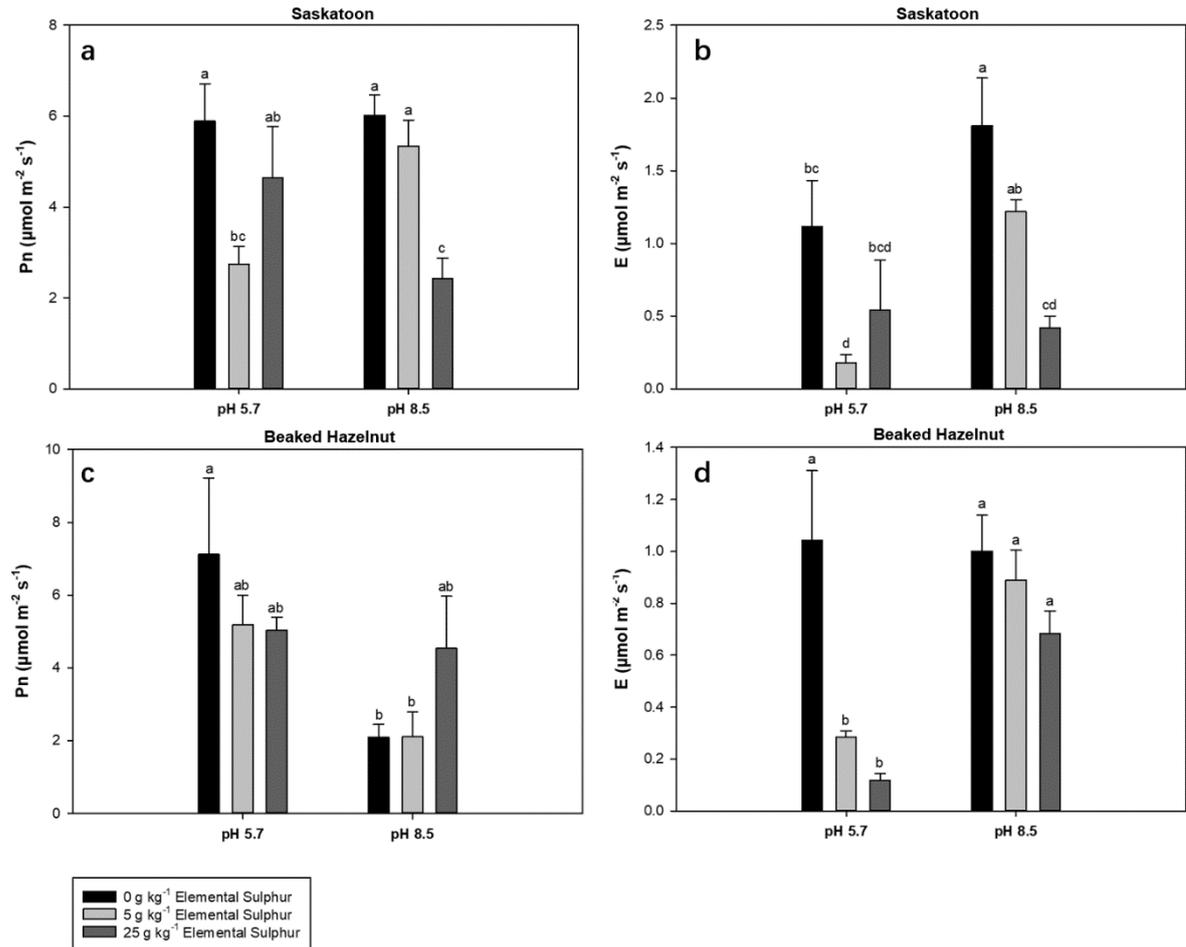


Figure 2.7 Net photosynthesis (Pn) and transpiration (E) rates in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

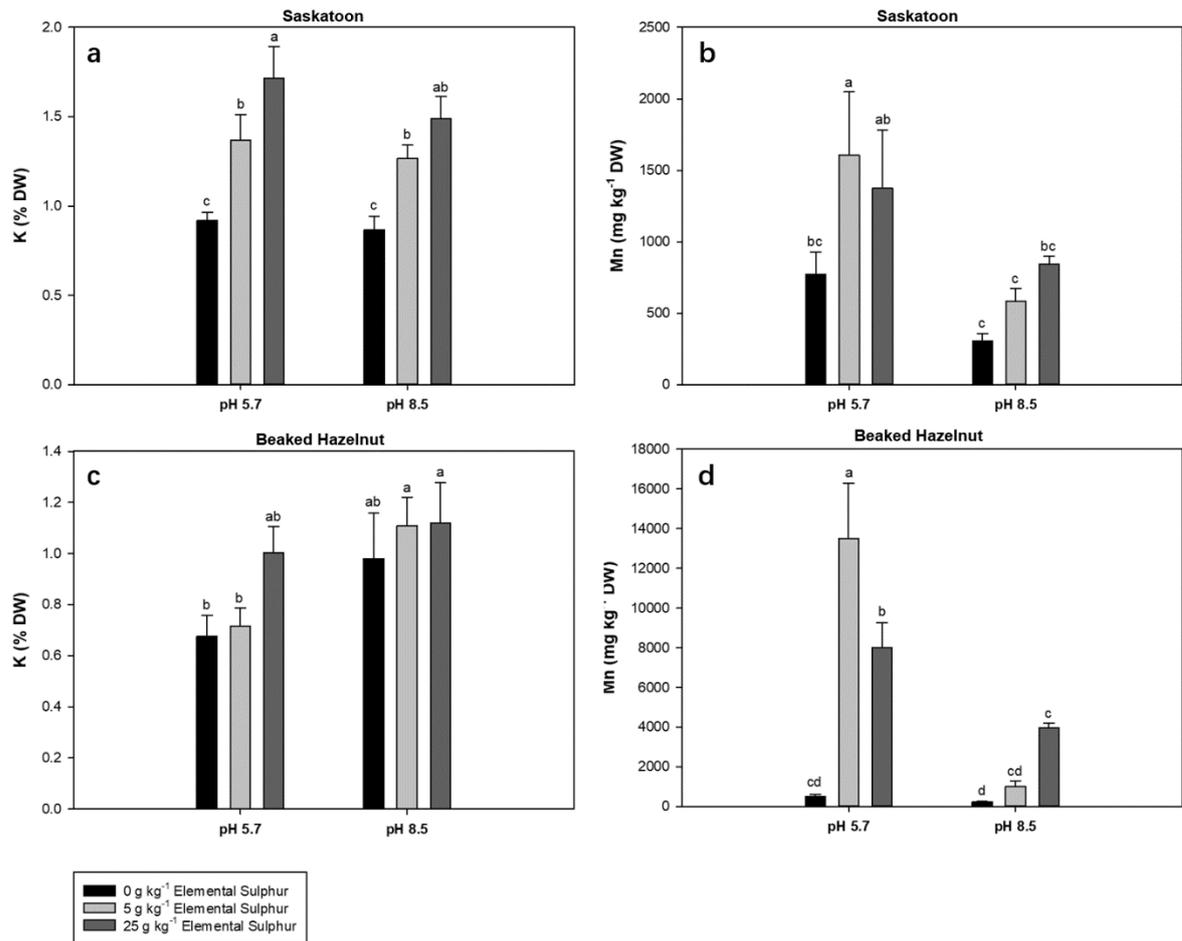


Figure 2.8 Leaf K and Mn concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

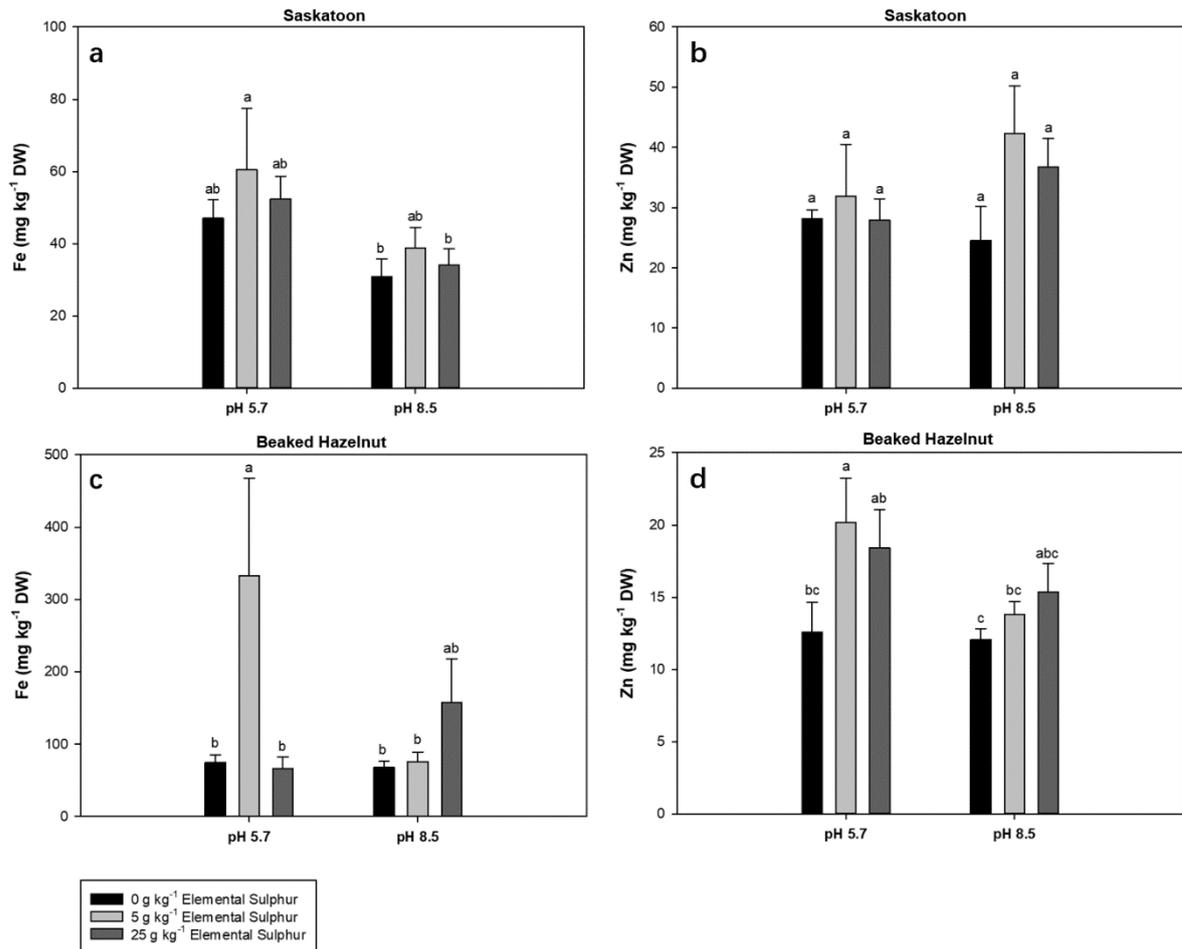


Figure 2.9 Leaf Fe and Zn concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

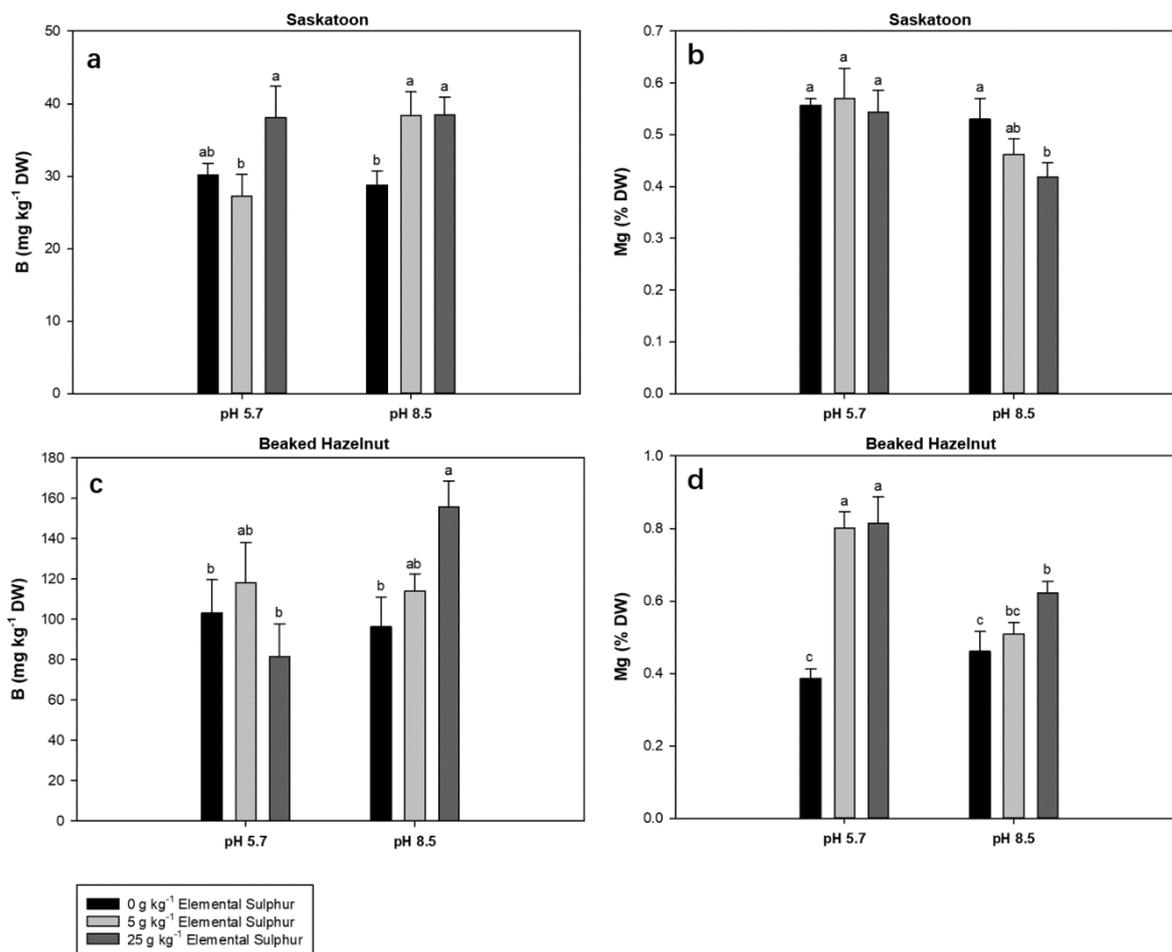


Figure 2.10 Leaf B and Mg concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments according to Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

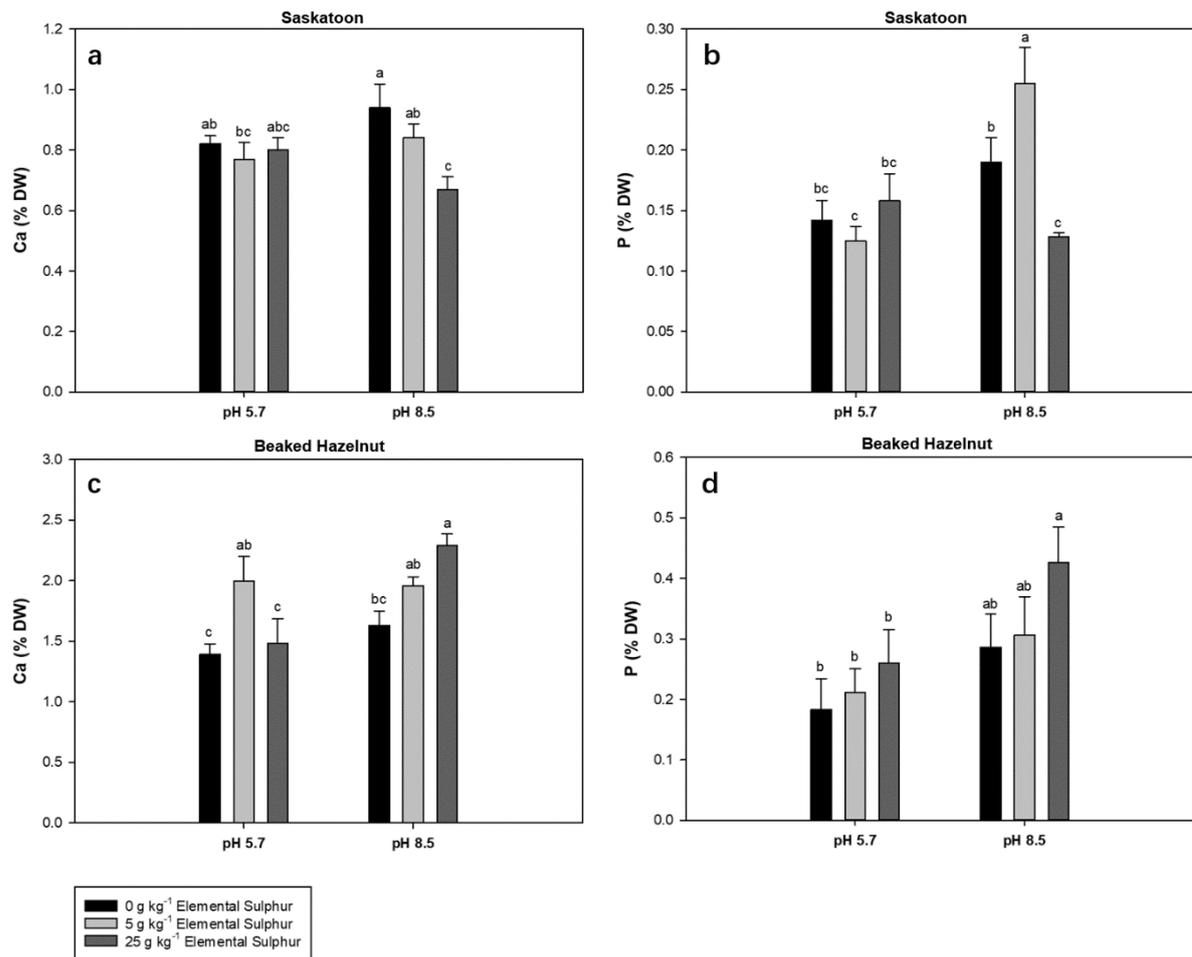


Figure 2.11 Leaf Ca and P concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means (n=6) \pm SE are shown.

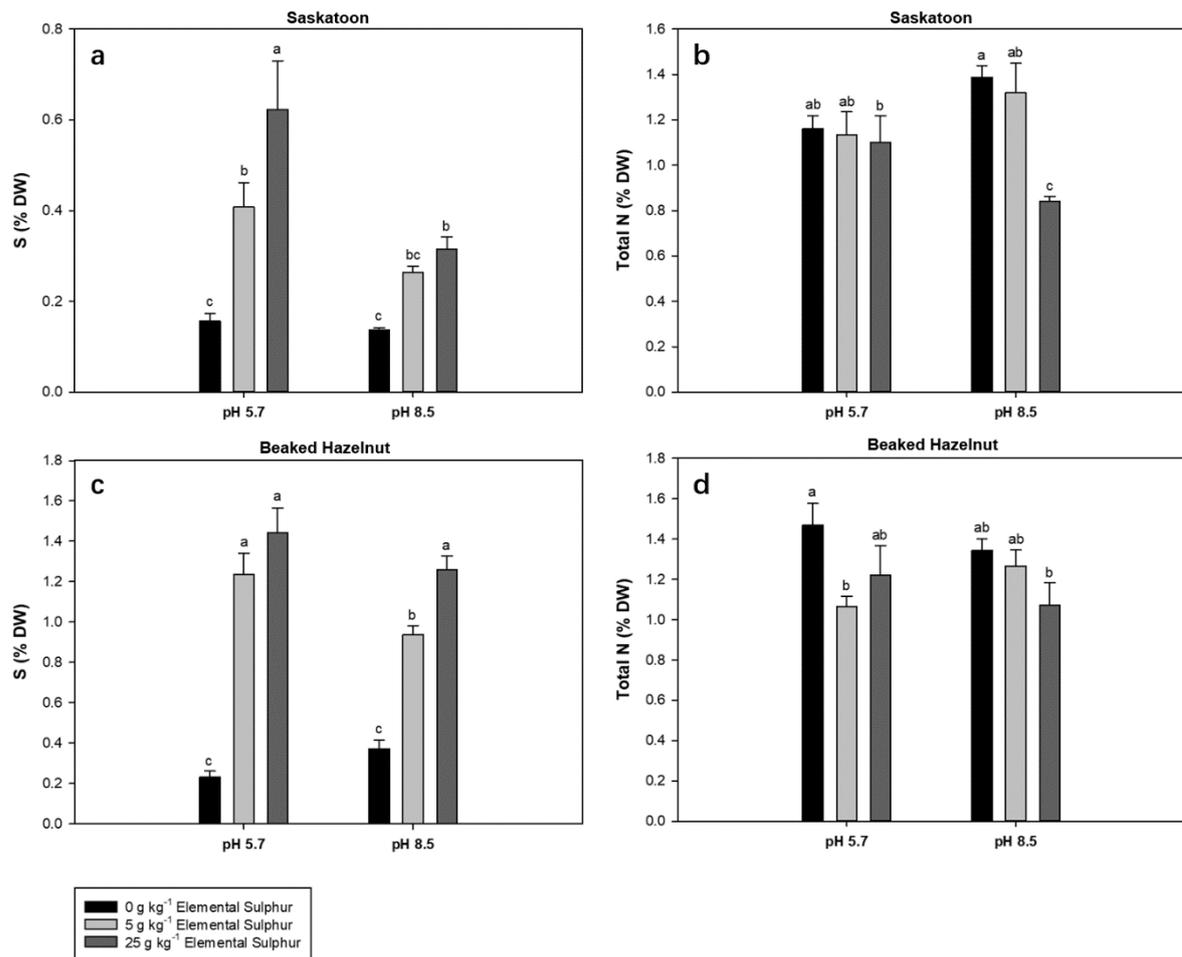


Figure 2.12 Leaf S and Total N concentrations in Saskatoon (a, b) and beaked hazelnut (c, d). Plants were grown for two months in the soil of the initial pH 5.7 and 8.5 that was supplemented with 0, 5, and 25 g kg⁻¹ elemental S. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means (n=6) \pm SE are shown.

2.7 Tables

Table 2.1 ANOVA table showing effects of pH and S treatments on the parameters measured in Saskatoon (n = 6).

p-value	Height	Diameter	LDW	SDW	RDW	St: Rt	TDW	Chl-a	Chl-b	Chl-a: Chl-b	Total Chl	Pn	E
pH	0.8725	0.2361	0.0053 **	0.0004 ***	1.06e- 06 ***	0.0027 **	1.54e- 05 ***	0.3791	0.2606	0.0268 *	0.5765	0.8920	0.0147 *
S	0.3155	0.0070 **	0.0027 **	0.0611	0.0456 *	0.7228	0.0408 *	0.0365 *	0.7824	0.0871	0.2335	0.0104 *	0.0014 **
pH*S	0.9782	0.3129	0.0223 *	0.4468	0.8213	0.1441	0.8102	0.1759	0.2123	0.0046 **	0.3489	0.0056 **	0.0615

p-value	K	Mn	Fe	Zn	B	Mg	Ca	P	S	Total N
pH	0.1772	0.0029 **	0.0087 **	0.2852	0.1866	0.0085 **	0.1126	0.0879	0.7828	0.0792
S	2.36e-06 ***	0.0519	0.4370	0.1979	0.0230 *	0.2691	0.7618	0.4855	1.90e-06 ***	0.8907
pH*S	0.7452	0.5005	0.9464	0.4401	0.0792	0.3932	0.047 *	0.0012 **	0.0279 *	0.0171 *

Abbreviations: LDW – leaf dry weight; SDW – shoot dry weight; RDW – root dry weight; St: Rt – shoot: root dry weight ratio; TDW – total dry weight; Chl-a – chlorophyll-a concentration; Chl-b – chlorophyll-b concentration; Chl-a: Chl-b – chlorophyll-a: chlorophyll-b ratio; Total Chl – total chlorophyll concentration; Pn – Net photosynthetic rate; E – Transpiration rate.

Table 2.2 ANOVA table showing effects of pH and S treatments on the parameters measured in beaked hazelnut (n = 6).

p-value	Height	Diameter	LDW	SDW	RDW	St: Rt	TDW	Chl-a	Chl-b	Chl-a: Chl-b	Total Chl	Pn	E
pH	0.1731	0.0202 *	0.0010 **	0.0042 **	0.0015 **	0.5312	0.0017 **	0.1884	0.8480	0.9739	0.1801	0.0055 **	0.8289
S	0.2854	0.0072 **	0.0396 *	0.2122	0.1400	0.8215	0.1490	7.92e- 05 ***	0.0035 **	0.0001 ***	0.0001 ***	0.5872	9.17e- 05 ***
pH*S	0.7857	0.0630	0.2713	0.4182	0.6374	0.7721	0.4792	0.1298	0.3763	0.0345 *	0.2034	0.1579	0.0438 *

p-value	K	Mn	Fe	Zn	B	Mg	Ca	P	S	Total N
pH	0.0105 *	0.8827	0.9411	0.0590	0.7480	0.2672	0.2413	0.0082 **	0.2032	0.7742
S	0.1694	1.88e-07 ***	0.0054 **	0.0499 *	0.2514	2.07e-07 ***	0.0111 *	0.1151	3.27e-12 ***	0.0261 *
pH*S	0.5302	0.0001 ***	0.0214 *	0.3846	0.0191 *	0.0014 **	0.0191 *	0.7711	0.0202 *	0.1546

Abbreviations: LDW – leaf dry weight; SDW – shoot dry weight; RDW – root dry weight; St: Rt – shoot: root dry weight ratio; TDW – total dry weight; Chl-a – chlorophyll-a concentration; Chl-b – chlorophyll-b concentration; Chl-a: Chl-b – chlorophyll-a: chlorophyll-b ratio; Total Chl – total chlorophyll concentration; Pn – Net photosynthetic rate; E – Transpiration rate.

Chapter 3 Effects of nitrogen and phosphorus nutrition on growth of trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) in reclamation soils affected by non-segregated tailings

3.1 Introduction

Oil sands mining in northeastern Alberta, Canada, has disturbed large areas of the northern boreal forest (Government of Alberta, 2017). As of 2017, these mining activities had disturbed about 1,000 km² of the boreal forests (Government of Alberta, 2017). The government of Alberta requires that these disturbed areas must be restored to equivalent land capability existed pre-disturbance levels after mining closure (Oil Sands Vegetation Reclamation Committee, 1998; Province of Alberta, 2014). During the process of oil bitumen extraction, large volumes of mine tailings are produced and need to be reclaimed. The extraction of bitumen is carried out with recycled hot water containing NaOH and, as a result, the oil sands tailings have high pH and elevated levels of Na⁺ (Howat, 2000), which are harmful to plants. To accelerate tailings consolidation, other chemicals may be added, which additionally affect tailings chemistry and make them more harmful to plants. To alleviate this concern, Canadian Natural Resources Limited (CNRL) has invested in the development of novel tailings technologies to consolidate fine tailings and produce non segregated tailings (NST) using thickeners in combination with CO₂ (CNRL, 2019)

To prevent root contact of plants with tailings deposits, overburden materials and coarse tailings sands are placed on the top tailings before revegetation (Fung and Macyk, 2000). For the soil placement, forest floor mineral soil mix (FMM) or peat mineral soil mix (PMM) are commonly used (Deput, 1984; Sydnor and Redente, 2002; Naeth et al., 2013). These two types of soil have high organic matter content and high water holding capacity and provide the source of nutrients, propagules and soil microorganisms (Deput, 1984; Lanoue and Qualizza, 2000; Sydnor and Redente, 2002; MacKenzie and Naeth, 2010). FMM has a relatively large propagule bank, therefore, plant abundance

and diversity are higher in areas reclaimed with FMM compared with PMM (MacKenzie and Naeth, 2010; MacKenzie, 2013). In addition, FMM is more readily decomposable due to the lower ratios of carbon to nitrogen, greater microbial biomass and enzyme activities (McMillan et al., 2007; MacKenzie and Naeth, 2010; Brown, 2010; Alberta Environment and Water, 2012; Hahn and Quideau, 2013; Jamro et al., 2014). However, PMM is more readily available in northern Alberta in larger quantities compared with FMM (Fung and Macyk, 2000), Therefore, PMM is an option when FMM is not available.

Nitrogen and phosphorus are major essential elements that are required in relatively large quantities for plant growth. In boreal forests, nitrogen availability is among the most significant factors affecting growth of plants and nitrogen turnover rate is related to forest productivity (Gundersen et al., 2009; Yan et al., 2012; Duan et al., 2015). In addition, nitrogen can affect canopy cover density as well as species richness and composition (Frelich et al., 2003; Moreno-Penaranda et al., 2004; Rowe et al., 2006; Walker and del Moral, 2009). Phosphorus is another macroelement that is essential to all plants and its soil availability may be limiting growth in many ecosystems (Jing et al., 2010). Phosphorous affects many processes in plants including leaf expansion, leaf number (Fredeen et al., 1989; Lynch et al., 1991), shoot to root ratios (Marschner, 2012), and root hydraulic conductivity (Clarkson et al., 2000). Both nitrogen and phosphorus availability are low in oil sands areas reclaimed with both PMM and FMM soils (Zhang et al., 2020).

In the present study, I examined the growth and physiological responses of trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) plants, which are commonly used for oil sands reclamation. The objectives of this study were to investigate the responses of these plants growing in soils with different tailings amendments and supplied with different N and P levels. The following hypotheses were tested: 1) presence of non-segregated tailings (NST) in the soil has detrimental effects on plant growth and the effects vary depending on the type of soil, and 2) soil supplementation with N and P is more effective in enhancing growth and physiological

parameters in trembling aspen compared with white spruce.

3.2 Materials and methods

3.2.1 Soil preparation

Soil substrates that were used in this study included the forest mineral mix (FMM), peat-mineral mix (PMM), non-segregating tailings (NST), 1:1 (by volume) mixture of NST amended with FMM by volume ratio of 1:1, NST amended with PMM by volume ratio of 1:1. For FMM, approximately 30 cm of the surface soil layer was collected from the boreal forest site near Fort McMurray, Alberta, Canada. Peat-mineral mix (PMM) and non-segregating tailings (NST) were collected from CNRL oil sands mining areas. All of these different substrates were sealed in pails and delivered to the University of Alberta. Large aggregates, stones, grass and tree branches were removed from PMM and FMM, then the soil was air dried for 3-4 days in order to mix together. The original soil pH value of each substrates was measured with the Orion STAR A111 pH meter (Thermo Fisher Scientific Inc., Waltham, MA) before growing plants.

3.2.2 Plant material and experimental set-up

One-year-old greenhouse-grown trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) dormant seedlings were obtained from Tree Time Services Inc., Edmonton, Alberta, Canada. The seedlings were stored for two weeks at 4°C prior to the experiment. After removing from cold storage, the plants were grown in 2 L pots with the above five soil substrates for two months in the growth room at 22/18°C (day/night) temperature, 65±10% relative humidity and 16-h photoperiod with 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPDF) at the top of the seedlings provided by the full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

Two levels of N & P nutrient solutions were prepared in 10% Hoagland's mineral

solution (Epstein, 1972). The 10% N & P nutrient solution was 10% Hoagland's mineral solution and the 100% N & P nutrient solution was 10% Hoagland's mineral solution with N and P maintained at the levels of the 100% Hoagland's solution (Table 3.1).

The experiment was a 5 x 2 completely randomized factorial design (fully randomized factorial design) with five soil substrates (NST, NST+PMM, NST+FMM, PMM and FMM) and two N & P levels (10% and 100%). There were ten replicates for each treatment combination. All plants were watered every two days and provided with 500 ml of 10% or 100% N & P nutrient solution once a week.

3.2.3 Growth parameters

Seedling height and stem diameter were measured at the time of planting and at harvest after two months of growth. Seedling height was measured from the base of the stem to the shoot tip in six seedlings randomly selected from ten replicates of each treatment combination. Stem diameter was measured at the base of the stem using calipers.

To determine leaf, shoot and root dry weights, six seedlings of each species were randomly removed from each soil substrate and N & P nutrient treatment combination. Leaves were separated from stems and placed in an ultra-low temperature freezer at -80°C before freeze-drying for 72 h. Roots and stems were dried in an oven at 70°C for 72 h. Shoot dry weights were determined by adding dry weights of stems and leaves. For the total dry weights, stem, shoot, and root dry weights from each plant were combined.

3.2.4 Leaf chlorophyll concentrations

Leaf chlorophyll-a, chlorophyll-b and total chlorophyll concentrations were determined in fully-expanded leaves in the mid-parts of the shoots in six randomly selected seedlings per treatment combination per species. The leaves were dried in a freeze-drier (Freeze Dry Lyph-Lock, Labconco, Kansas, USA) for 72 h and ground in a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted

from the leaf samples (10 mg dry weight) with 8 ml dimethyl sulfoxide (DMSO) at 65 °C for 22 h. After filtering, chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific, Massachusetts, USA), at 648 nm and 665 nm for chlorophyll-a and chlorophyll-b concentrations, respectively. Total chlorophyll concentration was calculated using the Arnon's equation (Sestak et al., 1971).

3.2.5 Net photosynthesis (Pn) and transpiration (E) rates

Six seedlings per species were randomly taken from each treatment combination for the measurements of gas exchange after two months of treatments. Pn and E rates were measured in the uppermost fully-developed leaves using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, Nebraska USA) at 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPDF) at approximately 4 - 9 hours after the onset of photoperiod.

3.2.6 Leaf elemental concentrations

Leaf elemental concentrations were determined in six plants of each species from each treatment combination. Ground dry leaf samples (0.2 g dry weight) were digested with 10 ml 70% HNO₃ and diluted with Milli-Q water to 40 ml. The concentrations of K, Mn, Fe, Zn, B, Mg, Ca, P and S in extracts were determined by Thermo iCAP6300 Duo (N. America) inductively coupled plasma-optical emission spectrometer (ICP-OES) (Thermo Fisher Corp., Cambridge, United Kingdom). After ground dry leaf samples (3 mg dry weight) were packed into a tin or silver capsule, total N was analyzed by the Thermo FLASH 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., Bremen, Germany 2016). All elemental analyses were conducted in the Natural Resources Analytical Laboratory of the University of Alberta, Edmonton, AB, Canada.

3.2.7 Statistical analysis

All data were analyzed by two-way ANOVA using the R software (Version 3.5.2, R Core Team, R Foundation for Statistical Computing, Vienna, Austria) to determine

statistically significant ($p \leq 0.05$) differences between treatments. Soil types and N&P levels were the main factors. Comparisons between the treatment means were conducted using the Fisher's LSD test.

3.3 Results

3.3.1 Soil pH

The initial pH values of NST, NST+PMM, NST+FMM, PMM and FMM substrates were 8.5, 7.5, 7.2, 5.8 and 5.9, respectively. The pH of NST was the highest of all examined substrates (Fig. 3.1).

3.3.2 Plant heights and stem diameters

There were significant interactions between substrate and nutrition on diameter increments of white spruce (Table 3.2, Table 3.3).

In trembling aspen, the height increments at 100% N & P level were significantly greater in FMM compared with NST (Fig. 3.2a). The diameter increments measured in NST+FMM, PMM and FMM were significantly greater compared with NST and NST+PMM at 10% N&P level. The diameter increments measured in NST+PMM, PMM and FMM were significantly greater compared with NST at the 100% N & P level (Fig. 3.2b).

In white spruce, the diameter increments measured in NST+FMM, PMM and FMM were significantly higher than measured in NST at the 10% N & P level (Fig. 3.2d).

3.3.3 Dry weights

There were no significant interactions between substrate and nutrition on leaf dry weights, shoot dry weights, root dry weights, shoot: root ratios and total dry weights of both plants (Table 3.2, Table 3.3).

In trembling aspen, the leaf dry weights measured in NST+FMM and FMM were

significantly higher compared with NST, NST+PMM and PMM at 10% N & P level. The leaf dry weights measured in NST+PMM, NST+FMM and FMM were significantly higher than in PMM at 100% N & P level (Fig. 3.3a). The shoot dry weights of plants growing in FMM were significantly higher than in NST, NST+PMM and PMM at 10% N & P level (Fig. 3.3b). The total dry weights measured in NST+FMM and FMM were significantly higher compared with NST at the 10% N & P level (Fig. 3.4b).

In white spruce, the results were not significant.

3.3.4 Chlorophyll concentrations

There were significant interactions substrate and nutrition on chlorophyll-a: chlorophyll-b ratios of trembling aspen, total chlorophyll concentrations of white spruce (Table 3.2, Table 3.3).

In trembling aspen, chlorophyll-a concentrations measured in NST+FMM and FMM were significantly higher than in NST, NST+PMM and PMM at the 10% N & P level. Chlorophyll-a concentrations measured in plants growing in FMM were significantly higher compared with the other substrates at the 100% N & P level (Fig. 3.5a). Chlorophyll-b concentrations measured in NST+FMM, PMM and FMM were significantly higher compared with the plants growing in NST at the 10% N & P level. Chlorophyll-b concentrations in trembling aspen growing in NST were significantly lower compared with the plants growing in the other types of substrates at the 10% N & P level (Fig. 3.5b). Chl-a: chl-b ratios measured in plants growing in FMM were significantly higher compared with NST, NST+PMM and PMM at the 10% N & P level. The ratios measured in trembling aspen growing in FMM were significantly higher than compared with plants growing in NST, NST+FMM and PMM at the 100% N & P level (Fig. 3.6a). The total chlorophyll concentrations measured in NST, NST+PMM and PMM were significantly lower than in NST+FMM and FMM at the 10% N & P level. The total chlorophyll concentrations measured in trembling aspen growing in NST were significantly lower than in the other types of substrate at the 100% N & P level (Fig.

3.6b).

In white spruce, chlorophyll-a concentrations measured in plants growing in NST were significantly lower than in the other types of substrates at the 10% N & P level. Chlorophyll-a concentrations measured in FMM were significantly higher compared with other types of substrates at the 100% N & P level (Fig. 3.5c). Chlorophyll-b concentration measured in plants growing in FMM were significantly higher compared with the other substrate types at both nutrient levels (Fig. 3.5d). Chl-a: chl-b ratio measured in NST-grown plants were significantly lower than in the other types of substrate at the 10% N&P level. The ratios measured in NST-grown plants were significantly lower than in NST+PMM, NST+FMM and FMM at the 100% N & P level (Fig. 3.6c). The total chlorophyll concentrations measured in NST were significantly lower than in the other four types of substrate at the 10% N & P level. The total chlorophyll concentrations in plants growing in FMM were significantly higher compared with NST, NST+FMM and PMM at the 100% N & P level (Fig. 3.6d).

3.3.5 Net photosynthesis (Pn) and transpiration (E) rates

There were no significant interactions between substrate and nutrition on Pn and E of both plants (Table 3.2, Table 3.3).

In trembling aspen, Pn measured in plants growing in NST was significantly lower compared with NST+PMM, NST+FMM and FMM at 100% N & P level (Fig. 3.7a). E measured in plants growing in NST and NST+PMM were significantly lower compared with NST+FMM and PMM at 10% N & P level, it measured in NST-grown plants was significantly lower than in the other types of substrate at the 100% N & P level (Fig. 3.7b).

In white spruce, Pn measured in plants growing NST was significantly lower than in other types of substrate at the 10% N & P level, it measured in plants growing NST was significantly lower compared with FMM at the 100% N & P level (Fig. 3.7c). E measured in plants growing in NST+FMM was significantly higher compared with NST, NST+PMM and PMM at the 10% N & P level, it measured in NST+FMM and FMM-

grown plants were significantly higher than in other three types of substrate at the 100% N & P level (Fig. 3.7d).

3.3.6 Leaf elemental concentrations

There were significant interactions between substrate and nutrition on leaf K concentrations of white spruce (Table 3.2, Table 3.3).

In trembling aspen, leaf K concentrations measured in plants growing in NST was significantly higher than measured in other types of substrate at the 10% N & P level, it measured in NST-grown plants was significantly higher compared with NST+FMM and FMM at the 100% N & P level (Fig. 3.8a). Leaf Mn concentrations of aspen measured in NST+FMM were significantly higher compared with NST, NST+PMM and PMM at both N & P levels (Fig. 3.8b). Leaf Fe concentrations measured in plants growing in NST+FMM were significantly higher than measured in other types of substrate at the 10% N & P level, leaf Fe concentrations measured in NST+FMM-grown plants were significantly higher compared with NST+PMM and PMM at the 100% N & P level (Fig. 3.9a). Leaf Zn concentrations measured in NST-grown plants were significantly lower compared with NST+FMM and FMM at both N & P levels (Fig. 3.9b). Leaf B concentrations measured in trembling aspen growing in NST were significantly higher compared with other types of substrate at both N & P levels (Fig. 3.10a). Leaf Mg concentrations measured in NST was significantly lower than in NST+PMM at the 10% N & P level (Fig. 3.10b). Leaf Ca concentrations measured in trembling aspen growing in NST were significantly lower than in other types of substrate without FMM at the 10% N&P level. At 100% N & P level, leaf Ca concentrations measured in NST-grown plants were significantly lower than in PMM (Fig. 3.11a). Leaf P concentrations measured in trembling aspen growing in NST were significantly higher compared with PMM and FMM at the 10% N & P level (Fig. 3.11b). Leaf total N concentrations measured in plants growing in NST was significantly lower compared with PMM soil at the 100% N & P level (Fig. 3.12b).

In white spruce, leaf K concentrations measured in plants growing in NST were

significantly lower compared with FMM at the 10% N & P level, the concentrations measured in NST-grown plants were significantly higher than other types of substrate at the 100% N & P level (Fig. 3.8c). Leaf Mn concentrations of white spruce measured in NST+FMM and FMM were significantly higher than other types of substrate at both N & P levels (Fig. 3.8d). Leaf B concentrations measured in NST-grown plants were significantly higher compared with other types of substrate at the 10% N & P level, the concentrations measured in white spruce growing in NST were significantly higher than in NST+PMM, PMM and FMM at the 100% N & P level (Fig. 3.10c). Leaf Mg concentrations measured in NST was significantly lower compared with NST+PMM, PMM and FMM at the 10% N & P level (Fig. 3.10d). Leaf Ca concentrations measured in white spruce growing in NST was significantly lower than in NST+PMM, PMM and FMM at the 10% N & P level, the concentrations measured in NST-grown plants were significantly lower than measured in FMM at the 100% N & P level (Fig. 3.11c). Leaf P concentrations measured in NST-grown plants were significantly higher than in PMM and FMM at the 10% N & P level (Fig. 3.11d). Leaf total N concentrations measured in white spruce growing in NST were significantly lower than in FMM at the 10% N & P level, the concentrations in NST-grown plants were significantly lower than in NST+PMM and FMM at the 100% N & P level (Fig. 3.12d).

3.4 Discussion

In the present study, growth and physiological responses to different levels of N & P in the NST, NST+PMM, NST+FMM, PMM and FMM growth substrates were examined in trembling aspen and white spruce. The soil pH of NST was the highest, likely due to the process of oil extraction in which NaOH is added (Howat, 2000). As expected, the pH values of NST+PMM and NST+FMM were lower compared with NST.

When fertilized with the 100% N & P level, The results illustrated that NST had negative effects on growth and physiological responses of plants likely due to high pH and elevated salinity of NST. Previous studies demonstrated that trembling aspen was

less tolerant of high pH than white spruce (Zhang et al., 2013) and that conifers trees were more sensitive to salt stress than deciduous trees (Renault et al., 1998). Therefore, the stress responses in trembling aspen were likely largely due to the high pH of NST, while in white spruce were likely due to the elevated salinity of NST. However, in trembling aspen, shoot: root ratio measured in NST-grown plants was significantly higher than in PMM. This was likely due to a greater limitation of NST on root growth compared with shoot growth when taking into consideration the results of lower dry weights in trembling aspen (Zhang et al., 2020). The results of shoot and root dry weights in trembling aspen growing in NST also demonstrated that high root substrate pH is inhibitory to both shoot and root growth (Tang et al., 1993; Zhang et al., 2013; Zhang et al., 2015). The decrease of root dry weights in trembling aspen growing in NST was likely due to the high pH of NST, which may reduce shoot water potential (Tang et al., 1993; Zhang et al., 2013) and the aquaporin-mediated root water flux (Kamaluddin and Zwiazek, 2004; Tournaire-Roux et al., 2003; Zhang and Zwiazek, 2016). In addition, the high pH of NST can also limit the formation of lateral roots and root hairs, and inhibit cell elongation in roots (Tang et al., 1993; Canmore-Neumann et al., 1996). However, due to the fine texture of NST, poor root aeration could be another factor that negatively affected the root growth of plants (Zhang et al., 2020). The highest dry weights of trembling aspen growing in FMM were likely due to the lower pH of FMM which was beneficial to plants (Mackenzie, 2007; MacKenzie and Naeth, 2010; Brown, 2010; Hahn, 2012; MacKenzie, 2012). In white spruce, chlorophyll-a: chlorophyll-b ratios measured in NST-grown plants were the lowest, indicating that chlorophyll-a was more affected by NST than chlorophyll-b, likely because it is less stable than chlorophyll-b (Koca et al., 2007; Zhang et al., 2015). The decrease of Pn and E observed in white spruce in NST were mainly due to the decrease of total chlorophyll concentrations which limited Pn and E. The limitations of Pn and E were also likely due to effects of high pH on root water transport and its delivery to leaves resulting in a decrease of stomatal conductance and reduced CO₂ uptake (Tang et al., 1993; Kamaluddin and Zwiazek, 2004).

When fertilized with the 100% N & P level, these decreases were sufficiently significant to affect photosynthesis in both plants.

In addition, the results also showed that PMM and FMM added to NST had positive effects on trembling aspen and white spruce under the two different levels of N & P treatments. The results were likely due to the improvement of physical properties of NST as a growth substrate and lowering the pH (Howat, 2000; Zhang et al., 2020). However, NST+FMM was a better growth substrate compared with NST+PMM for trembling aspen at the 10% N & P level, which could be due to the lower carbon to nitrogen ratios of FMM than PMM, providing more N for plants (Mackenzie and Naeth, 2010). The less effects of NST+PMM and NST+FMM at both levels of N & P on white spruce were likely due to the slower growth of white spruce compared with trembling aspen (Munson et al., 1995; Claveau et al., 2002).

Since high soil pH commonly reduces the availability of Fe, Mn, P, and Zn (George et al., 2012; Zhang et al., 2013), the high pH of NST may affect the availability of these essential elements. When fertilized with the 10% N & P level, the results indicate that NST had negative effects on leaf element concentrations of trembling aspen and white spruce mentioned above. However, the higher concentrations of leaf K, B and P in trembling aspen and higher concentrations of leaf B and P in white spruce observed in NST were likely due to biomass dilution effect (Jarrell and Beverly, 1981). When provided with the 100% N & P, the results were also mainly due to the high pH of NST. In addition, Fe and Mn are the most commonly deficient nutrients in plants growing in soil with high pH, which can inhibit chlorophyll synthesis and photosynthetic processes in plants (George et al., 2012). In this study, the leaf Mn concentration in both plant species and with both levels of N&P significantly decreased in NST. The results suggest that high pH of NST inhibited Mn uptake, which affected chlorophyll synthesis and photosynthesis.

N and P availability are the main nutritional factors limiting growth of boreal trees due to their high demand and important roles in many processes in plants (Jing et al., 2010; Duan et al., 2018). Therefore, the availability of N and P in soil may quickly

affect plant growth. In this study, two levels of N&P treatment were applied to trembling aspen and white spruce. In trembling aspen, leaf dry weights measured in plants growing in NST+FMM, chlorophyll-a concentrations in NST+PMM and PMM, chlorophyll-b concentrations in NST+PMM, total chlorophyll concentrations in NST+PMM, NST+FMM and PMM, Pn and E in NST+PMM, NST+FMM and FMM were significantly higher when 100% N & P was provided compared with 10% N & P. In white spruce, diameter increments and total chlorophyll concentrations measured in plants growing in NST, as well as Pn and E in FMM were significantly higher at the 100% N & P level than the 10% N & P level. The results indicated that trembling aspen was more affected by N & P levels than white spruce. The results were likely due to the rapid growth of trembling aspen compared with white spruce, which creates higher nutritional demand (Boyle, 1973; Perala, 1977; Alban, 1982; Debyle, 1985). White spruce grows more slowly compared with trembling aspen and nutrients stored in the tissues of white spruce seedlings at the time of growth in the tree nursery prior to the study, could make a significant contribution to later seedling growth (Greenway et al., 1992; Munson et al., 1995; Claveau et al., 2002).

In conclusion, the results of this study demonstrate that the growth and physiological responses to NST of trembling aspen and white spruce significantly improved in PMM and FMM-amended substrates. FMM was better than PMM when used with NST. Trembling aspen was more affected by NST compared with white spruce. Soil with 100% N & P nutrient level was more effective in enhancing growth and physiological effects in trembling aspen compared with white spruce.

3.5 References

- Alban, D.H., 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. *Soil Science Society of America Journal* 46, 853–861.
- Alberta Environment and Water, 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Prepared by

- D. MacKenzie for the Terrestrial Subgroup, Best Management Practices Task Group, Reclamation Working Group, Cumulative Environmental Management Association. Fort McMurray, AB, Canada.
- Boyle, J.R., Phillips, J.J., Ek, A.R., 1973. "Whole tree" harvesting: nutrient budget evaluation. *Journal of Forestry* 71, 760–762.
- Brown, R.L., 2010. Use of woody debris as an amendment for reclamation after oil sands mining. M.Sc. Thesis. University of Alberta, Department of Renewable Resources, Edmonton, Alberta, Canada.
- Canmore-Neumann, R., Silber, A., Mitchnick, B., Gilad, S., Ben-Jaacov, J., 1996. Effect of pH on the development of *Leucadendron* 'Safari Sunset'. IV International Protea Working Group Symposium 453, 47–52.
- Clarkson, D.T., Carvajal, M., Henzler, T., Waterhouse, R.N., Smyth, A.J., Cooke, D.T., Steudle, E., 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* 51, 61–70.
- Claveau, Y., Messier, C., Comeau, P.G., Coates, K.D., 2002. Growth and crown morphological responses of boreal conifer seedlings and saplings with contrasting shade tolerance to a gradient of light and height. *Canadian Journal of Forest Research* 32, 458–468.
- CNRL (Canadian Natural Resources Limited), 2019. Managing Tailings. <https://www.cnrl.com/corporate-responsibility/advancements-in-technology/managing-tailings>.
- DeByle, N.V., Winokur, R.P., 1985. Aspen: ecology and management in the western United States. USDA Forest Service General Technical Report RM-119. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.
- DePuit, E.J., 1984. Potential topsoiling strategies for enhancement of vegetation diversity on mined lands. *Minerals and the Environment* 6, 115–120.
- Duan, M., House, J., Chang, S.X., 2015. Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed

- sites in the Athabasca oil sands region. *Ecological Engineering* 75, 323–331.
- Duan, M., House, J., Liu, Y., Chang, S.X., 2018. Contrasting responses of gross and net nitrogen transformations to salinity in a reclaimed boreal forest soil. *Biology and Fertility of Soils* 54, 385–395.
- Epstein, E. 1972. *Mineral nutrition of plants: principles and perspectives*. Wiley, New York, USA.
- Fredeen, A.L., Rao, I.M., Terry, N., 1989. Influence of phosphorus nutrition on growth and carbon partitioning in glycine max. *Plant Physiology* 89, 225–230.
- Frelich, L.E., Machado, J.L., Reich, P.B., 2003. Fine-scale environmental variation and structure of understorey plant communities in two old-growth pine forests. *Journal of Ecology* 91, 283–293.
- Fung, M.Y., Macyk, T.M., 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands* 41, 755–774.
- George, E., Horst, W.J., Neumann, E., 2012. Adaptation of plants to adverse chemical soil conditions. *Marschner's Mineral Nutrition of Higher Plants* 409–472. Academic Press. London, UK.
- Government of Alberta, 2017. Oil Sands Overview. <https://www.alberta.ca/oilsands-overview.aspx>.
- Greenway, K.J., Macdonald, S.E., Lieffers, V.J., 1992. Is long-lived foliage in *Picea mariana* an adaptation to nutrient-poor conditions? *Oecologia* 91, 184–191.
- Gundersen, P., Sevel, L., Christiansen, J.R., Vesterdal, L., Hansen, K., Bastrup-Birk, A., 2009. Do indicators of nitrogen retention and leaching differ between coniferous and broadleaved forests in Denmark? *Forest Ecology and Management* 258, 1137–1146.
- Hahn, A.S., 2012. Soil microbial communities in early ecosystems. MSc Thesis. University of Alberta, Department of Renewable Resources. Edmonton, AB, Canada.
- Hahn, A.S., Quideau, S.A., 2013. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the

- Canadian boreal forest. *Plant and Soil* 363, 331–344.
- Howat, D., 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Alberta Environment. Environmental Sciences Division, Edmonton, Alberta.
- Jamro, G.M., Chang, S.X., Naeth, M.A., 2014. Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada. *Ecological Engineering* 73, 92–101.
- Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* 34, 197–224.
- Jing, J., Rui, Y., Zhang, F., Rengel, Z., Shen, J., 2010. Localized application of phosphorus and ammonium improves growth of maize seedlings by stimulating root proliferation and rhizosphere acidification. *Field Crops Research* 119, 355–364.
- Kamaluddin, M., Zwiazek, J.J., 2004. Effects of root medium pH on water transport in paper birch (*Betula papyrifera*) seedlings in relation to root temperature and abscisic acid treatments. *Tree Physiology* 24, 1173–1180.
- Koca, N., Karadeniz, F., Burdurlu, H.S., 2007. Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry* 100, 609–615.
- Lanoué, A., Qualizza, C., 2000. LFH and shallow mineral horizons as inoculants on reclaimed areas to improve native species catch. 1999 Status Report. Environmental Affairs, Syncrude Canada Ltd. Fort McMurray, AB, Canada.
- Lynch, J., Lauchli, A., Epstein, E., 1991. Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Science* 31, 380–387.
- MacKenzie, D., 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Alberta Environment and Water. Edmonton, AB, Canada.
- MacKenzie, D.D., 2013. Oil sands mine reclamation using boreal forest surface soil (LFH) in northern Alberta. Ph.D. dissertation. University of Alberta, Department of Renewable Resources. Edmonton, AB, Canada.

- MacKenzie, D.D., Naeth, M.A., 2007. Assisted natural recovery using a forest soil propagule bank in the Athabasca oil sands. *Seeds Biology, Development and Ecology* 374–382. Cromwell Press, Trowbridge, UK.
- Mackenzie, D.D., Naeth, M.A., 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. *Restoration Ecology* 18, 418–427.
- Marschner, H., 2012. *Marschner's mineral nutrition of higher plants*. Academic press, London, UK.
- McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O., 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. *Journal of Environmental Quality* 36, 1470–1478.
- Moreno-Peñaranda, R., Lloret, F., Alcaniz, J.M., 2004. Effects of sewage sludge on plant community composition in restored limestone quarries. *Restoration Ecology* 12, 290–296.
- Munson, A.D., Margolis, H.A., Brand, D.G., 1995. Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. *Tree Physiology* 15, 141–149.
- Naeth, M.A., Wilkinson, S.R., Mackenzie, D.D., Archibald, H.A., Powter, C.B., 2013. Potential of LFH mineral soil mixes for reclamation of forested lands in Alberta. OSRIN Report No. TR-35. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, AB, Canada.
- Oil Sands Vegetation Reclamation Committee, 1998. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. Alberta Environmental Protection. Report No. ESD/LM/99-1. Edmonton, AB, Canada.
- Perala, D.A., 1977. *Manager's handbook for aspen in the north-central states*. General Technical Report NC-36. St. Paul, MN: US Dept. of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota, USA.
- Province of Alberta, 2014. Conservation and reclamation regulation of environmental

- protection and enhancement act. Alberta Queen's Printer, Edmonton, AB, Canada.
- Renault, S., Lait, C., Zwiazek, J.J., MacKinnon, M., 1998. Effect of high salinity tailings waters produced from gypsum treatment of oil sands tailings on plants of the boreal forest. *Environmental Pollution* 102, 177-184.
- Rowe, E.C., Healey, J.R., Edwards-Jones, G., Hills, J., Howells, M., Jones, D.L., 2006. Fertilizer application during primary succession changes the structure of plant and herbivore communities. *Biological Conservation* 131, 510–522.
- Sestak, Z., Catský, J., Jarvis, P.G., 1971. Plant photosynthetic production. Manual of methods. Dr. W. Junk Publishers, The Hague, Netherlands.
- Sydnor, M.E., Redente, E.F., 2002. Reclamation of high-elevation, acidic mine waste with organic amendments and topsoil. *Journal of Environmental Quality* 31, 1528–1537.
- Tang, C., Cogley, B.T., Mokhtara, S., Wilson, C.E., Greenway, H., 1993. High pH in the nutrient solution impairs water uptake in *Lupinus angustifolius* L., in: *Plant Nutrition—from Genetic Engineering to Field Practice* 763–765. Springer, Dordrecht, Netherlands.
- Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.T., Bligny, R., Maurel, C., 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* 425, 393–397.
- Walker, L.R., del Moral, R., 2009. Lessons from primary succession for restoration of severely damaged habitats. *Applied Vegetation Science* 12, 55–67.
- Yan, E.-R., Hu, Y.-L., Salifu, F., Tan, X., Chen, Z.C., Chang, S.X., 2012. Effectiveness of soil N availability indices in predicting site productivity in the oil sands region of Alberta. *Plant and Soil* 359, 215–231.
- Zhang, W., Calvo-Polanco, M., Chen, Z.C., Zwiazek, J.J., 2013. Growth and physiological responses of trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*) and tamarack (*Larix laricina*) seedlings to root zone pH. *Plant and Soil* 373, 775–786.

Zhang, W., Fleuriel, K., Sherr, I., Vassov, R., Zwiazek, J.J., 2020. Growth and physiological responses of tree seedlings to oil sands non-segregated tailings. *Environmental Pollution* 259, 113945.

Zhang, W., Xu, F., Zwiazek, J.J., 2015. Responses of jack pine (*Pinus banksiana*) seedlings to root zone pH and calcium. *Environmental and Experimental Botany* 111, 32-41.

Zhang, W., Zwiazek, J.J., 2016. Effects of root medium pH on root water transport and apoplastic pH in red-osier dogwood (*Cornus sericea*) and paper birch (*Betula papyrifera*) seedlings. *Plant Biology* 18, 1001-1007.

3.6 Figures

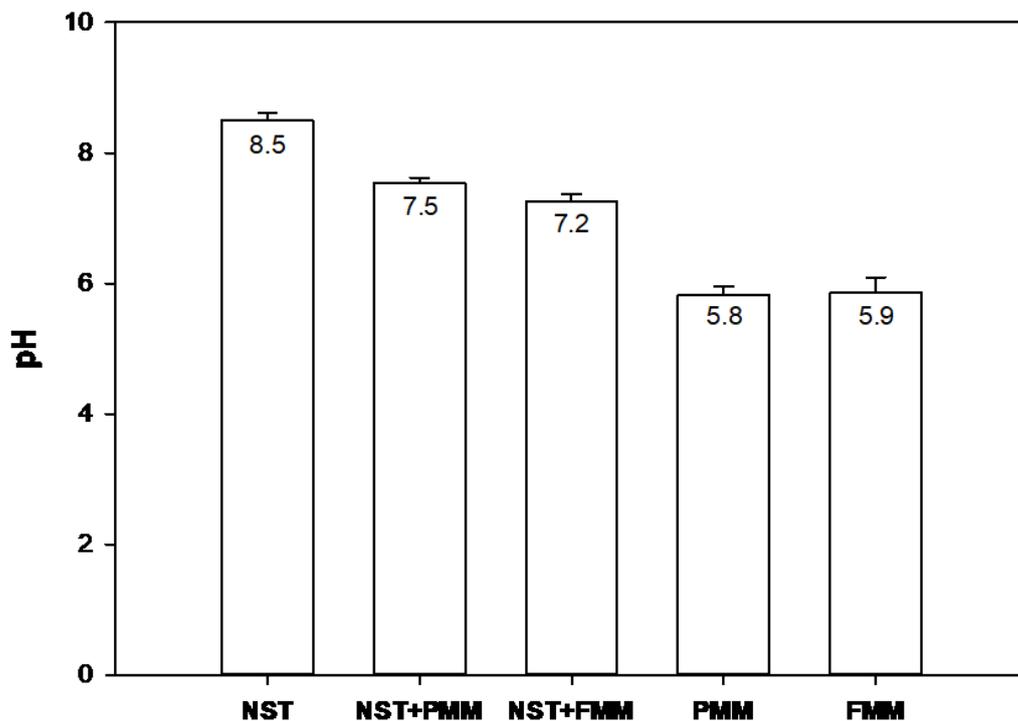


Figure 3.1 Initial soil pH of NST, NST+PMM, NST+FMM, PMM and FMM. Means (n = 6) ± SE are shown.

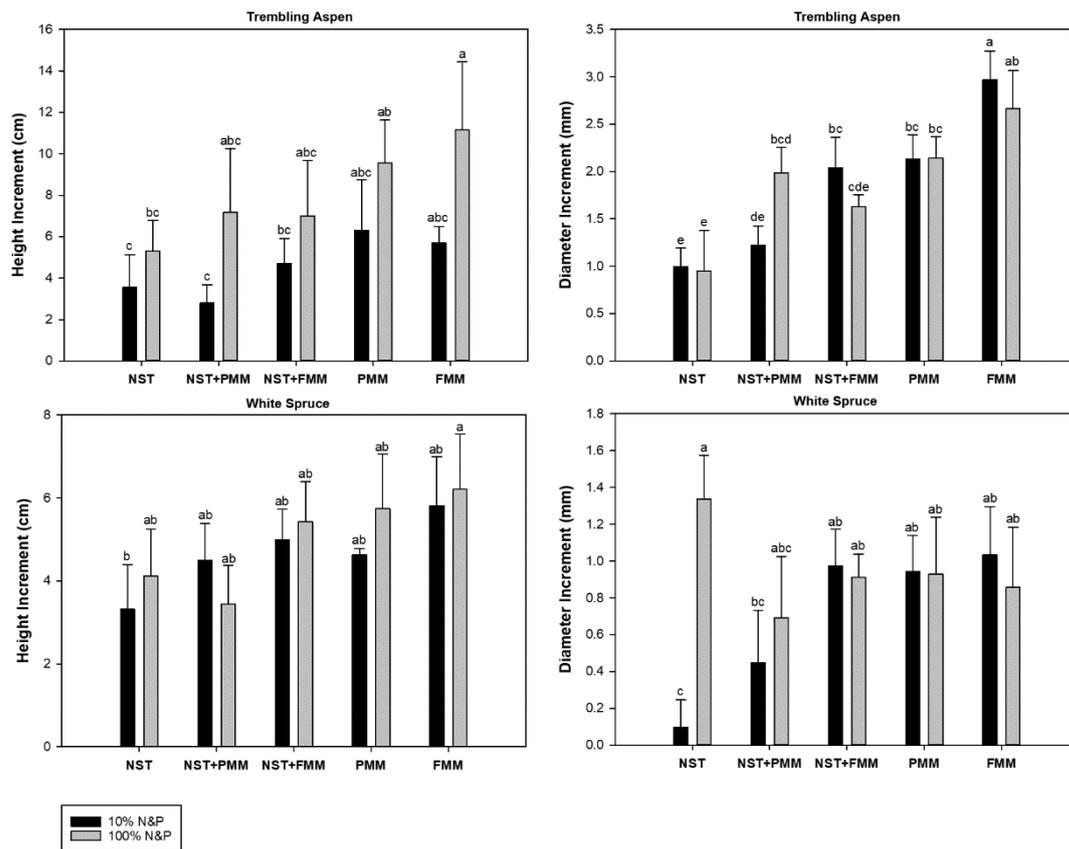


Figure 3.2 Height increment (cm) and diameter increment (mm) in trembling aspen (a, b) and white spruce (c, d) plants after two months of growth in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

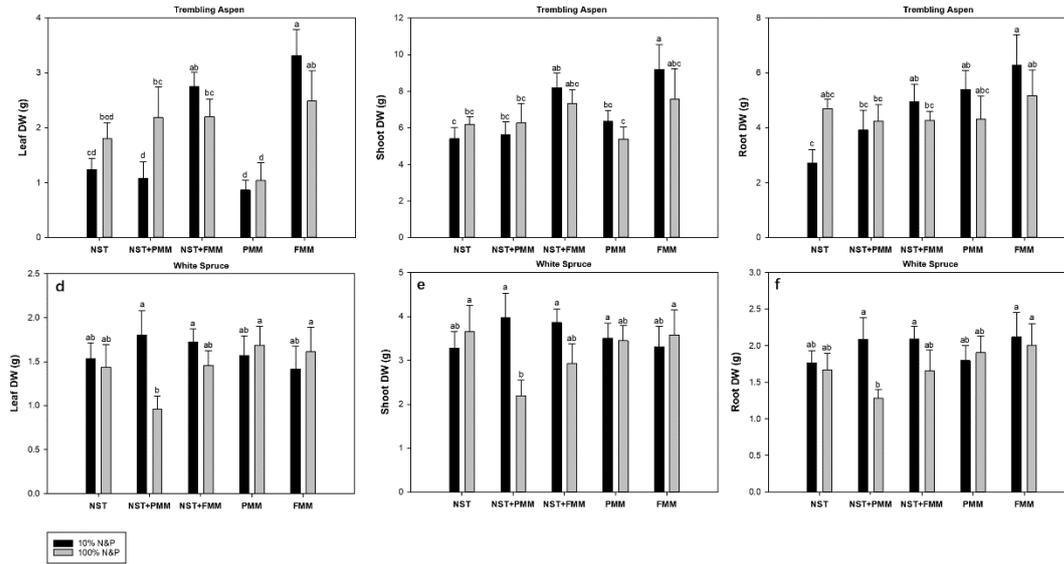


Figure 3.3 Leaf, shoot, and root dry weights in trembling aspen (a, b, c) and white spruce (d, e, f). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

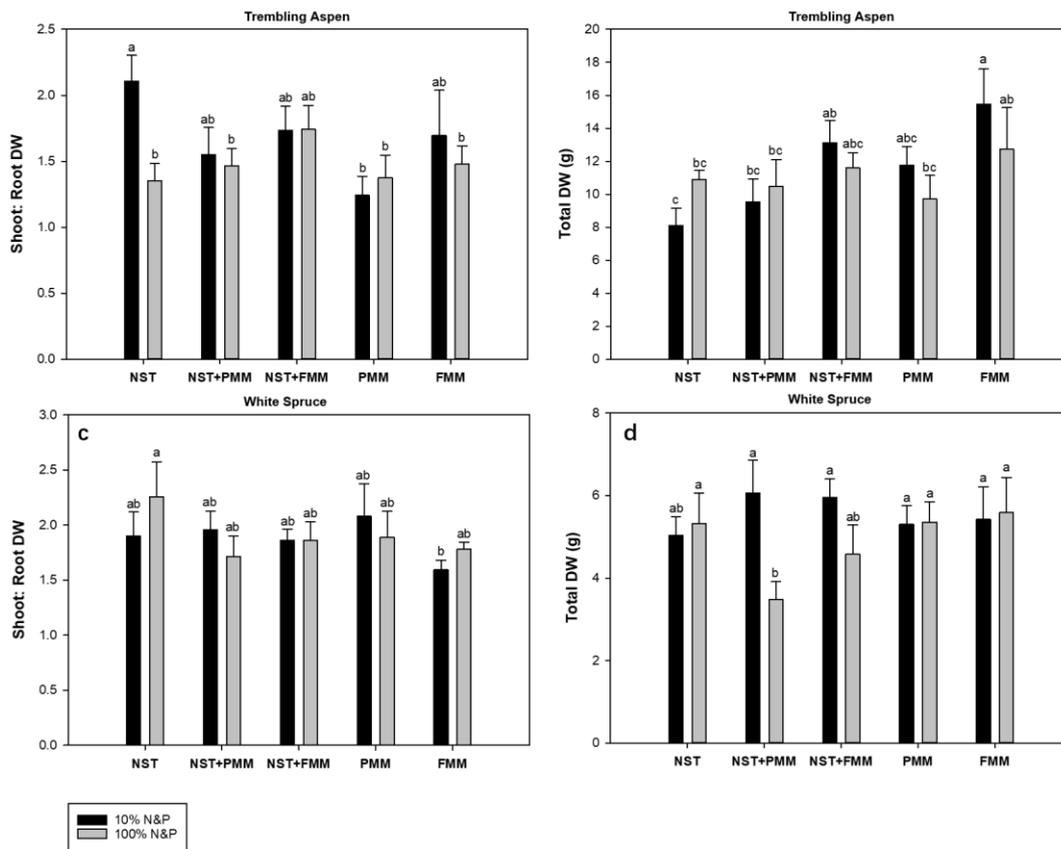


Figure 3.4 Shoot: Root dry weight ratios and total plant dry weights in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

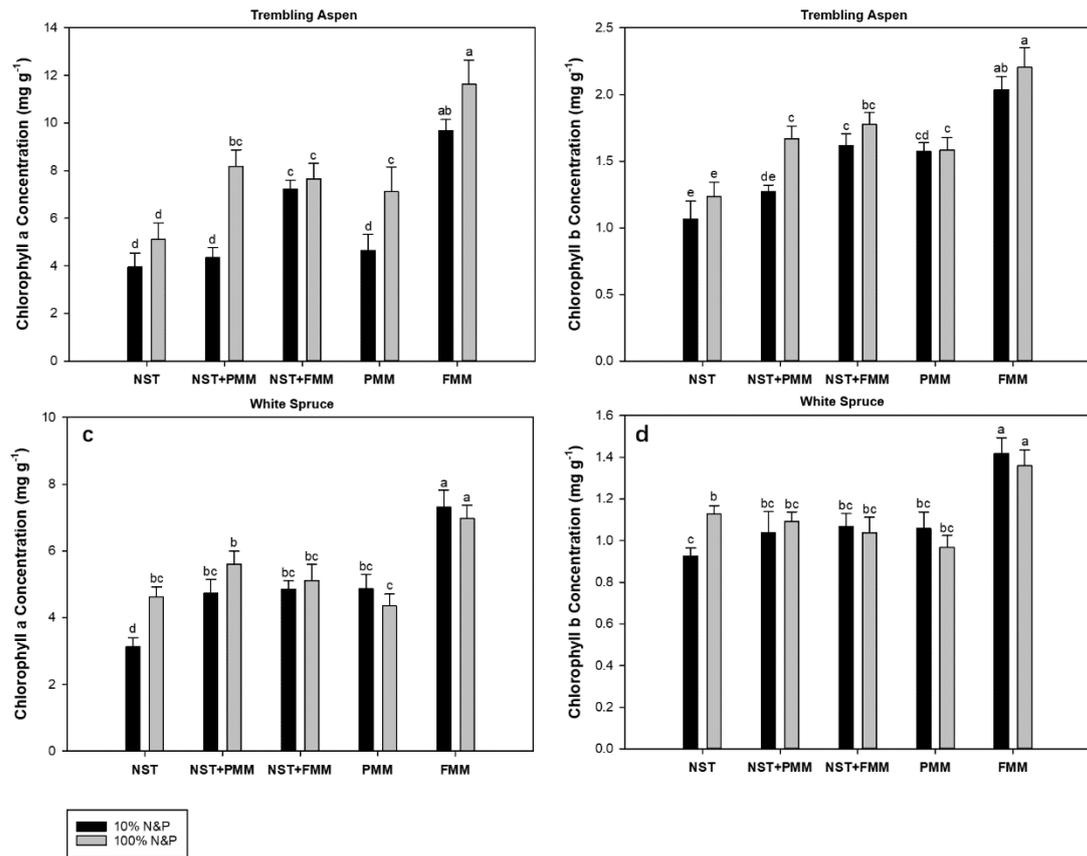


Figure 3.5 Leaf chlorophyll-a and chlorophyll-b concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

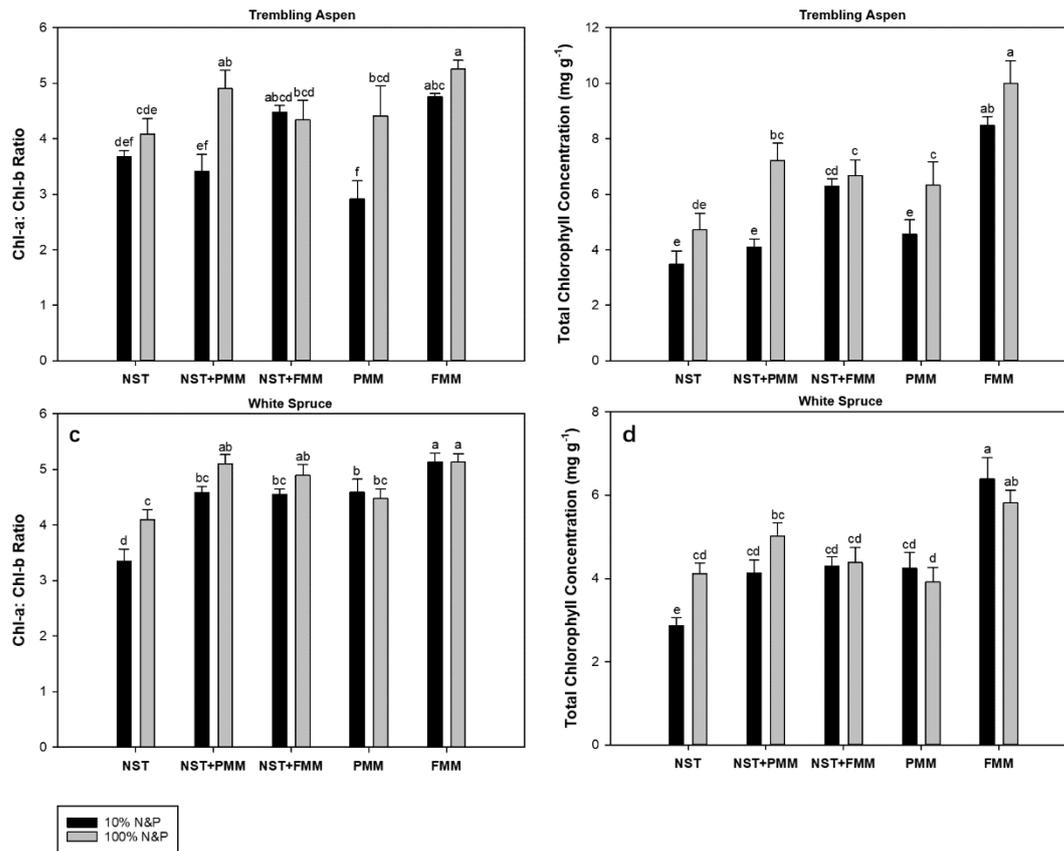


Figure 3.6 Leaf chlorophyll-a: chlorophyll-b ratios and total chlorophyll concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

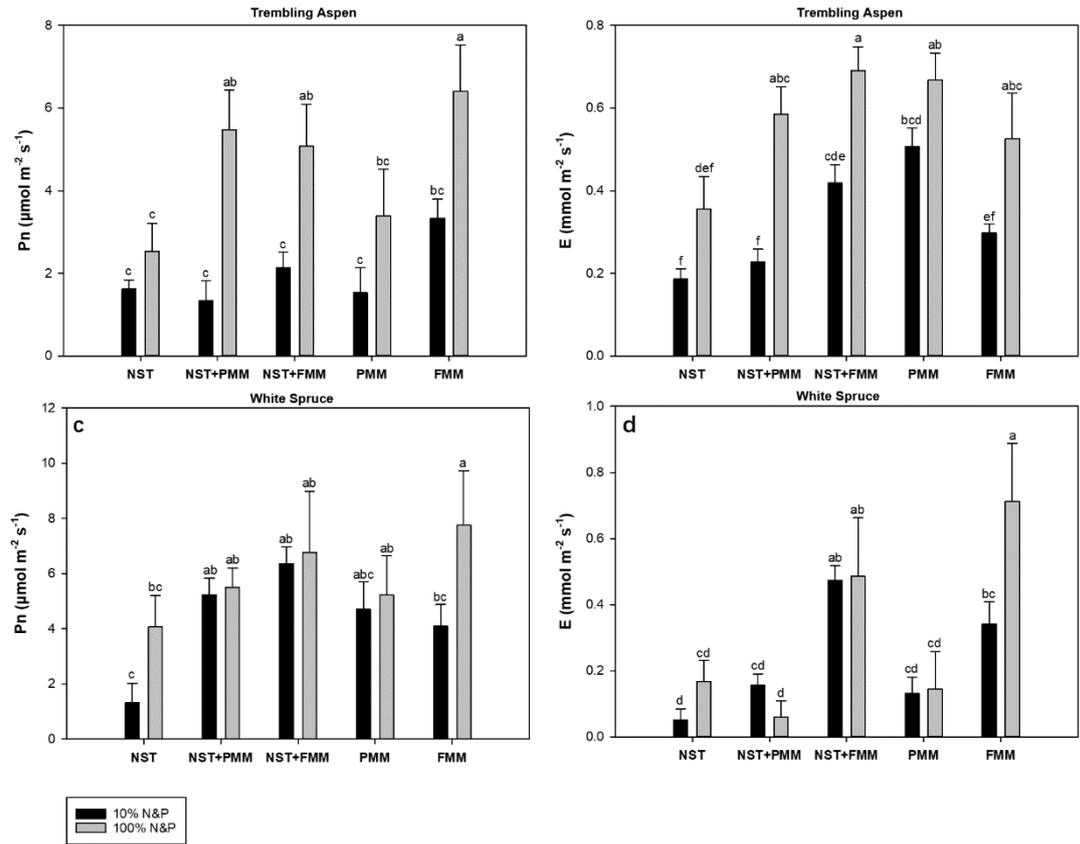


Figure 3.7 Net photosynthesis (Pn) and transpiration (E) rates in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

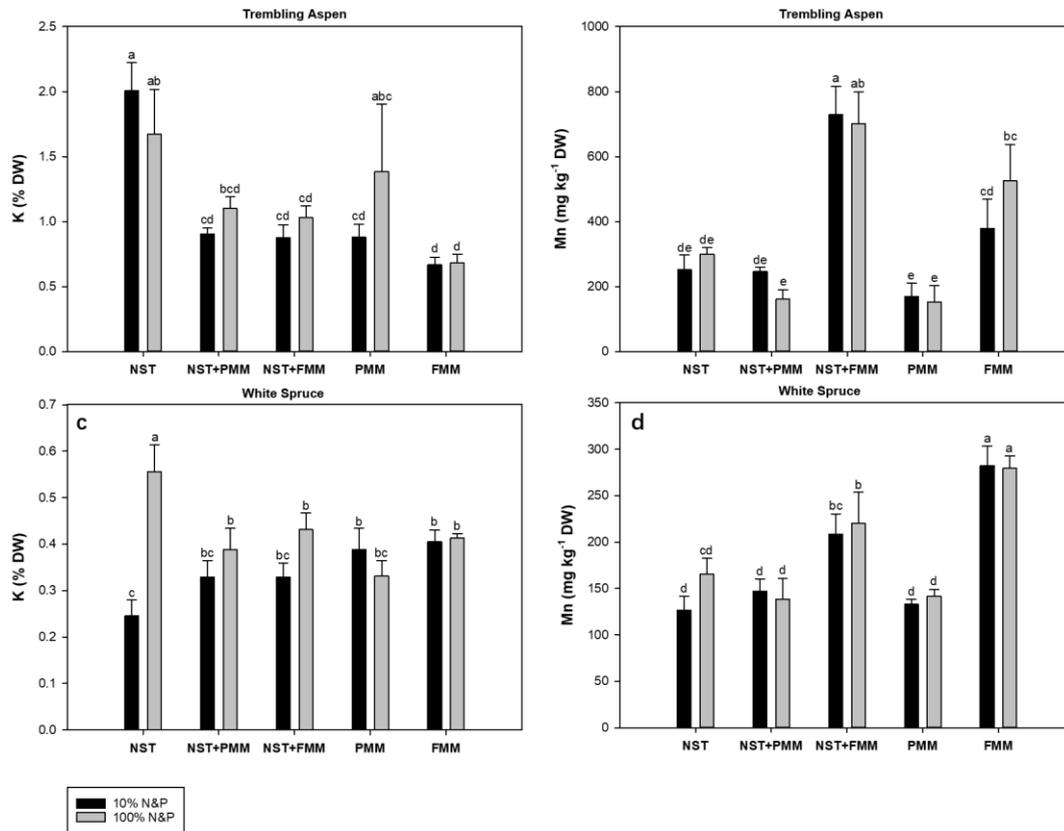


Figure 3.8 Leaf K and Mn concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

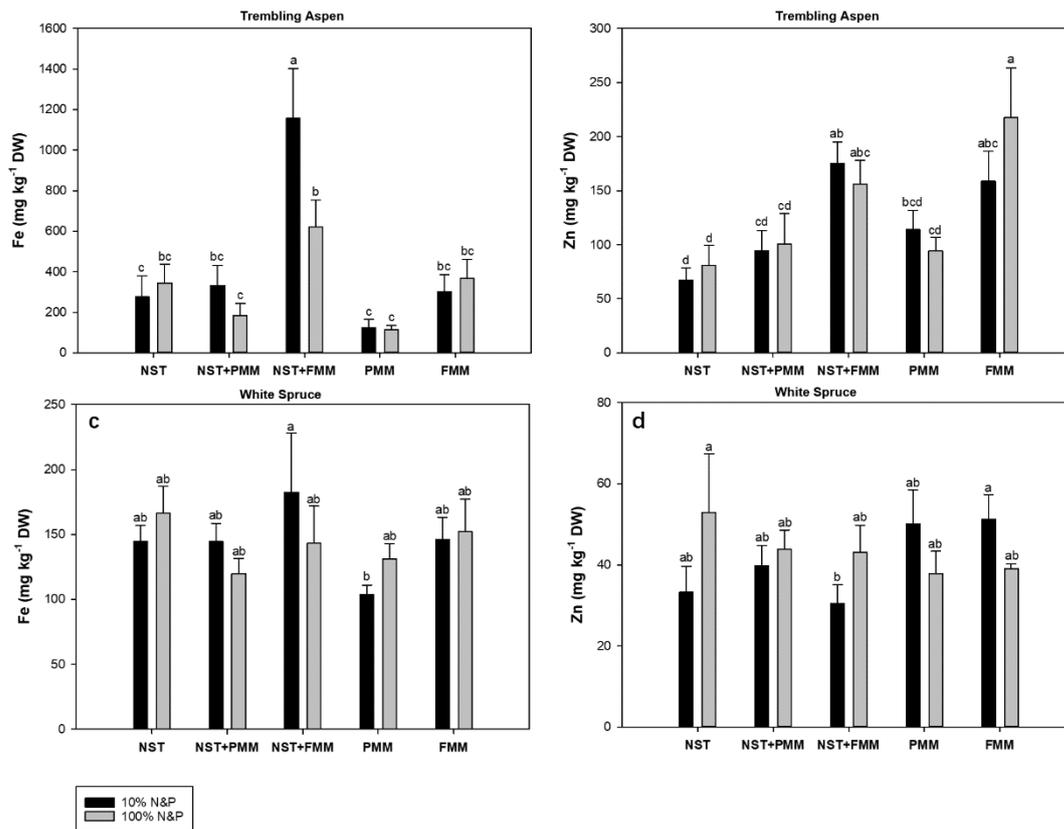


Figure 3.9 Leaf Fe and Zn concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

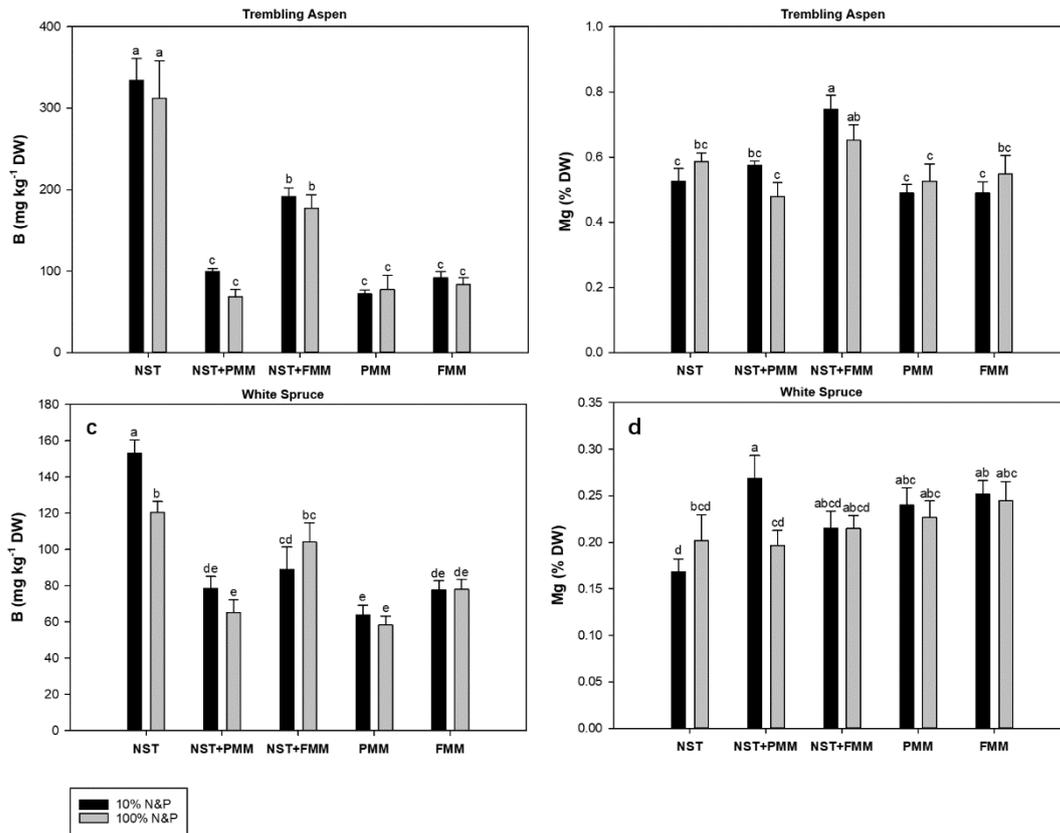


Figure 3.10 Leaf B and Mg concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments according to Fisher's LSD test. Means ($n = 6$) \pm SE are shown.

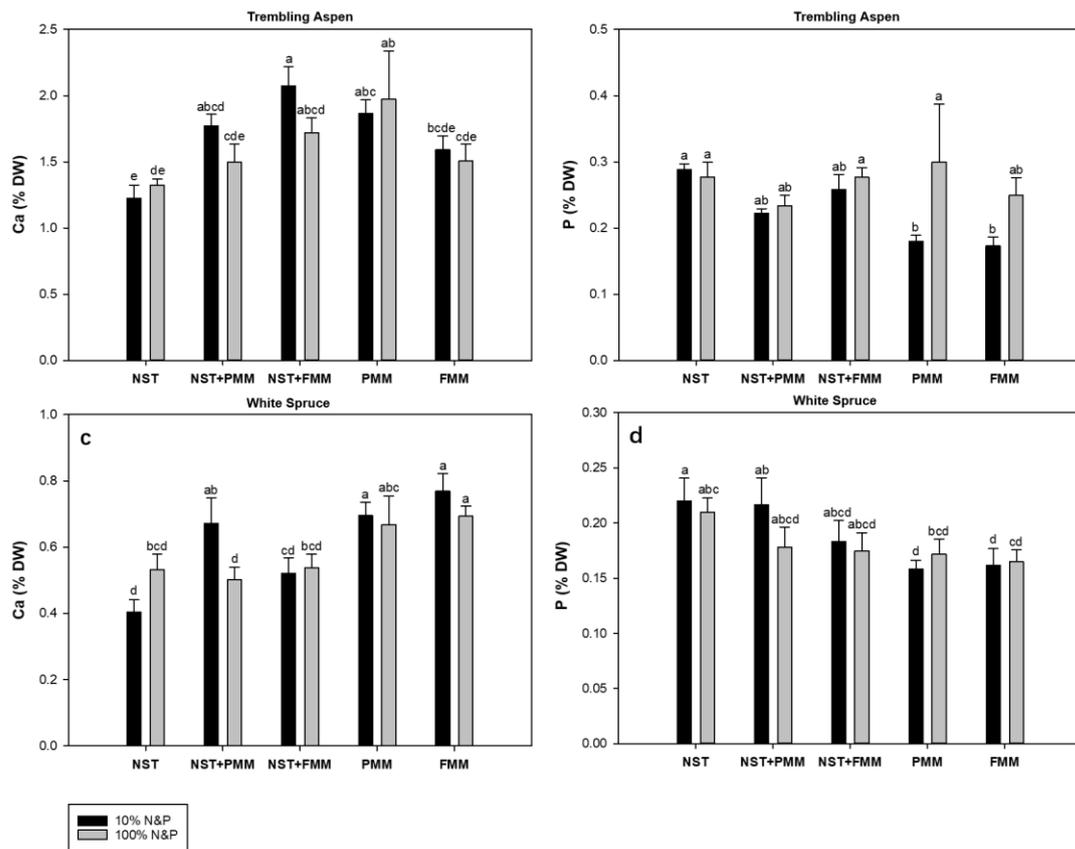


Figure 3.11 Leaf Ca and P concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n=6$) \pm SE are shown.

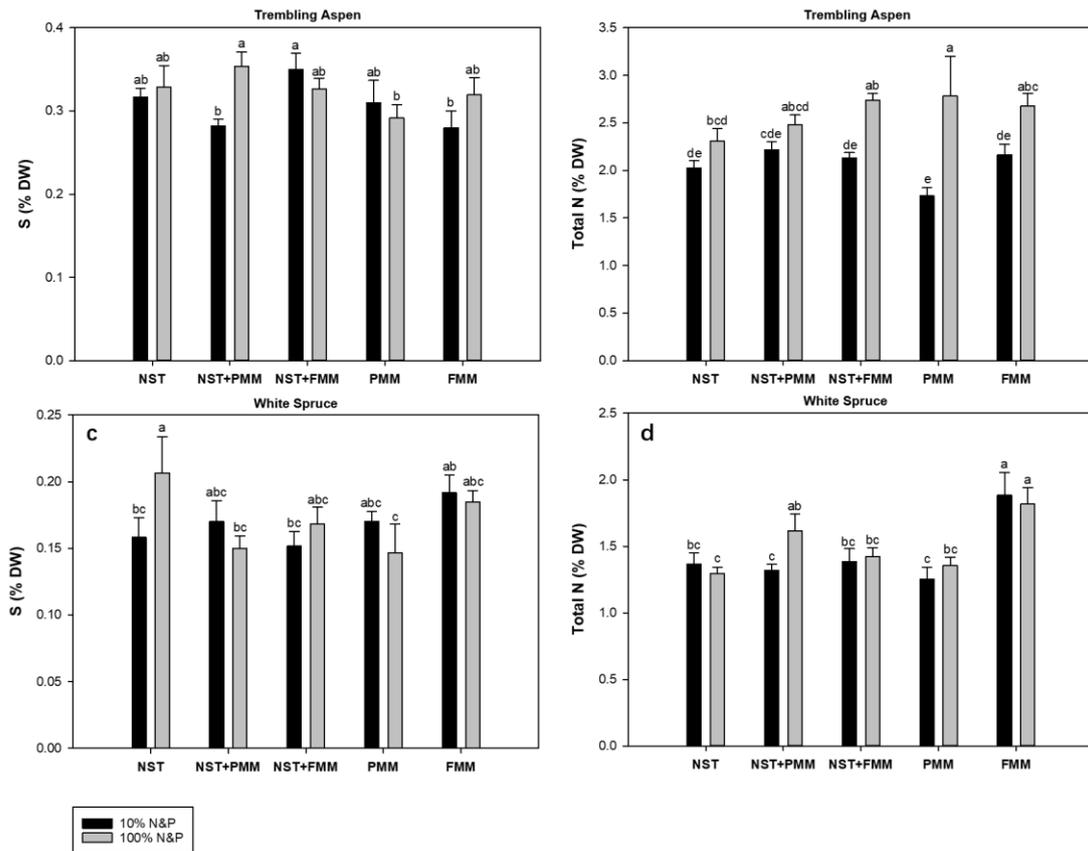


Figure 3.12 Leaf S and Total N concentrations in trembling aspen (a, b) and white spruce (c, d). Plants were grown for two months in the soil of NST, NST+PMM, NST+FMM, PMM and FMM. Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by Fisher's LSD test. Means ($n=6$) \pm SE are shown.

3.7 Tables

Table 3.1 The molar concentrations of all components in both treatment solutions ($\mu\text{mol L}^{-1}$).

	N	K	Ca	P	S	Mg	Cl	B	Mn	Zn	Cu	Mo	Fe
10%	1600	600	400	200	100	100	5	2.5	0.2	0.2	0.05	0.05	2
N&P													
100%	8400	6000	400	2000	100	100	5	2.5	0.2	0.2	0.05	0.05	2
N&P													

Table 3.2 ANOVA table showing effects of substrate and nutrition treatments on the parameters measured in trembling aspen (n = 6).

p-value	Height	Diameter	LDW	SDW	RDW	St: Rt	TDW	Chl-a	Chl-b	Chl-a: Chl-b	Total Chl	Pn	E
Substrate	0.193 6	4.16e-07 ***	3.62e-05 ***	0.01534 *	0.0760	0.4317	0.0257 *	2.09e-10 ***	1.89e-11 ***	0.0002 ***	1.26e-10 ***	0.0062 **	1.23e-05 ***
Nurition	0.010 0	0.9392	0.766 0	0.4748	0.7959	0.3216	0.5737	8.59e-05 ***	0.0089 **	0.32689	7.01e-05 ***	2.96e-06 ***	9.78e-08 ***
Substrate*Nutrition	0.895 5	0.3429	0.082 5	0.6458	0.1628	0.1794	0.3344	0.1706	0.4844	0.0249 *	0.2511	0.2872	0.5012

p-value	K	Mn	Fe	Zn	B	Mg	Ca	P	S	Total N
Substrate	5.49e-05 ***	7.93e-11 ***	3.99e-07 ***	3.36e-05 ***	<2e-16 ***	0.0001 ***	0.0005 ***	0.1946	0.2580	0.4703
Nurition	0.4557	0.7338	0.1498	0.6025	0.2618	0.8372	0.3290	0.0419 *	0.2268	4.55e-06 ***
Substrate*Nutrition	0.4458	0.5210	0.0515	0.4795	0.9123	0.1134	0.4890	0.2504	0.0855	0.1290

Abbreviations: LDW – leaf dry weight; SDW – shoot dry weight; RDW – root dry weight; St: Rt – shoot: root dry weight ratio; TDW – total dry weight; Chl-a – chlorophyll-a concentration; Chl-b – chlorophyll-b concentration; Chl-a: Chl-b – chlorophyll-a: chlorophyll-b ratio; Total Chl – total chlorophyll concentration; Pn – Net photosynthetic rate; E – Transpiration rate

Table 3.3 ANOVA table showing effects of pH and S treatments on the parameters measured in white spruce (n = 6).

p-value	Height	Diameter	LDW	SDW	RDW	St: Rt	TDW	Chl-a	Chl-b	Chl-a: Chl-b	Total Chl	Pn	E
Substrate	0.1344	0.4616	0.8218	0.9051	0.5517	0.3544	0.8428	3.11e-09 ***	2.71e-06 ***	2.86e-09 ***	8.15e-08 ***	0.0309 *	9.86e-06 ***
Nurition	0.5989	0.1299	0.2075	0.1581	0.0860	0.8612	0.1044	0.1733	0.7261	0.0111 *	0.0114 *	0.0543	0.1873
Substrate*Nurition	0.8524	0.0443 *	0.1403	0.0939	0.3687	0.5393	0.1211	0.0753	0.2226	0.0925	0.0403 *	0.5218	0.1630

p-value	K	Mn	Fe	Zn	B	Mg	Ca	P	S	Total N
Substrate	0.0358 *	3.42e-11 ***	0.2454	0.8157	1.86e-10 ***	0.0272 *	5.83e-05 ***	0.0101 *	0.1737	4.98e-06 ***
Nurition	4.13e-07 ***	0.4129	0.8921	0.6077	0.0035 **	0.3572	0.4765	0.4410	0.7654	0.3469
Substrate*Nurition	0.0002 ***	0.7433	0.4910	0.0979	0.0390 *	0.1142	0.0837	0.5998	0.1256	0.3463

Abbreviations: LDW – leaf dry weight; SDW – shoot dry weight; RDW – root dry weight; St: Rt – shoot: root dry weight ratio; TDW – total dry weight; Chl-a – chlorophyll-a concentration; Chl-b – chlorophyll-b concentration; Chl-a: Chl-b – chlorophyll-a: chlorophyll-b ratio; Total Chl – total chlorophyll concentration; Pn – Net photosynthetic rate; E – Transpiration rate

Chapter 4 General discussion and conclusion

4.1 General discussion

The results of Study 1 showed that elemental S was effective in lowering soil pH, the results were mainly due to the oxidization process of elemental S, which released H^+ into soil (Zhang et al., 2007; Zhao et al., 2008; Cao et al., 2010; Sun et al., 2014). The results also showed that the addition of 5 and 25 g kg^{-1} elemental S at pH 5.7 soil resulted in high soil acidity (pH 3.7 and pH 2.7, respectively), the addition of 5 and 25 g kg^{-1} elemental S at pH 8.5 soil did not bring pH to the desirable level (pH 7.7 and pH 2.7, respectively), but both Saskatoon and beaked hazelnut grown better in the pH 8.5 soil with no addition of S in this study. In addition, previous studies had illustrated that plants growing in low pH soil may face a variety of stresses, such as ion toxicity, nutrient deficiencies, limitation of enzyme activities (Rout et al., 2001; Brady and Weil, 2002; Kopittke et al., 2016). Therefore, careful measurements of soil pH in reclamation sites should be carried out before S is added, due to the negative effects of excessive soil acidity after the addition of elemental S. However, the results of growth and physiological responses of Saskatoon and beaked hazelnut growing in the soil of pH 5.7 and 8.5 with the addition of different levels of elemental S performed differently. The results were likely due to the different nutrient uptake strategy of these two species, Saskatoon may be less tolerant to much lower soil pH when compared with beaked hazelnut.

The results of Study 2 showed that NST had negative effects on growth and physiological responses of plants, the results were likely due to the high pH of NST caused by the addition of NaOH during the oil extraction process (Howat, 2000), the fine texture of NST which would result in poor root aeration which negatively affected the root growth of plants (Zhang et al., 2020), and low nutrients in NST (Zhang et al., 2020). The results also showed that growth and physiological responses of trembling aspen and white spruce were significantly improved in NST amended with PMM and

FMM compared with NST, the results were likely due to PMM and FMM lowered the high pH of NST, which changed from 8.5 to 7.5 and 7.2, respectively. PMM and FMM have high organic matter content, high water holding capacity and provide the source of nutrients, propagules and soil microorganisms (Lanoue and Qualizza, 2000; Sydnor and Redente, 2002; MacKenzie and Naeth, 2010). Therefore, the results were also likely due to the positive effects of PMM and FMM on NST. In addition, the results also showed that NST+FMM was a better growth substrate compared with NST+PMM for trembling aspen at the 10% N & P level, the results were likely due to the lower carbon to nitrogen ratios of FMM than PMM, providing more N for plants (Mackenzie and Naeth, 2010). The results also showed that white spruce was less affected by NST+PMM and NST+FMM at both levels of N & P compared with trembling aspen, the results were likely due to slower growth of white spruce compared with trembling aspen (Munson et al., 1995; Claveau et al., 2002). The results also indicated that trembling aspen was more affected by N & P levels than white spruce, the results were likely due to the rapid growth of trembling aspen compared with white spruce, which creates higher nutritional demand (Boyle, 1973; Perala, 1977; Alban, 1982; Debyle, 1985).

4.2 General conclusions

The results of Study 1 revealed that elemental S was effective in lowering soil pH, but the addition of S negatively affected growth and physiological performance of Saskatoon and beaked hazelnut plants when applied to the pH 5.7 soil. Saskatoon and beaked hazelnut seedlings growing in the pH 8.5 soil did not substantially benefit from the addition of 5 and 25 g kg⁻¹ S to the soil. Saskatoon and beaked hazelnut grew better in the pH 8.5 soil with no addition of S. However, the results clearly demonstrated the effectiveness of using S to lower the soil pH, which could have potential beneficial effects to plants in reclamation sites.

The results of Study 2 indicate that NST had a detrimental effect on growth and

physiological processes in trembling aspen and white spruce. The growth and physiological responses of these plants were significantly improved when NST was mixed with PMM and FMM. However, FMM was better to use with NST compared with PMM. Supplementing the growth substrates with 100% N & P level was more effective in enhancing growth and physiological effects in trembling aspen compared with white spruce.

4.3 Suggestions for oil sands reclamation

The research in this thesis benefits land reclamation process for restoring boreal forest in oil sands mining area, by demonstrating the effectiveness of S supplementation for lowering soil pH and the effects of a potential root contact of white spruce and trembling aspen plants with NST. The results demonstrated that N and P fertilization could alleviate some of the effects of NST when present in the soil, especially for trembling aspen.

4.4 Suggestions for future research

Although the results of Study 1 in this thesis indicate that elemental S was effective in lowering soil pH, the applied concentrations of S were not beneficial to plant growth. Saskatoon and beaked hazelnut were suitable for growing in soil with no S added in this study. Therefore, future research should be conducted to examine the effects of different levels of elemental S on the pH of different types of soils with different initial pH levels. In addition, the common pH levels in targeted ecosystem should be determined before conducting studies.

While the results of Study 2 in this thesis revealed that plants growth under 100% N & P nutrient level was improved more compared with 10% N & P, different concentration and compositions of mineral nutrients should be studied to determine the optimum fertilization treatments. In addition, more plants species should be studied in

the future to find out the most suitable plants for various land reclamation scenarios.

4.5 Application to oil sands reclamation sites

These two studies were conducted in growth chamber, therefore, there will be some limitations in the application of the results to oil sands reclamation sites. When applying the results of these two studies to reclamation sites, a field experiment should be conducted with the same treatment levels and plant species, to see if it works in real land reclamation scenarios, then the treatment levels based on the results of field experiment can be adjusted and the best levels of each treatment can be found, finally the results of field experiment can be widely applied to oil sands reclamation sites.

In study 1, Saskatoon and beaked hazelnut grew better in pH 8.5 soil with no S added. Therefore, more levels of S should be tested to find the most suitable level of S in field experiment. Saskatoon and beaked should also be tested to see if they perform well in field experiment.

In study 2, FMM and PMM can help improve NST and FMM was better than PMM to amend NST. Field experiment is easily conducted, but large volume of NST mixed with large volume of FMM and PMM will never happen in reclamation operations. Therefore, if NST capped with cover soil directly still works need to be tested before application.

Four plant species were selected for these two studies, more plants such as wetland and salt tolerant species should be selected and tested in field experiment, to provide more options for oil sands reclamation.

4.6 References

Alban, D.H., 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. *Soil Science Society of America Journal* 46, 853–861.

Brady, N.C., Weil, R.R., 2002. *The nature and properties of soils*. 13th Edition.

Prentice-Hall Inc., New Jersey, USA.

Boyle, J.R., Phillips, J.J., Ek, A.R., 1973. "Whole tree" harvesting: nutrient budget evaluation. *Journal of Forestry* 71, 760–762.

Cao, Y., Zhao, Y., Zhao, W., Lv, J., 2010. Research on effect of sulfur application on saline-alkaline soil pH and availability of trace elements in Huanghe River irrigation district in Ningxia. *Agricultural Research in the Arid Areas* 28, 199–201.

Claveau, Y., Messier, C., Comeau, P.G., Coates, K.D., 2002. Growth and crown morphological responses of boreal conifer seedlings and saplings with contrasting shade tolerance to a gradient of light and height. *Canadian Journal of Forest Research* 32, 458–468.

DeByle, N.V., Winokur, R.P., 1985. *Aspen: ecology and management in the western United States*. USDA Forest Service General Technical Report RM-119. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

Howat, D., 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Alberta Environment. Environmental Sciences Division, Edmonton, AB, Canada.

Kopittke, P.M., Menzies, N.W., Wang, P., Blamey, F.P.C., 2016. Kinetics and nature of aluminium rhizotoxic effects: a review. *Journal of Experimental Botany* 67, 4451–4467.

Lanoue, A., Qualizza, C., 2000. LFH and shallow mineral horizons as inoculants on reclaimed areas to improve native species catch. 1999 Status Report. Environmental Affairs, Syncrude Canada Ltd. Fort McMurray, AB, Canada.

Mackenzie, D.D., Naeth, M.A., 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. *Restoration Ecology* 18, 418–427.

Munson, A.D., Margolis, H.A., Brand, D.G., 1995. Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. *Tree*

- Physiology 15, 141–149.
- Rout, G., Samantaray, S., Das, P., 2001. Aluminium toxicity in plants: a review. *Agronomie* 21, 4–5.
- Sun, L., Duan, D., Peng, C., He, J., Shi, J., 2014. Influence of sulfur on the speciation transformation and phyto-availability of heavy metals in soil: A review. *Chinese Journal of Applied Ecology* 25, 2141–2148.
- Sydnor, M.E., Redente, E.F., 2002. Reclamation of high-elevation, acidic mine waste with organic amendments and topsoil. *Journal of Environmental Quality* 31, 1528–1537.
- Zhang, C., Zhang, M., Zeng, Y., 2007. Effects of sulphur on chemical properties of calcareous soil. *Chinese Journal of Applied Ecology* 7, 1453–1458.
- Zhang, W., Fleurial, K., Sherr, I., Vassov, R., Zwiazek, J.J., 2020. Growth and physiological responses of tree seedlings to oil sands non-segregated tailings. *Environmental Pollution* 259, 113945.
- Zhao, X., Li, Y., Mai, W., Tian, X., Lai, H., Lv, J., 2008. A preliminary study on amendment of saline soil through addition of sulphur. *Agricultural Research in the Arid Areas* 4, 74–78.

References

- ACRRE, 2016. *Corylus cornuta*. Alberta Centre for Reclamation and Restoration Ecology. University of Alberta [WWW Document]. URL http://accre.ualberta.ca/Portals/14/ACRREDocuments/Corylus_cornuta.pdf.
- Alban, D.H., 1982. Effects of nutrient accumulation by aspen, spruce, and pine on soil properties. *Soil Science Society of America Journal* 46, 853–861.
- Alberta Chamber of Resources, 2015. Caring for the land [WWW Document]. URL <https://www.acralberta.com/app/uploads/Reclamation-Brochure-Caring-For-The-Land.pdf>.
- Alberta Environment, 2010. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region, 2nd Edition. Prepared by the Terrestrial Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, Fort McMurray, AB, Canada.
- Alberta Environment and Water, 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Prepared by D. MacKenzie for the Terrestrial Subgroup, Best Management Practices Task Group, Reclamation Working Group, Cumulative Environmental Management Association. Fort McMurray, AB, Canada.
- Aroca, R., Ferrante, A., Vernieri, P., Chrispeels, M.J., 2006. Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Annals of Botany* 98, 1301–1310.
- Attiwill, P.M., Adams, M.A., 1993. Nutrient cycling in forests. *New Phytologist* 124, 561–582.
- Atwell, B.J., 1991. Factors which affect the growth of grain legumes on a solonized brown soil. II. Genotypic responses to soil chemical factors. *Australian Journal of Agricultural Research* 42, 107–119.
- Baath, E., Berg, B., Lohm, U., Lundgren, B., Lundkvist, H., Rosswall, T., Soderstrom, B., Wirén, A., 1980. Effects of experimental acidification and liming on soil

- organisms and decomposition in a Scots pine forest. *Pedobiologia* 20, 85–100.
- Banerjee, M.R., Chapman, S.J., 1996. The significance of microbial biomass sulphur in soil. *Biology and Fertility of Soils* 22, 116–125.
- Bertoni, G.M., Pissaloux, A., Morard, P., Sayag, D.R., 1992. Bicarbonate-pH relationship with iron chlorosis in white lupine. *Journal of Plant Nutrition* 15, 1509–1518.
- Boukhalfa, H., Crumbliss, A.L., 2002. Chemical aspects of siderophore mediated iron transport. *Biometals* 15, 325–339.
- Boyle, J.R., Phillips, J.J., Ek, A.R., 1973. “Whole tree” harvesting: nutrient budget evaluation. *Journal of Forestry* 71, 760–762.
- Brady N.C., Weil R.R., 1996. *The nature and properties of soils*. Prentice-Hall Inc., New Jersey, USA.
- Brady, N.C., Weil, R.R., 1999. *The nature and properties of soils*. 12th Edition. Prentice-Hall Inc., New Jersey, USA.
- Brady, N.C., Weil, R.R., 2002. *The nature and properties of soils*. 13th Edition. Prentice-Hall Inc., New Jersey, USA.
- Brand, D.G., Kehoe, P., Connors, M., 1986. Coniferous afforestation leads to soil acidification in central Ontario. *Canadian Journal of Forest Research* 16, 1389–1391.
- Brinkman, K.A., Roe, E.I., 1975. *Quaking aspen: silvics and management in the Lake States*. Agriculture Handbook, US Department of Agriculture. US Government Printing Office, Washington D.C., USA.
- Brown, R.L., 2010. Use of woody debris as an amendment for reclamation after oil sands mining. M.Sc. Thesis. University of Alberta, Department of Renewable Resources, Edmonton, Alberta, Canada.
- Cai, X., Ma, Y., Wang, W., Wei, C., Wang, X., Dun, S., 2008. Effects of applying sulphur on alkali soil pH and its consequence on yield and quality of flue-cured Tobacco. *Acta Tabacaria Sinica* 14, 19–22.
- Calvo-Polanco, M., Zhang, W., Macdonald, S.E., Señorans, J., Zwiazek, J.J., 2017.

- Boreal forest plant species responses to pH: ecological interpretation and application to reclamation. *Plant and Soil* 420, 195–208.
- Canmore-Neumann, R., Silber, A., Mitchnick, B., Gilad, S., Ben-Jaacov, J., 1996. Effect of pH on the development of *Leucadendron* ‘Safari Sunset’. IV International Protea Working Group Symposium 453, 47–52.
- Cakmak, I., Sari, N., Marschner, H., Ekiz, H., Kalayci, M., Yilmaz, A., Braun, H.J., 1996. Phytosiderophore release in bread and durum wheat genotypes differing in zinc efficiency. *Plant and Soil* 180, 183–189.
- Cao, Y., Zhao, Y., Zhao, W., Lv, J., 2010. Research on effect of sulfur application on saline-alkaline soil pH and availability of trace elements in Yellow River irrigation district in Ningxia. *Agricultural Research in the Arid Areas* 28, 199–201.
- Chalaturnyk, R.J., Don Scott, J., Özüüm, B., 2002. Management of oil sands tailings. *Petroleum Science and Technology* 20, 1025–1046.
- Chen, Y., Barak, P., 1982. Iron nutrition of plants in calcareous soils. *Advances in Agronomy* 35, 217–240.
- Clarkson, D.T., Carvajal, M., Henzler, T., Waterhouse, R.N., Smyth, A.J., Cooke, D.T., Steudle, E., 2000. Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *Journal of Experimental Botany* 51, 61–70.
- Claussen, W., Lenz, F., 1999. Effect of ammonium or nitrate nutrition on net photosynthesis, growth, and activity of the enzymes nitrate reductase and glutamine synthetase in blueberry, raspberry and strawberry. *Plant and Soil* 208, 95–102.
- Claveau, Y., Messier, C., Comeau, P.G., Coates, K.D., 2002. Growth and crown morphological responses of boreal conifer seedlings and saplings with contrasting shade tolerance to a gradient of light and height. *Canadian Journal of Forest Research* 32, 458–468.
- CNPS, 2020. Saskatoon Serviceberry, *Amelanchier alnifolia* [WWW Document].

- URL [https://calscape.org/Amelanchier-alnifolia-\(\)](https://calscape.org/Amelanchier-alnifolia-()).
- CNRL, 2019. Managing Tailings. <https://www.cnrl.com/corporate-responsibility/advancements-in-technology/managing-tailings>.
- Comerford, N.B., 2005. Soil factors affecting nutrient bioavailability. Nutrient Acquisition by Plants 1–14. Springer, Berlin, Germany.
- COSIA, 2020. Carbon Dioxide Amended Tailings. <https://cosia.ca/initiatives/tailings/projects/carbon-dioxide-amended-tailings#:~:text=In%202015%2C%20Canadian%20Natural%20began,use%20of%20cyclones%20and%20thickeners>.
- Cui, Y., Wang, Q., Dong, Y., Li, H., Christie, P., 2004. Enhanced uptake of soil Pb and Zn by Indian mustard and winter wheat following combined soil application of elemental sulphur and EDTA. *Plant and Soil* 261, 181–188.
- DeByle, N.V., Winokur, R.P., 1985. Aspen: ecology and management in the western United States. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. Fort Collins, Colorado, USA.
- Dede, G., Ozdemir, S., 2016. Effects of elemental sulphur on heavy metal uptake by plants growing on municipal sewage sludge. *Journal of Environmental Management* 166, 103–108.
- DePuit, E.J., 1984. Potential topsoiling strategies for enhancement of vegetation diversity on mined lands. *Minerals and the Environment* 6, 115–120.
- Dietrich, S.T., MacKenzie, M.D., 2018. Comparing spatial heterogeneity of bioavailable nutrients and soil respiration in boreal sites recovering from natural and anthropogenic disturbance. *Frontiers in Environmental Science* 6, 126.
- Dong, B., Rengel, Z., Graham, R.D., 1995. Root morphology of wheat genotypes differing in zinc efficiency. *Journal of Plant Nutrition* 18, 2761–2773.
- Downing, D.J., Pettapiece, W.W., 2006. Natural regions and subregions of Alberta. Natural Regions Committee, Edmonton.
- Duan, M., House, J., Chang, S.X., 2015. Limiting factors for lodgepole pine (*Pinus*

- contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region. *Ecological Engineering* 75, 323–331.
- Duan, M., House, J., Liu, Y., Chang, S.X., 2018. Contrasting responses of gross and net nitrogen transformations to salinity in a reclaimed boreal forest soil. *Biology and Fertility of Soils* 54, 385–395.
- Engelhardt, R., Todirescu, M., 2005. An introduction to development in Alberta's oil sands. University of Alberta. AB, Canada.
- Eriksen, J., Mortensen, J.V., 2002. Effects of timing of sulphur application on yield, S-uptake and quality of barley. *Plant and Soil* 242, 283–289.
- EPCOR, 2020. Daily Water Quality [WWW Document].
URL <https://www.epcor.com/products-services/water/water-quality/Pages/daily-water-quality.aspx>.
- Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. Wiley, New York, USA.
- Epstein, E., Bloom, A.J., 2005. Mineral nutrition of plants: principles and perspectives, 2nd Edition. Sinauer, Sunderland, UK.
- Ewens M., Leigh, R.A., 1985. The effect of nutrient solution composition on the length of root hairs of wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* 36, 713–724.
- Fageria, N.K., Baligar, V.C., 2003. Fertility management of tropical acid soils for sustainable crop production. *Handbook of Soil Acidity*, 359–385. Marcel Dekker Inc., New York, USA.
- Farjon, A., 1990. Pinaceae. Drawings and descriptions of the genera *Abies*, *Cedrus*, *Pseudolarix*, *Keteleeria*, *Nothotsuga*, *Tsuga*, *Cathaya*, *Pseudotsuga*, *Larix* and *Picea*. Koeltz Scientific Books, Königstein, Germany.
- Felle, H.H., Hanstein, S., 2002. The apoplastic pH of the substomatal cavity of *Vicia faba* leaves and its regulation responding to different stress factors. *Journal of Experimental Botany* 53, 73–82.
- Forsch, K.B.C., 2014. Oil sands reclamation using woody debris with LFH mineral

- soil mix and peat mineral soil mix cover soils: impacts on select soil and vegetation properties. M.Sc. Thesis. Department of Renewable Resources, University of Alberta. Edmonton, AB, Canada.
- Fredeen, A.L., Rao, I.M., Terry, N., 1989. Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. *Plant Physiology* 89, 225–230.
- Frelich, L.E., Machado, J.-L., Reich, P.B., 2003. Fine-scale environmental variation and structure of understorey plant communities in two old-growth pine forests. *Journal of Ecology* 91, 283–293.
- Frey, B.R., Lieffers, V.J., Landhäusser, S.M., Comeau, P.G., Greenway, K.J., 2003. An analysis of sucker regeneration of trembling aspen. *Canadian Journal of Forest Research* 33, 1169–1179.
- Fryer, Janet, L., 2007. *Corylus cornuta*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). [WWW Document].
URL <https://www.fs.fed.us/database/feis/plants/shrub/corcor/all.html>.
- Fung, M.Y., Macyk, T.M., 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands* 41, 755–774.
- George, E., Horst, W.J., Neumann, E., 2012. Adaptation of plants to adverse chemical soil conditions. *Marschner's Mineral Nutrition of Higher Plants* 409–472. Academic Press. London, UK.
- Gifford, G.F., Humphries, W., Jaynes, R.A., 1984. A preliminary quantification of the impacts of aspen to conifer succession on water yield. *Journal of the American Water Resources Association* 20, 181–186.
- Gill, J.D., Healy, W.M., 1974. Shrubs and vines for northeastern wildlife. USDA Forest Service, Northeastern Forest Experiment Station, Upper Darby, Pennsylvania, USA.
- Goldberg, S., 1997. Reactions of boron with soils. *Plant and Soil* 193, 35–48.
- Goldberg, S., Glaubig, R.A., 1986. Boron adsorption and silicon release by the clay minerals kaolinite, montmorillonite, and illite. *Soil Science Society of*

- America Journal 50, 1442–1448.
- Government of Alberta, 2017. Oil sands – Overview [WWW Document]. URL <https://www.alberta.ca/oil-sands-overview.aspx>.
- Government of Alberta, 2015. Alberta’s oil sands: reclamation [WWW Document]. URL <https://www.oilsands.alberta.ca/reclamation.html>.
- Government of Alberta, 2014. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region (2nd edition). Fort McMurray, AB, Canada.
- Graham, M.J., Stephens, P.A., Widholm, J.M., Nickell, C.D., 1992. Soybean genotype evaluation for iron deficiency chlorosis using sodium bicarbonate and tissue culture. *Journal of Plant Nutrition* 15, 1215–1225.
- Graham, R.D., 2008. Micronutrient deficiencies in crops and their global significance. *Micronutrient Deficiencies in Global Crop Production* 41-61. Springer, Dordrecht, Netherlands.
- Greenway, K.J., Macdonald, S.E., Lieffers, V.J., 1992. Is long-lived foliage in *Picea mariana* an adaptation to nutrient-poor conditions? *Oecologia* 91, 184–191.
- Gundersen, P., Sevel, L., Christiansen, J.R., Vesterdal, L., Hansen, K., Bastrup-Birk, A., 2009. Do indicators of nitrogen retention and leaching differ between coniferous and broadleaved forests in Denmark? *Forest Ecology and Management* 258, 1137–1146.
- Hahn, A.S., 2012. Soil microbial communities in early ecosystems. MSc Thesis. University of Alberta, Department of Renewable Resources. Edmonton, AB, Canada.
- Hahn, A.S., Quideau, S.A., 2013. Long-term effects of organic amendments on the recovery of plant and soil microbial communities following disturbance in the Canadian boreal forest. *Plant and Soil* 363, 331–344.
- Hewitt, E. J., Smith, T. A., et al. 1974. *Plant mineral nutrition*. English Universities Press Ltd., London, UK.
- Howat, D., 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Alberta Environment. Environmental Sciences Division,

Edmonton, AB, Canada.

- Iles, J., 2001. Community Trees: Community tree steward program. Requirements for plant growth. Department of Horticulture, Iowa State University. Ames, IA, USA.
- Jamro, G.M., Chang, S.X., Naeth, M.A., 2014. Organic capping type affected nitrogen availability and associated enzyme activities in reconstructed oil sands soils in Alberta, Canada. *Ecological Engineering* 73, 92–101.
- Janzen, H.H., Bettany, J.R., 1984. Sulfur nutrition of rapeseed: I. Influence of fertilizer nitrogen and sulfur rates. *Soil Science Society of America Journal* 48, 100–107.
- Jarrell, W.M., Beverly, R.B., 1981. The dilution effect in plant nutrition studies. *Advances in Agronomy* 34, 197–224.
- Jauregui, M.A., Reisenauer, H.M., 1982. Dissolution of oxides of manganese and iron by root exudate components 1. *Soil Science Society of America Journal* 46, 314–317.
- Jing, J., Rui, Y., Zhang, F., Rengel, Z., Shen, J., 2010. Localized application of phosphorus and ammonium improves growth of maize seedlings by stimulating root proliferation and rhizosphere acidification. *Field Crops Research* 119, 355–364.
- Kamaluddin, M., Zwiazek, J.J., 2004. Effects of root medium pH on water transport in paper birch (*Betula papyrifera*) seedlings in relation to root temperature and abscisic acid treatments. *Tree Physiology* 24, 1173–1180.
- Karimizarchi, M., Aminuddin, H., Khanif, M.Y., Radziah, O., 2014. Elemental sulphur application effects on nutrient availability and sweet maize (*Zea mays* L.) response in a high pH soil of Malaysia. *Malaysian Journal of Soil Science* 18, 75–86.
- Keshta, N., Elshorbagy, A., Barbour, S.L., 2010. Comparative probabilistic assessment of the hydrological performance of reconstructed and natural watersheds. *Hydrological Processes* 24, 1333–1342.

- Koca, N., Karadeniz, F., Burdurlu, H.S., 2007. Effect of pH on chlorophyll degradation and colour loss in blanched green peas. *Food Chemistry* 100, 609–615.
- Kopittke, P.M., Menzies, N.W., 2005. Control of nutrient solutions for studies at high pH. *Plant and Soil* 266, 343–354.
- Kopittke, P.M., Menzies, N.W., Wang, P., Blamey, F.P.C., 2016. Kinetics and nature of aluminium rhizotoxic effects: a review. *Journal of Experimental Botany* 67, 4451–4467.
- Kosegarten, H., Hoffmann, B., Mengel, K., 2001. The paramount influence of nitrate in increasing apoplastic pH of young sunflower leaves to induce Fe deficiency chlorosis, and the re-greening effect brought about by acidic foliar sprays. *Journal of Plant Nutrition and Soil Science* 164, 155–163.
- Lambers, H., Chapin III, F.S., Pons, T.L., 2008. *Plant physiological ecology*. Springer Science & Business Media, New York, USA.
- Lanoue, A., Qualizza, C., 2000. LFH and shallow mineral horizons as inoculants on reclaimed areas to improve native species catch. 1999 Status Report. Environmental Affairs, Syncrude Canada Ltd. Fort McMurray, AB, Canada.
- Li, C., Liu, Y., He, T., Yang, H., Huang, L., Teng, Y., 1999. Relation between soil pH and potassium nutrition of flue-cured tobacco. *Acta Pedologica Sinica* 2, 276–282.
- Lindsay, W.L., 1984. Soil and plant relationships associated with iron deficiency with emphasis on nutrient interactions. *Journal of Plant Nutrition* 7, 489–500.
- Liu, G., Li, X., Zhang, Y., Lv, J., Wei, Y., 2008. Primary study on function of sulphur on saline-alkali soil in Yinbei Area. *Agricultural Research in the Arid Areas* 4, 79–82.
- Lynch, J., Lauchli, A., Epstein, E., 1991. Vegetative growth of the common bean in response to phosphorus nutrition. *Crop Science* 31, 380–387.
- MacKenzie, D., 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Alberta Environment and

- Water. Edmonton, AB, Canada.
- MacKenzie, D., Alberta, Alberta Environment and Water, 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. Alberta Environment and Water, Edmonton, Alberta.
- MacKenzie, D.D., 2013. Oil sands mine reclamation using boreal forest surface soil (LFH) in northern Alberta. Ph.D. dissertation. University of Alberta, Department of Renewable Resources. Edmonton, AB, Canada.
- MacKenzie, D.D., Naeth, M.A., 2007. Assisted natural recovery using a forest soil propagule bank in the Athabasca oil sands. *Seeds Biology, Development and Ecology* 374–382. Cromwell Press, Trowbridge, UK.
- Mackenzie, D.D., Naeth, M.A., 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. *Restoration Ecology* 18, 418–427.
- Maini, J.S., 1966. Phytoecological study of sylvotundra at Small Tree Lake, NWT. *Arctic* 19, 220–243.
- Marschner, H., 2011. *Marschner's mineral nutrition of higher plants*. Academic Press, London, UK.
- Marschner, H., 2012. *Marschner's mineral nutrition of higher plants*. Academic press, London, UK.
- Marschner, P., 2012. *Marschner's mineral nutrition of higher plants*. Elsevier Ltd., 225 Wyman Street, Waltham, MA 02451, USA.
- Marschner, H., 1995. *Mineral nutrition of higher plants*. Academic Press, London, UK.
- Marschner, H., Römheld, V., Kissel, M., 1986. Different strategies in higher plants in mobilization and uptake of iron. *Journal of Plant Nutrition* 9, 695–713.
- Masliyah, J., Zhou, Z.J., Xu, Z., Czarnecki, J., Hamza, H., 2004. Understanding water-based bitumen extraction from Athabasca oil sands. *The Canadian Journal of Chemical Engineering* 82, 628–654.
- McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O., 2007. Nitrogen

- mineralization and microbial activity in oil sands reclaimed boreal forest soils. *Journal of Environmental Quality* 36, 1470–1478.
- Mengel, K., 1994. Iron availability in plant tissues-iron chlorosis on calcareous soils. *Plant and Soil* 165, 275–283.
- Mengel, K., Kosegarten, H., Kirkby, E.A., Appel, T., 2001. Principles of plant nutrition. Springer, New York, USA.
- Merhaut, D.J., 1993. Effects of nitrogen form on vegetative growth, and carbon/nitrogen assimilation, metabolism, and partitioning in blueberry. Ph.D. Dissertation, University of Florida, USA.
- Ministry of Natural Resources and Forestry, 2014. Trembling aspen [WWW Document]. URL <https://www.ontario.ca/page/trembling-aspen>.
- Modaihsh, A.S., Al-Mustafa, W.A., Metwally, A.I., 1989. Effect of elemental sulphur on chemical changes and nutrient availability in calcareous soils. *Plant and Soil* 116, 95–101.
- Moreno-Peñaranda, R., Lloret, F., Alcaniz, J.M., 2004. Effects of sewage sludge on plant community composition in restored limestone quarries. *Restoration Ecology* 12, 290–296.
- Munson, A.D., Margolis, H.A., Brand, D.G., 1995. Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. *Tree Physiology* 15, 141–149.
- Naeth, M.A., Wilkinson, S.R., Mackenzie, D.D., Archibald, H.A., Powter, C.B., 2013. Potential of LFH mineral soil mixes for reclamation of forested lands in Alberta. OSRIN Report No. TR-35. Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, Edmonton, AB, Canada.
- Native Plants PNW, 2016. Beaked Hazelnut, *Corylus cornuta*. Native Plants PNW. URL <http://nativeplantspnw.com/beaked-hazelnut-corylus-cornuta/>.
- Natural Regions Committee, 2006. Natural regions and subregions of Alberta. Compiled by DJ Downing and WW Pettapiece. Government of Alberta, AB,

Canada.

- Natural Resources Canada, N.R., 2017. Oil Sands Processes [WWW Document].
URL <https://www.nrcan.gc.ca/energy/energy-sources-distribution/crude-oil/technical-overview/oil-sands-processes/5853>.
- Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. *Environmental Microbiology* 10, 2966–2978.
- Nienstaedt, H., Teich, A., 1972. The genetics of white spruce. Forest Service Research Paper, (WO-15). US Department of Agriculture, USA.
- Nienstaedt, H., Zasada, J.C., 1990. *Picea glauca* (Moench) Voss. Pages: 204–226. In: Burns, R.M. and Honkala, B.H. (eds.). *Silvics of North America: 1. Conifers*. Agriculture Handbook 654. U.S. Department of Agriculture, Forest Service, Washington D.C., USA.
- Nommik, H., Vahtras, K., 1982. Retention and fixation of ammonium and ammonia in soils. *Nitrogen in Agricultural Soils* 22, 123–171.
- Oaks, A., Aslam, M., Boesel, I., 1977. Ammonium and amino acids as regulators of nitrate reductase in corn roots. *Plant Physiology* 59, 391–394.
- Ohmann, L.F., Batzer, H.O., Buech, R.R., Lothner, D.C., Perala, D.A., Schipper Jr, A.L., Verry, E.S., 1978. Some harvest options and their consequences for the aspen, birch and associated conifer forest types of the Lake States. North Central Forest Experiment Station, St. Paul, Minnesota, USA.
- Oil Sands Vegetation Reclamation Committee, 1998. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. Alberta Environmental Protection. Report No. ESD/LM/99-1. Edmonton, AB, Canada.
- Orman, S., 2012. Effects of elemental sulphur and farmyard manure applications to calcareous saline clay loam soil on growth and some nutrient concentrations of tomato plants. *Journal of Food, Agriculture & Environment* 10, 720–725.
- Parker, M.B., Walker, M.E., 1986. Soil pH and manganese effects on manganese nutrition of peanut 1. *Agronomy Journal* 78, 614–620.

- Patton, D.R., Jones, J.R., 1977. Managing aspen for wildlife in the Southwest. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.
- Perala, D.A., 1977. Manager's handbook for aspen in the north-central states. General Technical Report NC-36. US Department of Agriculture, Forest Service, North Central Forest Experiment Station, St. Paul, Minnesota, USA.
- Perry, L., 2003. pH for the Garden. URL <http://pss.uvm.edu/ppp/pubs/oh34.htm>.
- Province of Alberta, 2014. Conservation and reclamation regulation of environmental protection and enhancement act. Alberta Queen's Printer, Edmonton, Canada.
- Ramos-Padrón, E., Bordenave, S., Lin, S., Bhaskar, I.M., Dong, X., Sensen, C.W., Fournier, J., Voordouw, G., Gieg, L.M., 2011. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environmental Science & Technology* 45, 439–446.
- Rao, P.S., Karunasagar, Iddya, Otta, S.K., Karunasagar, Indrani, 2000. Incidence of bacteria involved in nitrogen and sulphur cycles in tropical shrimp culture ponds. *Aquaculture International* 8, 463-472.
- Renault, S., Lait, C., Zwiazek, J.J., MacKinnon, M., 1998. Effect of high salinity tailings waters produced from gypsum treatment of oil sands tailings on plants of the boreal forest. *Environmental Pollution* 102, 177-184.
- Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D., 1999. Responses of boreal plants to high salinity oil sands tailings water. *Journal of Environmental Quality* 28, 1957–1962.
- Rengel, Z., 2002. Handbook of Plant Growth pH as the Master Variable. CRC Press.
- Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied and Environmental Microbiology* 75, 1589–1596.
- Rout, G., Samantaray, S., Das, P., 2001. Aluminium toxicity in plants: a review. *Agronomie* 21, 4–5.
- Rowe, E.C., Healey, J.R., Edwards-Jones, G., Hills, J., Howells, M., Jones, D.L.,

2006. Fertilizer application during primary succession changes the structure of plant and herbivore communities. *Biological Conservation* 131, 510–522.
- Schooley, K., 2008. Introduction to Saskatoons [WWW Document]. URL https://web.archive.org/web/20081212072602/http://www.omafra.gov.on.ca/english/crops/facts/info_introduction_saskatoons.htm.
- Schramm, L.L., Smith, R.G., 1989. Some parametric studies of oil sand conditioning in the hot water flotation process. *AOSTRA Journal of Research* 5, 87–107.
- Sestak, Z., Catský, J., Jarvis, P.G., 1971. Plant photosynthetic production. Manual of methods. Dr. W. Junk Publishers, The Hague, Netherlands.
- Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F., 2011. Phosphorus dynamics: from soil to plant. *Plant Physiology* 156, 997–1005.
- Shepherd, W.D., 1986. Silviculture of Aspen Forests in the Rocky Mountains and in the Southwest. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.
- Siemens, J.A., Zwiazek, J.J., 2011. *Hebeloma crustuliniforme* modifies root hydraulic responses of trembling aspen (*Populus tremuloides*) seedlings to changes in external pH. *Plant and Soil* 345, 247–256.
- Slaton, N.A., Norman, R.J., Gilmour, J.T., 2001. Oxidation rates of commercial elemental sulfur products applied to an alkaline silt loam from Arkansas. *Soil Science Society of America Journal* 65, 239–243.
- Soaud, A.A., Al Darwish, F.H., Saleh, M.E., El-Tarabily, K.A., Sofian-Azirun, M., Rahman, M.M., 2011. Effects of elemental sulfur, phosphorus, micronutrients and ‘*Paracoccus versutus*’ on nutrient availability of calcareous soils. *Australian Journal of Crop Science* 5, 554.
- Spiers, J.M., Braswell, J.H., 1992. Soil-applied sulfur affects elemental leaf content and growth of ‘*Tifblue*’ rabbiteye blueberry. *Journal of the American Society for Horticultural Science* 117, 230–233.
- Starast, M., Karp, K., Vool, E., 2007. Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry. *Agricultural and Food*

- Science 16, 34–45.
- Steudle, E., Peterson, C.A., 1998. How does water get through roots? *Journal of Experimental Botany* 49, 775–788.
- Stiell, W.M., 1976. White spruce: artificial regeneration in Canada. Canadian Forestry Service, Department of the Environment, Canada.
- Stone, E.L., 1968. Microelement nutrition of forest trees: a review. *Forest Fertilization, Theory and Practice*, 132–175.
- Sun, L., Duan, D., Peng, C., He, J., Shi, J., 2014. Influence of sulfur on the speciation transformation and phyto-availability of heavy metals in soil: A review. *Chinese Journal of Applied Ecology* 25, 2141–2148.
- Sutton, R.F., 1969. Silvics of white spruce (*Picea glauca* (Moench) Voss). *Canadian Forestry Services* 1250, 57. Ottawa, ON, Canada.
- Svistoonoff, S., Creff, A., Reymond, M., Sigoillot-Claude, C., Ricaud, L., Blanchet, A., Nussaume, L., Desnos, T., 2007. Root tip contact with low-phosphate media reprograms plant root architecture. *Nature Genetics* 39, 792.
- Sydnor, M.E., Redente, E.F., 2002. Reclamation of high-elevation, acidic mine waste with organic amendments and topsoil. *Journal of Environmental Quality* 31, 1528–1537.
- Taiz, L., Zeiger, E., 2002. *Plant Physiology*, 3rd Edition. Sinauer, Sunderland, UK.
- Takahashi, H., Kawahara, A., Inoue, Y., 2003. Ethylene promotes the induction by auxin of the cortical microtubule randomization required for low-pH-induced root hair initiation in lettuce (*Lactuca sativa* L.) seedlings. *Plant and Cell Physiology* 44, 932–940.
- Tang, C., Cogley, B.T., Mokhtara, S., Wilson, C.E., Greenway, H., 1993. High pH in the nutrient solution impairs water uptake in *Lupinus angustifolius* L. *Plant Nutrition—from Genetic Engineering to Field Practice* 763–765. Springer, Dordrecht, Netherlands.
- Tang, C., Longnecker, N.E., Thomson, C.J., Greenway, H., Robson, A.D., 1992. Lupin (*Lupinus angustifolius* L.) and pea (*Pisum sativum* L.) roots differ in

- their sensitivity to pH above 6.0. *Journal of Plant Physiology* 140, 715–719.
- Tang, C., Turner, N.C., 1999. The influence of alkalinity and water stress on the stomatal conductance, photosynthetic rate and growth of *Lupinus angustifolius* L. and *Lupinus pilosus* Murr. *Australian Journal of Experimental Agriculture* 39, 457–464.
- Tang, C., Zheng, S.J., Qiao, Y.F., Wang, G.H., Han, X.Z., 2006. Interactions between high pH and iron supply on nodulation and iron nutrition of *Lupinus albus* L. genotypes differing in sensitivity to iron deficiency. *Plant and Soil* 279, 153–162.
- Tang, K., Zhu, W., Zhou, W., Yi, Z., Tu, N., 2013. Research progress on effects of soil pH on plant growth and development. *Crop Research* 27, 207–212.
- Tichý, R., Fajtl, J., Kužel, S., Kolář, L., 1996. Use of elemental sulphur to enhance a cadmium solubilization and its vegetative removal from contaminated soil. *Nutrient Cycling in Agroecosystems* 46, 249–255.
- Törnroth-Horsefield, S., Wang, Y., Hedfalk, K., Johanson, U., Karlsson, M., Tajkhorshid, E., Neutze, R., Kjellbom, P., 2006. Structural mechanism of plant aquaporin gating. *Nature* 439, 688–694.
- Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., Bligny, R., Maurel, C., 2003. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature* 425, 393–397.
- TSCEMA, 2009. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. Second edition - Open Government [WWW Document]. URL <https://open.alberta.ca/publications/9780778588252>.
- Tuttle, S., Powter, C.B., 1991. An overview of land reclamation at Suncor. *Land Reclamation of Oil Sands and Heavy Oil Developments*, 1–23.
- Valentine, D.W., Kielland, K., Chapin III, F.S., McGuire, A.D., Van Cleve, K., 2006. Patterns of biogeochemistry in Alaskan boreal forests. *Alaska's Changing Boreal Forest* 241–266. Oxford University Press, New York, USA.
- Vander, W. C., Verdoucq, L., Boursiac, Y. and Maurel, C. 2004. Aquaporins in plants.

- In: Blatt MR, ed. Membrane transport in plants. Annual Plant Reviews 8, Sheffield: Blackwell Publishing 221–250.
- Viani, R.A., Rodrigues, R.R., Dawson, T.E., Lambers, H., Oliveira, R.S., 2014. Soil pH accounts for differences in species distribution and leaf nutrient concentrations of Brazilian woodland savannah and seasonally dry forest species. *Perspectives in Plant Ecology, Evolution and Systematics* 16, 64–74.
- Voicu, M.C., Zwiazek, J.J., 2004. Cycloheximide inhibits root water flow and stomatal conductance in aspen (*Populus tremuloides*) seedlings. *Plant, Cell & Environment* 27, 199–208.
- Walker, L.R., del Moral, R., 2009. Lessons from primary succession for restoration of severely damaged habitats. *Applied Vegetation Science* 12, 55–67.
- Wang G.M., Stribley D., Tinker P., 1985. Soil pH and vesicular-arbuscular mycorrhizas. *Special Publications Series of the British Ecological Society* 4, 219–224.
- Wang, L., 2014. On the current research situation of *Amelanchier alnifolia*. *Journal of Agriculture* 4, 82–84.
- Wang, S., Hu, C., Cheng, D., Liao, W., Li, N., Chen, L., Fang, J., 2011. Effects of soil pH regulation on indigenous microflora and physiological groups in tea plantations. *Soils* 43, 76–80.
- Wang, W., Lu, A., Hou, D., Cao, Y., 2012. Cultivation technique of white spruce seedling. *Journal of Liaoning Forestry Science and Technology*, 60–62.
- Ward, J.T., Lahner, B., Yakubova, E., Salt, D.E., Raghothama, K.G., 2008. The effect of iron on the primary root elongation of *Arabidopsis* during phosphate deficiency. *Plant Physiology* 147, 1181–1191.
- Whitney, S., 1985. *Western forests*. New York: Knopf: Distributed by Random House.
- Wildflower Center, 2013. *Amelanchier alnifolia* (Saskatoon serviceberry serviceberry). *Native Plants of North America*. URL https://www.wildflower.org/plants/result.php?id_plant=AMAL2.
- Wildflower Center, 2017. *Corylus cornuta* (Beaked hazelnut). *Native Plants of North*

- America. URL
https://www.wildflower.org/plants/result.php?id_plant=COCO6.
- Wu, X., Chen, M.C., Yang, Z.P., 2007. Effects of sulfur application on the growth of cole, soil pH and available P in alkaline soil. *Plant Nutrition and Fertilizer Science* 13, 671–677.
- Xing, Z., Guo, J., Yan, Z., 1997. Behaviors of coated-urea in calcareous soil. *Journal of Plant Nutrition and Fertilizers* 1, 16–23.
- Xu, F., Tan, X., Zhang, W. Q., Zwiazek, J. J., 2019. Effects of iron and root zone pH on growth and physiological responses of paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*) and red-osier dogwood (*Cornus stolonifera*) seedlings in a split-root hydroponic system. *Acta Physiologiae Plantarum* 41, 142.
- Xu, F., Vaziriyeganeh, M., Zwiazek, J.J., 2020. Effects of pH and mineral nutrition on growth and physiological responses of trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and white spruce (*Picea glauca*) seedlings in sand culture. *Plants* 9, 682.
- Yan, E.-R., Hu, Y.-L., Salifu, F., Tan, X., Chen, Z.C., Chang, S.X., 2012. Effectiveness of soil N availability indices in predicting site productivity in the oil sands region of Alberta. *Plant and Soil* 359, 215–231.
- Yang, X., Römheld, V., Marschner, H., 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). *Plant and Soil* 164, 1–7.
- Yousfi, S., Mahmoudi, H., Abdelly, C., Gharsalli, M., 2007. Effect of salt on physiological responses of barley to iron deficiency. *Plant Physiology and Biochemistry* 45, 309–314.
- Zasada, J., 1986. Natural regeneration of trees and tall shrubs on forest sites in interior Alaska. *Forest Ecosystems in the Alaskan Taiga* 44–73. Springer, New York, USA.
- Zasada, J.C., Phipps, H.M., 1990. *Populus balsamifera* L. Balsam poplar. *Silvics of*

- North America 2, 518–529.
- Zhang, C., Zhang, M., Zeng, Y., 2007. Effects of sulphur on chemical properties of calcareous soil. *Chinese Journal of Applied Ecology* 7, 1453–1458.
- Zhang, W., Calvo-Polanco, M., Chen, Z.C., Zwiazek, J.J., 2013. Growth and physiological responses of trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*) and tamarack (*Larix laricina*) seedlings to root zone pH. *Plant and Soil* 373, 775–786.
- Zhang, W., Fleurial, K., Sherr, I., Vassov, R., Zwiazek, J.J., 2020. Growth and physiological responses of tree seedlings to oil sands non-segregated tailings. *Environmental Pollution* 259, 113945.
- Zhang, W., Xu, F., Zwiazek, J.J., 2015. Responses of jack pine (*Pinus banksiana*) seedlings to root zone pH and calcium. *Environmental and Experimental Botany* 111, 32–41.
- Zhang, W., Zwiazek, J.J., 2016. Effects of root medium pH on root water transport and apoplastic pH in red-osier dogwood (*Cornus sericea*) and paper birch (*Betula papyrifera*) seedlings. *Plant Biology* 18, 1001-1007.
- Zhang, W., Zwiazek, J.J., 2016. Responses of reclamation plants to high root zone pH: effects of phosphorus and calcium availability. *Journal of Environmental Quality* 45, 1652–1662.
- Zhao, F.J., Wood, A.P., McGrath, S.P., 1999. Effects of sulphur nutrition on growth and nitrogen fixation of pea (*Pisum sativum* L.). *Plant and Soil* 212, 207–217.
- Zhao, X., Li, Y., Mai, W., Tian, X., Lai, H., Lv, J., 2008. A preliminary study on amendment of saline soil through addition of sulphur. *Agricultural Research in the Arid Areas* 4, 74–78.
- Zhou, W., Lin, B., 1997. The progress in study on the behavior of sulphur in soil and plants. *Soils and Fertilizers* 5, 8–11.
- Zieslin, N., Snir, P., 1989. Responses of rose plants cultivar ‘Sonia’ and *Rosa indica major* to changes in pH and aeration of the root environment in hydroponic culture. *Scientia Horticulturae* 37, 339–349.