Effects of Enhanced Non-segregated Tailings, Root Hypoxia, and Salinity on the Growth of Commonly Used Oil Sands Reclamation Plant Species and Halophytes

by

Yexin Han

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Department of Renewable Resources
University of Alberta

Abstract

In Alberta, Canada, oil sands mining activities have disturbed many boreal forest habitats and produced large amounts of tailings. Recently, a novel oil sands tailings management technology of the enhanced non-segregated tailings (eNST) has been developed, which uses polymers to accelerate tailings consolidation and reduce their salinity levels. However, the effects of eNST on the growth of plants that are used for reclamation of oil sands have not been thoroughly studied. Additionally, some tailings reclamation areas may be prone to waterlogging since water released by the tailings may infiltrate the root zone and affect plants. In my thesis research, I carried out two studies to examine the effects of eNST on plants.

The first study examined the effects of eNST release water on the seedling growth and physiological responses of four tree species commonly used for the oil sands reclamation: paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*) under well-aerated and hypoxic conditions in hydroponics. The study showed that eNST water impaired the growth and physiological processes of all plants and that root hypoxia can aggravate the negative effects of eNST. After two months of treatments, paper birch and jack pine had significantly higher leaf Na concentrations compared with trembling aspen and black spruce. Moreover, jack pine and black spruce had higher leaf Na/K ratios compared with paper birch and trembling aspen. The eNST water significantly reduced chlorophyll concentrations, net photosynthesis, and transpiration rates in paper birch and trembling aspen, but not in jack pine and black spruce. Root hypoxia aggravated the negative effects of eNST water on paper birch, resulting in higher leaf Na/K, Na/Ca ratios, lower net photosynthesis, and impaired growth. Root hypoxia had a stronger inhibitory effect on the net photosynthesis rates in jack pine and black spruce compared with the eNST water. In summary, the first experimental results demonstrated that eNST water significantly reduced the growth and physiological parameters in paper birch and trembling aspen. The root hypoxia aggravated the negative effects of eNST water on the growth parameters and chlorophyll concentrations of paper birch, jack pine, and black spruce.

The second study examined the role of salinity in the phytotoxicity of eNST water. In this study, a halophytic grass (*Puccinellia nuttalliana*), a relatively salt-tolerant grass (*Poa juncifolia*), and a relatively salt-sensitive grass (*Poa pratensis*) were subjected in hydroponics to treatments with eNST supplemented with 0, 60, and 120 mM NaCl The results demonstrated that the growth and physiological processes in *Puccinellia nuttalliana* were unaffected by the 100% eNST water regardless of NaCl concentrations. The 50% eNST water did not affect the growth and physiological processes of *Poa juncifolia*. In 100% concentration of eNST water and in the treatments with supplemented NaCl, the growth of *Poa juncifolia* was significantly reduced. The growth of *Poa pratensis* was inhibited in all treatment groups. The study pointed to salinity as the major factor contributing to the phytotoxicity of eNST water. In summary, *Puccinellia nuttalliana* is more tolerant to eNST water than *Poa juncifolia* and *Poa pratensis*. The high eNST water tolerance of *Puccinellia nuttalliana* can be likely partly attributed to its salt exclusion ability. *Poa pratensis* was the most sensitive of the three studied grass species to all applied treatments. The addition of NaCl to 50% eNST water aggravated the negative effects of the eNST water on *Poa pratensis*.

Preface

This thesis is an original work by Yexin Han. No part of this work has been published previously.

“A journey of a thousand miles begins with a single step”

- Lao Tzu

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List of Abbreviations

ANOVA	analysis of variance
Chl	chlorophyll
CNRL	Canadian Natural Resources Ltd.
CT	composite tailing
DCM	Dichloromethane
DMSO	dimethyl sulfoxide
DO	dissolved oxygen
DW	dry weight
E	transpiration rate
EC	electrical conductivity
FMM	forest mineral soil mix
ICP-OES	inductively coupled plasma-optical emission spectroscopy
Kr	root hydraulic conductance
LFH	upland surface soil
Lpr	root hydraulic conductivity
Pn	net photosynthesis rate
PPFD	photosynthetic photon flux density
PVDF	polyvinyl difluoride
RELR	relative electrolyte leakage ratio
ROS	reactive oxygen species
RSHG	relative shoot height growth
RSDG	relative stem diameter growth

Chapter 1 Introduction and literature review

1.1 Introduction

Oil sand is a mixture of sand, clay, water, bitumen, and other minerals. It can be processed and used by refineries to produce a variety of hydrocarbon products including gasoline and diesel fuels. The province of Alberta in Canada has the second-largest bitumen deposit in the world (Dhar et al., 2018). According to the Alberta Energy Regulator's Outlook in 2018, the development of oil sands is predicted to increase from 2.8 million barrels per day in 2017 to 3.9 million barrels per day in 2027 (Alberta, 2023). Despite creating huge economic value for the local economy, the oil sands mining industry disturbed large areas of the boreal forest in northern Alberta, including the Athabasca, Cold Lake, and Peace River regions. Additionally, the oil sands mining and the bitumen extraction process lead to the accumulation of tailings and overburden materials (Fung and Macyk, 2000). The Alberta provincial government requires oil sands mining companies to restore all disturbed land to the natural equivalent land capability (Fung and Macyk, 2000, Alberta, 2022). In the reclamation process, tailings and overburden materials are placed at the bottom of mining areas with the peat soil mixture and forest mineral mix covering them to support the growth of plants (Fung and Macyk, 2000, Naeth et al., 2013, Dhar et al., 2018) Tailings must form trafficable surfaces before being used as a substrate for land reclamation (Fung and Macyk, 2000). However, the consolidation process of tailings may exceed one century (Mikula et al., 1996, Fung and Macyk, 2000). Therefore, Canadian Natural Resources Limited (CNRL) adds polymer flocculants (anionic polyacrylamides) to tailings to produce the enhanced non-segregated tailing (eNST), which can capture 80% or more of the fine particles in tailings and accelerate the consolidation process (Vedoy and Soares, 2015, Cnrl, 2017).

Tailings can continuously release water during the storage and reclamation processes, which is referred to as the tailings water (Fung and Macyk, 2000). The oil sands tailings are characterized by high pH ($pH > 8$) and elevated salinity (electrical conductivity > 4 mS cm^{-1}) due to the chemicals added in the extraction process and the salt contents of the oil sands ore. This is aggravated by recirculation of water that is used for bitumen extraction (Fung and Macyk, 2000, Redfield et al., 2003, Redfield et al., 2004, Macdonald et al., 2015). When the roots of plants in reclaimed areas reach the tailings, high pH and salinity of tailings can inhibit plant growth (Zhang et al., 2020). Besides, tailings water may infiltrate into the root zone and affect plant growth (Redfield et al., 2003, Fleurial et al., 2022). Furthermore, tailings have low porosity and water permeability, which may lead to waterlogging and create root hypoxia around the root zone (Redfield et al., 2003, Redfield et al., 2004, Fleurial et al., 2022).

High soil pH reduces the availability of mineral nutrients in the soil, including Fe, Zn, Mn, and P (Marschner, 2011). Deficiencies of these essential elements can inhibit plant growth by reducing photosystem efficiency (Carstensen et al., 2018), chlorophyll synthesis (Marschner, 2011, Farhat et al., 2016), enzyme activities (Farhat et al., 2016), and photosynthetic processes (Marschner, 2011). High pH of the growth medium can disrupt H^+ gradient across the root plasma membranes, which plays an essential role in nutrient uptake (Rengel, 2002). Furthermore, the high soil pH ($pH > 8$) also directly inhibits cell growth as the optimum pH for the activity of cell wall expansion protein (expansin) is from 3 to 4.5, which is essential for cell wall enlargement and cell growth (Rengel, 2002).

The high salinity of tailings also affect the growth and survival of plants (Redfield et al., 2003, Redfield et al., 2004). High electrical conductivity due to accumulated salts can induce osmotic stress around plant roots and affect plant water relations sand nutrient uptake (Jouyban, 2012b, Sheldon et al., 2017, Arif et al., 2020). Osmotic stress also induces the generation of abscisic acid, which can trigger stomatal closure hence reduces the availability of CO_2 for photosynthesis (Jouyban, 2012b, Upadhyaya et al., 2013b). Also, the high Na concentration in tailings can inhibit plant K uptake and affects the water relations, cell expansion, and enzyme activity (Çalişkan and Çalişkan, 2019, Rawat et al., 2022). Other effects of high Na concentrations include a displacement of Ca in the cell membranes, leading to an increase in membrane permeability, and an accumulation of reactive oxygen species (ROS) (Shahid et al., 2020, Tomar et al., 2021). Root hypoxia reduces the oxygen availability for mitochondrial respiration, resulting in limited ATP production (Parent et al., 2008b). Energy deficiency affects the activity of the Rubisco enzyme and plasma membrane H^+ ATPase, resulting in lower net photosynthesis rate and nutrient uptake (Steffens et al., 2005, Parent et al., 2008b). The oxygen deficiency also disrupts electron transport in photosystems, inhibiting the photosynthetic process (Parent et al., 2008b). Furthermore, root hypoxia affects plant water uptake and transpiration by inducing stomatal closure, cytosol acidosis, and reducing root aquaporin activity (Tournaire-Roux et al., 2003b, Parent et al., 2008b, Tan et al., 2018).

Some studies demonstrated that polymer flocculants can affect plant growth (Düzyol, Kuboi and Fujii, 1985b, Wallace et al., 1986). Cationic flocculants reduced the germination rate of Chinese cabbage (Düzyol). Additionally, cationic flocculants induced chlorosis and growth reduction in hydroponically-grown plants (Kuboi and Fujii, 1985b). The anionic polymers can reduce the accumulations of P, Si, Mn, and B in plants (Wallace et al., 1986). Furthermore, polyacrylamide can degrade to acrylamide monomer, which is a known neurotoxin and a potential carcinogen (Xiong et al., 2018).

Most terrestrial plants suffer from growth reductions even under low salinity conditions (Sarath et al., 2021a). Relatively a few plant species can tolerate, grow, and complete their life cycle in environments with high salt concentrations. These plants are referred to as halophytes, and account for only one percent of the world's flora (Mishra and Tanna, 2017a, Sarath et al., 2021a). All plant species have evolved some ability to exclude, eliminate or sequester salt (Munns and Tester, 2008). Compared with other plant species, halophytes can maintain this ability under higher salinity levels (Munns and Tester, 2008, Mishra and Tanna, 2017a). In some halophytes, the presence of Na can even enhance their growth (Sarath et al., 2021a). Using halophytes to reclaim salt-affected soils is considered low-cost and sustainable (Sarath et al., 2021a). Therefore, halophytes have a high potential to improve the oil sands tailings reclamation in Alberta.

This thesis research examined under the controlled-environment conditions the effects of eNST water, root hypoxia, and salinity on the growth and physiological processes of some boreal forest plant species native to northeastern Alberta. The first study examined the effects of eNST water and root hypoxia on seedlings of paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*). The second study examined the effects of eNST water and salinity on three northern grasses varying in salt tolerance: *Puccinellia nuttalliana*, *Poa juncifolia*, and *Poa pratensis*.

The objectives of the thesis studies were to:

1) Examine the effects of aerated eNST and hypoxic eNST water on four common boreal reclamation tree species.
2) Examine the effects of eNST water on halophyte species.
3) Identify the main factors in eNST water that are detrimental to reclamation plants.

1.2 Literature review

1.2.1 Oil sands mining and reclamation

Oil sands are a type of a sedimentary rock, composed of sand, water, and bitumen (Geology, 2023). The bitumen can be recovered from the oil sands and processed to produce crude oil, which can be farther refined into various fuels, such as gasoline and diesel (Producers, 2023). Athabasca, Cold Lake, and Peace River regions in Alberta, Canada have the largest amount of oil sands in the world accounting for about 14% of the world's oil resources (Geology, 2023). Depending on the depth of the oil sands, oil sands companies recover bitumen by surface mining or in situ recovery (Dhar et al., 2018, Regulator, 2023).

About 20% of the oil sands are buried within 70 meters from the surface, and can be easily recovered by surface mining (Dhar et al., 2018, Producers, 2023). Before surface mining, the vegetative cover, upland surface soil, peat mineral mix, and overburden layer above the oil sands are stripped and stored separately as the substrates for future land reclamation (Fung and Macyk, 2000, Mackenzie, 2012, Dhar et al., 2018). Then, the excavation equipment is used to dig out oil sands ores from the land. Finally, the ores are sent to extraction plants to separate the bitumen from oil sands (Fung and Macyk, 2000). The remaining 80% of oil sands are buried too deep (deeper than 75 m) to acquire by surface mining (Dhar et al., 2018). Therefore, in-situ technology is used to remove the bitumen from the oil sands deposit (Dhar et al., 2018, Producers, 2023). In this method, the bitumen is heated to make it flowable, and is pumped to the ground (Producers, 2023).

In surface mining, the bitumen is extracted from oil sands by the mixture of hot water and sodium hydroxide (Fung and Macyk, 2000, Giesy et al., 2010). After recovering the bitumen from the oil sands, the remaining materials are tailings, consisting of sand, silt, water, clay, residual bitumen, dissolved metals, and naphthenic acids (Fung and Macyk, 2000, Giesy et al., 2010, Vedoy and Soares, 2015). The tailings are stored in tailing ponds for long-term storage (Fung and Macyk, 2000, Vedoy and Soares, 2015). In tailing ponds, tailings separate into coarse tailings and fine tailings (Fung and Macyk, 2000, Vedoy and Soares, 2015). Coarse tailings can quickly consolidate and settle to the pond bottom, while fine tailings can maintain in suspension for a long period of time (about one century) (Fung and Macyk, 2000). During that time, fine tailings continuously release entrapped water, which is referred to as the tailings water or tailings release water (Vedoy and Soares, 2015). Before being reclaimed, tailings need to form a trafficable surface (Fung and Macyk, 2000). To accelerate the tailings consolidation and reclamation processes, Canadian Natural Resources Limited (CNRL) developed the non-segregated tailings (NST) technique, which uses thickener underflow and cyclone underflow and injects carbon dioxide into tailings to increase the fine particle capture (Zhang et al., 2020). In 2017, CNRL developed enhanced non-segregated tailings (eNST) based on NST (Cnrl, 2017). In eNST, flocculants (anionic polyacrylamide) are added to tailings to clump solid particles together (Vedoy and Soares, 2015, Cnrl, 2017). After that, large metal plates lined with filter materials (geotextile) mechanically press tailings into clay fine cakes, which are dense and strong enough to be shipped to reclamation areas. The filter materials can filter, dewater, and consolidate fines (Cnrl, 2017).

Until 2013, about 895 km^2 of the land surface was disturbed by oil sands mining activities (Alberta, 2013). The total volume of tailings in tailing ponds was around 976 million m^3 by 2013, and is expected to increase to two billion m^3 in 2034 (Vedoy and Soares, 2015, Alberta, 2023). More than 31,500 ha of boreal forest were estimated in 2010 to be affected by the oil sands mining activities in Alberta (Mackenzie and Naeth, 2010). This area is likely much larger now. Therefore, local regulations require oil sands companies to reclaim the disturbed land and oil sands tailings to a natural equivalent capability, which means that the land should be returned to the original state to support the land uses and activities, but the individual land uses are not necessary to be identical to the original land uses (Fung and Macyk, 2000, Alberta, 2010, MacKenzie and Naeth, 2010, Dhar et al., 2018, Alberta, 2022). For land reclamation, the overburden materials and oil sands tailings are placed as a substrate at the reclamation sites on top of the mining pits. The peat, peat mineral mix, forest floor mineral mix, and upland surface soil (LFH mineral soil) are covered on the substrate to support the growth of reclamation plants (Fung and Macyk, 2000, McMillan et al., 2007, MacKenzie, 2012, Howell, 2015). Land reclamation is considered successful when the reclaimed land meets the revegetation success indicator, including plant community composition, ecosystem net primary productivity, and soil salinity (Alberta, 2010).

Bitumen extraction requires the addition of sodium hydroxide (NaOH), which significantly increases the pH (8 to 10) and salinity of tailings and tailings water (4.6-5.5 mS cm^{-1}) (Fung and Macyk, 2000). Moreover, tailings have low porosity and water permeability properties, which can induce the accumulation of tailings water and precipitation, resulting in waterlogging (Howat, 2000, Redfield et al., 2003). Therefore, when plant roots are in contact with tailings, they may suffer from hypoxia (Redfield et al., 2003, Fleurial et al., 2022). High pH, high salinity, and root hypoxia can threaten the survival of reclamation plants (Zhang et al., 2020, Fleurial et al., 2022).

1.2.2 Biology of studied plant species

1.2.2.1 Paper birch (*Betula papyrifera*)

Paper birch, which is native to northern North America, is a medium-sized deciduous tree. It reaches up to 30 meters high and 60 cm in diameter with 4-9 cm toothed diamond-shaped leaves (Burns and Honkala, 1990). In northern and central Alberta, paper birch is widely spread in the mountains, boreal forests, and is occasionally found in the parkland (Burns and Honkala, 1990). Paper birch has a high disturbance tolerance and can tolerate waterlogged or dry soils (Limited, 1989). Its root system is large in proportion to the aboveground mass of the tree and has a high soil penetrating ability (Limited, 1989). However, it shows low tolerance to shade and oil spills (Limited, 1989). Paper birch can also tolerate acidic soils with heavy metals such as Ni, Cu, and Al (Limited, 1989). Its growth requires low levels of P and Ca and moderate levels of S and N (Limited, 1989).

Paper birch is considered to be an aggressive pioneer species, which can colonize disturbed land and is one of the main tree species of the boreal mixedwood forest (Feis, 2023a). In the mixedwood forest, paper birch is always co-dominant with black spruce (*Picea mariana*), white spruce (*Picea glauca*), jack pine (*Pinus banksiana*), trembling aspen (*Populus tremuloides*), and balsam fir (*Abies balsamifera*) (Limited, 1989, Feis, 2023a). Paper birch also provides an important source of food for wildlife, including moose, hare, birds, and small mammals (Feis, 2023a).

1.2.2.2 Trembling aspen (*Populus tremuloides*)

Trembling aspen is the most widely distributed and abundant tree species in North America, including Alberta. It is a tall and fast-growing tree species with 15-18 meters in height. Usually up to 25 centimeters in diameter (Arno and Hammerly, 2020). The wood of trembling aspen is widely used to produce particleboard, like waferboard and strandboard (Feis, 2023f). Trembling aspen can adapt to a wide variety of soil types, such as rocky soil, heavy clay, well-drained soil, and poorly-drained soil. However, it grows best in well-drained, moist, and loamy soil with rich nutrient content (Limited, 1989, Arno and Hammerly, 2020, Feis, 2023f).

Trembling aspen is also a pioneer species, and is found as the first tree species to colonize land disturbed by fire, mining, and heavy metals (Limited, 1989). The roots of trembling aspen trees can widely spread over a long distance around a single stand. In the growth process, the roots generate root suckers to expand wider into the disturbed land (Limited, 1989, Feis, 2023f). The root suckers can not only produce more trees, but also improve the soil conditions. Therefore, trembling aspen is a good nursery tree species on disturbed land to promote the growth of other tree species, resulting in mixedwood forests (Limited, 1989). On the other hand, trembling aspen is also considered to be sensitive to drought and shade, and prefers a high light intensity environment (Limited, 1989).

1.2.2.3 Jack pine (*Pinus banksiana*)

Jack pine is a widely spread evergreen native tree species in Canada. It is a small to medium size coniferous tree species, whose height and diameter range from 10 to 20 meters and 64 centimeters in stem diameter depending on the growth conditions. Its needles are mostly 2-5 centimeters long (Limited, 1989, Feis, 2023c). The wood of jack pine is an important raw material for pulp and the construction of telephone poles and rail ties. Jack pine also provides food and habitats for birds, bears, and some small mammals (Limited, 1989, Feis, 2023c).

Jack pine trees grow in various types of soils, including rocky and sandy soils, however, they prefer well-drained and sandy soils (Limited, 1989). The trees can grow well in acidic and neutral soils (pH 3.5-6.5) and in most cases they cannot survive in a highly alkaline soils unless the roots are colonized with proper types of mycorrhizal fungi (Limited, 1989, Feis, 2023c). Jack pine has low nutrient requirements. It is always the first succession tree species following forest fire. Its root system is shallow and widely distributed, and the trees are highly sensitive to shading. The high light intensity is necessary for jack pine to grow well and on a large scale (Limited, 1989, Wendel and Smith, 1990, Feis, 2023c).

1.2.2.4 Black spruce (*Picea mariana*)

Black spruce is widely distributed in northern parts of North America, including northern and central Alberta. It is one of the most common coniferous evergreen tree species in Canadian boreal forests. The habitats of black spruce are mostly in cold environments with below 0°C annual mean temperature, while the moisture of habitats ranges from humid to dry sub-humid (Wendel and Smith, 1990). Across Canada, black spruce can grow on many types of soil. It grows best in well-drained brown to blackish peat, which always contains large amounts of decayed plant materials (Wendel and Smith, 1990). In Alberta, black spruce grows in peat bogs and swamps with pH 4 to 7. In some cases, it is also found in the calcareous bogs with pH 8 (Wendel and Smith, 1990. Feis, 2023b).

Black spruce is well adapted to the permafrost soil in northern Alberta, whose annual thaw depth zone is only 40 centimeters deep. The shallow root system of black spruce is suitable for these areas (Wendel and Smith, 1990, Feis, 2023b). The tree species associated with black spruce growing in mineral soil are mainly hardwood species, such as trembling aspen and paper birch. Black spruce tends to form a pure stands in the organic soil (Wendel and Smith, 1990, Feis, 2023b). Black spruce is more tolerant to shade than most of its associated tree species except for balsam fir (*Abies balsamea*) and northern white cedar (*Thuja occidentalis*). It is considered a pioneer species on fire-disturbed land, where communities of black spruce can establish quickly with enough seeds (Wendel and Smith, 1990, Feis, 2023b).

1.2.2.5 *Poa pratensis*

Poa pratensis (Kentucky bluegrass) is a cool-season perennial sod-forming grass, which is used as turf amenity grass and forage for livestock and wildlife (Feis, 2023d). *Poa pratensis* has a shallow root system within 8 cm of soil surface. Its stems are 30 to 90 cm tall. Leaves are flat blades, 2 to 5 mm wide, and 10 to 40 cm long (Wennerberg and Skinner, 2007). *Poa pratensis* has strong competitiveness compared to other herbaceous plants. It can not only reproduce by seeds, but also through rhizome expansion (Feis, 2023d). Its reproduction process contributes to its growth on a disturbed land (Feis, 2023d). Among cool-season grasses, *Poa pratensis* is considered relatively sensitive to salt stress (Vaziriyeganeh et al., 2018); however, an earlier study also showed that plants can exhibit significant individual differences in their salt tolerance (Feis, 2023d).

Poa pratensis is widespread in North America and grows on various sites with numerous vegetation types. *Poa pratensis* has the best growth conditions in full sunlight, cool, and humid environments. It can tolerate light shading if the precipitation and nutrients are sufficient (Feis, 2023d). *Poa pratensis* may be abundant in the lowlands and uplands of tallgrass prairie due to the high amount of annual precipitation (Wennerberg and Skinner, 2007, Feis, 2023d). It is a dominant species in the understory of aspen habitat in the intermountain region. *Poa pratensis* is also dominant in the ponderosa pine (*Pinus ponderosa*), sagebrush/bunchgrass, bunchgrass habitats, and riparian habits in the American west mountain areas, and midwestern prairies (Wennerberg and Skinner, 2007, Feis, 2023d).

Poa pratensis is considered not suitable for site restoration since its shallow root system does not make it a good soil stabilizer (Feis, 2023d). The optimal soil pH for *Poa pratensis* is between 5.8 and 8.2 (Feis, 2023d). It prefers well-drained loams or clay loams rich in humus and soils with limestone parent materials (Wennerberg and Skinner, 2007, Feis, 2023d). It has high soil fertility requirements such as a high amount of nitrogen during the active growth stage and is not considered drought tolerant. It needs two to three years to establish after its seeds are planted (Feis, 2023d).

1.2.2.6 *Poa juncifolia*

Poa juncifolia is a halophytic, pioneer, shallow-rooted cool-season perennial. It is one of the first species to colonize disturbed land. *Poa juncifolia* distributes from southeastern Alaska to southern Canada. It is also found in the west, great plain states, and the Great Lakes region in the US. *Poa juncifolia* has a small size with 30 to 70-cm tall stems Its leaf blade is rolled to less than two mm in width (Usda, 2023, Feis, 2023e). The seed production of *Poa juncifolia* cannot be carried out on a dry land with annual precipitation of less than 380 mm and in areas with a high probability of killing frost after mid-May (Usda, 2023).

Poa juncifolia can be found in saline and alkaline meadows, chaparral, foothill woodland, grasslands, sagebrush scrub, dry meadows, and sub-alpine areas up to 3,800 m in elevation (Feis, 2023e). It grows in rich clays, loams, harsh sands, serpentines, and scablands. It tends to grow in moist environments (400 to 500 mm in precipitation). It has moderate shading and excellent cold tolerance, however, the plants do not tolerate waterlogging (Usda, 2023). *Poa juncifolia* can tolerate heavy grazing and trampling due to its early maturity and dormancy in the summer and fall grazing periods (Usda, 2023, Feis, 2023e). *Poa juncifolia* is widely used for land reclamation and rehabilitation in coal mining and soil erosion areas (Feis, 2023e). In land reclamation areas, it is a minor component of the plant community. Therefore, the plant community management should focus on other key plant species (Usda, 2023).

1.2.2.7 *Puccinellia nuttalliana*

Puccinellia nuttalliana is a halophytic, perennial, and tufted bunchgrass. It produces 40 to 100-cm tall stems. Its leaf blade is in-rolled with leaves 1-2.5 mm wide and 1-3 mm long (E-Flora, 2023). *Puccinellia nuttalliana* is widespread across the western and central North America from

Wisconsin to British Columbia, and south to California and New Mexico. It is also found in Alaska (E-Flora, 2023).

Puccinellia nuttalliana is considered one of the most salt-tolerant grass species in North America (Tarasoff et al., 2007). It always occupies low-productivity land, such as arid, high-pH soils (higher than 8), saline, or alkaline meadows (Tarasoff et al., 2007). It also grows in ditches, and shorelines in the lowland, steppe, and montane zones (E-Flora, 2023). Records demonstrate that *Puccinellia nuttalliana* was found in saline depressions, saline lake margins, heavily salted roadsides, and ruderal areas (Tarasoff et al., 2007).

1.2.3 High soil pH

1.2.3.1 Introduction

High-pH soil covers about 25% of the Earth's surface (López-Bucio et al., 2000). High-pH soil can be classified as calcareous or alkaline soil (Gupta and Abrol, 1990). Most crop species can reach optimal growth at pH 5.5 to 6.5 (Islam et al., 1980). The calcareous soil usually has a pH higher than 7, while the pH value of the alkaline soil is higher than 8.5 (Gupta and Abrol, 1990, Marschner, 2011). Both alkaline and calcareous soil can be classified as alkali soil, which also refers to salt-affected soils (Gupta and Abrol, 1990). The soil pH is considered the master soil variable, as it can affect the biological, physical, and chemical properties of the soil (Neina, 2019). High soil pH can directly reduce plant root growth by increasing the OH^- concentration (Kopittke and Menzies, 2005). High soil pH also inhibits plant growth indirectly by affecting functions of different proteins (Rengel, 2002), nutrient availability (Läuchli and Grattan, 2012, Marschner, 2011), and soil microbial community (Rengel, 2002, Lopes et al., 2021).

1.2.3.2 High soil pH and nutrient availability

The high-pH soil environment reduces the availability of various soil nutrients, such as iron, phosphorus, and nitrogen, which are essential for the growth of plants (Marschner, 2011). Fe is involved in many crucial biochemical processes, such as photosynthesis, and plays key roles in maintaining the normal functions of proteins and enzymes (Jones Jr, 2012). In the high pH soil, Fe deficiency is induced by the low availability of Fe ions. Compared with mineral soils, the concentration of Fe^{2+}, Fe^{3+}, and other inorganic species in a high pH soil is significantly lower than in mineral soils (Boukhalfa and Crumbliss, 2002, Marschner, 2011). The concentration of soluble Fe and iron hydroxide are dramatically reduced with the increasing soil pH (Robin et al., 2008). The availability of Fe ions reaches its minimum amount when the pH is 8.5, which is almost the same as the pH of calcareous soils (Robin et al., 2008).

Zinc is also an essential microelement in plants. Zinc ensures a normal operation of protein folding and catalytic activity of enzymes (Broadley et al., 2007). Zinc deficiency is common in alkaline soils and in high calcareous carbonate content soil (Alloway, 2008). Besides, the availability of zinc is also low in high pH soil (pH > 7), especially when the soil contains free calcium carbonate (Alloway, 2008). When the soil pH is above 5.5, the increasing soil pH increases the zinc absorption by soil components (Moraghan and Mascagni Jr, 1991). Also, the self-diffusion coefficient of zinc is significantly inhibited in the calcareous soil compared with the acidic soil (Melton et al., 1973).

Soil pH can also affect the availability of manganese (Andresen et al., 2018), which is crucial for maintaining plant growth (Alejandro et al., 2020). Mn is the catalyst of many enzymes and plays an important role in transforming water into protons and oxygen in light photosynthetic reactions (Andresen et al., 2018). High-pH soil can reduce the availability of Mn^{2+}, which is the only form that plants can absorb, by causing the formation of Mn oxides, such as MnO_2 (Andresen et al., 2018). Calcareous soils can also inhibit the reaction between hydrous oxides of manganese and root exudate, reducing the release of Mn^{2+} in soil (Jauregui and Reisenauer, 1982).

Boron is not a metal micronutrient element. It is crucial for maintaining the cell wall structure in higher plants (O'neill et al., 2004). In calcareous soils, boron can be significantly absorbed by calcium carbonate (Goldberg and Forster, 1991). Besides, clay minerals can also absorb boron (Marschner, 2011). Their boron absorption amount increases from pH 6 and reaches the maximum at pH 9.5 (Goldberg and Forster, 1991).

Phosphorus is one of the most important mineral nutrients participating in various biological processes in plants. It plays a key role in maintaining the structures of nucleic acids and membranes (Raghothama, 2005, Sims and Sharpley, 2005). Phosphorus must be in a soluble form to be absorbed by plants. However, it readily reacts with Ca minerals, resulting in low solubility and low availability to plants (Penn and Camberato, 2019). The maximum solubility of P is near the neutral pH (pH 6.5), which has the lowest P fixation degree by Ca, Al, and Fe minerals (Penn and Camberato, 2019).

Nitrogen is a key essential macroelement that forms various biological molecules, such as proteins, enzymes, and nucleic acids (Lewis and Lewis, 1991). Since N can be absorbed by most plants as a cation or an anion, it can be available to plants under a relatively wide range of soil pH (Marschner, 2011).

1.2.3.3 Effects of high soil pH on plant root proteins

Plant cells are surrounded by cell walls, which restrict the protoplast volume and maintain cell shape (Cosgrove, 1996). To grow, plant cells must remodel the cell wall to adapt to the cell volume expansion (Cosgrove, 1996, Rengel, 2002). Earlier studies demonstrated that cell wall extensibility is the key factor regulating plant cell wall enlargement processes (Rengel, 2002). The cell wall extensibility depends on the activity of cell wall-loosening proteins, expansins (Cosgrove, 1996, Rengel, 2002, Cosgrove, 2005). The optimal pH for the activity of expansins is between 4.5 to 6 (Cosgrove, 2005). Cell wall acidification can be achieved by controlling the H^+ ATPase, which can release protons from cells to acidify apoplasts (Cosgrove, 2005). On the contrary, cell wall alkalization inhibits plant growth (Cosgrove, 2022). For example, plants that do not adapt to the alkaline stress have limited root activity and root cell H^+ influx in high soil pH environments ($pH > 8$) (Chen et al., 2022b). This plant growth mechanism is referred to as acid growth theory (Rengel, 2002). Moreover, waterlogging can increase the apoplastic pH by 1.5 units (Felle and Hanstein, 2002). Therefore, the high pH of oil sands tailings and tailings water may inhibit plant root growth by alkalizing the plant root apoplasts and inhibiting the function of expansins.

High soil pH also upsets plant water relations, partly by affecting the function of aquaporins. Aquaporins are channel proteins that are involved in the transmembrane water transport (Grondin et al., 2015). The high pH of the growth medium can reduce the leaf water potential and root hydraulic conductivity by inhibiting root growth and root aquaporin activity (Tang et al., 1993, Kamaluddin and Zwiazek, 2004). Moreover, plants can generate abscisic acid to regulate water relations under high soil pH conditions (Turner and Begg, 1981, Passioura, 2010). Abscisic acid may regulate stomatal conductance, root hydraulic conductivity, and root water flow by regulating the activity of aquaporins (Kamaluddin and Zwiazek, 2004). However, in the high pH medium (pH 8), abscisic acid may be ineffective in regulating plant water relations, likely because the optimum pH for abscisic acid to bind to channel proteins is close to 6 (Wilkinson, 1999, Kamaluddin and Zwiazek, 2004).

1.2.3.4 Salt stress and plant growth

Salt-affected soils are classified into saline, sodic, and saline-sodic. The formation of salt-affected soils is called soil salinization or sodification according to the soil composition (Sajal and Nasrin, 2020). The formation of salt-affected soils can be attributed to natural causes or human-induced causes. The human-induced causes include land deforestation, improper drainage, and overuse of fertilizers (Sajal and Nasrin, 2020).

Salt stress mainly inhibits plant growth through ion toxicity and osmotic stress (Jouyban, 2012a). Sodium chloride is the most common salt source (Zhu, 2007). Since Na and K have similar chemical natures, salt stress caused by the high Na concentration inhibits the root uptake of K ions (Jouyban, 2012a). The K deficiency results in plant growth inhibition, as potassium ions are essential for maintaining cell turgor, membrane potential, and enzyme activities (Murat et al., 2009, Jouyban, 2012a). Besides, sodium can displace the calcium ions from binding sites (Jouyban, 2012a, Shahid et al., 2020). The decrease of calcium ion concentration affects membrane integrity and permeability, which can also aggravate potassium leakage (Jouyban, 2012a, Shahid et al., 2020). Furthermore, the high soil salinity can inhibit the growth of plants and jeopardize the physiological parameters in salt-sensitive species (Andrews et al., 1998). High soil salinity can make plant roots develop higher tissue osmotic potential and cause osmotic stress (Murat et al., 2009), which inhibits plant water uptake and induces physiological drought. Moreover, salt stress induces the synthesis of abscisic acid, which can trigger stomatal closure, leading to the inhibition of leaf gas exchange and plant growth (Murat et al., 2009, Jouyban, 2012a, Upadhyaya et al., 2013a).

The presence of salt stress often accompanies alkali stress (Yang et al., 2008, Guo et al., 2017). The combination of alkali stress and salt stress showed greater inhibition effects on plant growth than the salt stress alone (Yang et al., 2008, Guo et al., 2017). The high pH around the plant root zone can result in plant nutrient deficiencies by causing precipitation of nutrient elements, such as Fe, Mn, Zn, and P (Marschner, 2011, Guo et al., 2017). Besides, the alkalized salt stress reduces the NO_3^- contents in plant roots and converts NH_4^+ to NH_3^-, which leads to N deficiency (Wang et al., 2012). Plants can exclude sodium ions from root cells through Na^+/H^+ antiporter (Zhu, 2007, Guo et al., 2017). The alkali stress might decrease the root apoplastic proton concentrations, which inhibits the activity of Na^+/H^+ antiporters and decreases plant salt tolerance (Zhu, 2007).

1.2.3.5 Root hypoxia and plant growth

Excessive precipitation and water infiltration cause waterlogging and create hypoxic conditions (oxygen deficiency) around the root zone (Ashraf, 2012). Oxygen deficiency limits mitochondrial respiration and electron transport through the respiratory pathway, resulting in low ATP production and inhibition of physiological processes (Parent et al., 2008a). One of the plant salt tolerance mechanisms depends on the activity of H^+ ATPase, which is an energy-demanding process (Malakar and Chattopadhyay, 2021). Therefore, oxygen deficiency may disrupt the activity of H^+ ATPase and reduce plant salt tolerance (Lopes et al., 2020), resulting in a leakage of protons to the cytoplasm and cytosol acidosis (Drew, 1997). Cytosol acidosis can inhibit the activity of water channel proteins (aquaporins) (Tournaire-Roux et al., 2003a), resulting in the reduction of root hydraulic conductivity (Parent et al., 2008a). Aquaporins play crucial roles in maintaining root water uptake and root hydraulic conductivity (Tournaire-Roux et al., 2003a, Parent et al., 2008a, Tan et al., 2021). Root hypoxia can also directly upset plant water relations by inducing stomatal closure, leading to reductions of stomatal conductance and transpiration rates (Bradford and Hsiao, 1982, Parent et al., 2008a, Bansal and Srivastava, 2015).

1.2.3.6 Polymers and plant growth

Polymer flocculants in oil sands tailing water may affect the growth of reclamation plants. A previous study showed that cationic flocculants may adhere to the negatively charged plant roots, resulting in root growth reductions (Kuboi and Fujii, 1985a). The cationic flocculants can also hold or absorb Cu and Fe from the culture medium. These metal elements can accumulate around plant roots with cationic flocculants and may reach toxic levels causing chlorosis and plant growth inhibition (Kuboi and Fujii, 1985a). The anionic polymers can induce decreases in P, Si, K, Ca, Mg, and Mn concentrations in plants, which may be attributed to the anionic properties of the polymers (Wallace et al., 1986). Polyacrylamides can degrade to acrylamide monomers, which are neurotoxins and a potential carcinogens (Wallace et al., 1986). However, acrylamide can be degraded in soil with only a one-day half-life (Xiong et al., 2018).

1.2.3.7 Halophytes and land reclamation

As opposed to glycophytes, halophytes can grow and complete their life cycle in high-salinity environments with 200 mM or higher NaCl concentrations (Flowers and Colmer, 2008, Sarath et al., 2021b). Halophytes are usually classified into obligate and facultative halophyte categories (Mishra and Tanna, 2017b, Sarath et al., 2021b). Obligate halophytes need salts to grow, while facultative halophytes can grow in the soil without salts (Mishra and Tanna, 2017b, Sarath et al., 2021b). Although halophytes can survive in high-salinity environments, their cytosolic enzymes are still sensitive to high sodium concentrations (Jouyban, 2012a). To overcome the high concentrations of sodium ions, halophytes can exclude sodium ions into vacuoles to prevent the salt concentration from reaching a toxic level in the cytoplasm (Mishra and Tanna, 2017b, Sarath et al., 2021b). Meanwhile, the sodium ions in vacuoles can help halophytes maintain water potential and balanced water relations (Ali and Yun, 2017). Although most plant species have this salt exclusion ability, halophytes can exclude sodium ions into vacuoles more effectively than other plant species (Barros et al., 2021).

Apart from salt exclusion, halophytes developed many morphological traits to tolerate salt stress. For example, they can regulate the stomatal length and density to control transpiration, reduce water loss, maintain water potential, and increase carbon dioxide uptake to maintain plant growth under salt stress (Chen et al., 2022a). Some halophytes can also increase the succulence of their leaves, resulting in larger mesophyll cells and vacuoles (Mishra and Tanna, 2017b, Sarath et al., 2021b). The increase in leaf succulence provides a thicker epidermis, which provides more space to maintain water and store sodium ions (Sarath et al., 2021b). Apart from stomatal regulation and leaf succulence, some halophytes have evolved special structures on leaf surfaces and stems to secret excessive salts, which may be either salt glands or bladders (Mishra and Tanna, 2017b, Sarath et al., 2021b). Halophytes can secrete salts through these structures and reduce sodium ion concentrations in photosynthetically active mesophyll cells (Kiani-Pouya et al., 2019, Sarath et al., 2021b, Chen et al., 2022a). As salt glands are larger than epidermal cells, they can accumulate large amounts of salt (Kiani-Pouya et al., 2019). Due to the high salt accumulation in vacuoles and salt glands of halophytes, some researchers suggested that removing the harvested halophytes from the original soil can desalinate the saline soil (Sarath et al., 2021b).

1.3 References

Alberta, G. O. 2010. Guidelines for reclamation to forest vegetation in the Athabasca Ooil Ssands Rregion. *Second edition* [Online]. Available: https://open.alberta.ca/publications/9780778588252 [Accessed 2022 September]. 43 p.

Alberta, G. O. 2013. Oil sands Mmine Rreclamation and Ddisturbance Ttracking by Yyear [Online]. Available: http://osip.alberta.ca/library/Dataset/Details/27 [Accessed 2023 February].

Alberta, G. O. 2023. Oil Sands Overview [Online]. Available: https://www.alberta.ca/oil-sands-overview.aspx [Accessed 2023 May].

Alberta, P. O. 2022. Environmental protection and enhancement act, conservation and reclamation regulation. 134 p.

Alejandro, S., Höller, S., Meier, B. & Peiter, E. 2020. Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science,* 11**,** 300. doi: 10.3389/fpls.2020.00300.

Ali, A. & Yun, D.-J. 2017. Salt stress tolerance; what do we learn from halophytes. *Journal of Plant Biology,* 60**,** 431-439.

Alloway, B. J. 2008. Micronutrient deficiencies in global crop production. *Springer Science & Business Media*. 230 p. doi: https://doi.org/10.1007/978-1-4020-6860-7.

Andresen, E., Peiter, E. & Küpper, H. 2018. Trace metal metabolism in plants. *Journal of Experimental Botany,* 69**,** 909-954.

Andrews, J. A., Johnson, J. E., Torbert, J. L., Burger, J. A. & Kelting, D. L. 1998. Minesoil and site properties associated with early height growth of eastern white pine. *Journal of Environmental Quality,* 27**,** 192-199.

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. & Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: an omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156**,** 64-77.

Arno, S. F. & Hammerly, R. P. 2020. Northwest trees: identifying and understanding the region's native trees. *Mountaineers Books.* 601 p.

Ashraf, M. 2012. Waterlogging stress in plants: a review. *African Journal of Agricultural Research,* 7 (13), 1976-1981.

Bansal, R. & Srivastava, J. P. 2015. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. *Russian Journal of Plant Physiology,* 62**,** 322-327.

Barros, N. L. F., Marques, D. N., Tadaiesky, L. B. A. & De Souza, C. R. B. 2021. Halophytes and other molecular strategies for the generation of salt-tolerant crops. *Plant Physiology and Biochemistry,* 162**,** 581-591.

Boukhalfa, H. & Crumbliss, A. L. 2002. Chemical aspects of siderophore mediated iron transport. *Biometals,* 15**,** 325-339.

Bradford, K. J. & Hsiao, T. C. 1982. Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiology,* 70**,** 1508-1513.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. & Lux, A. 2007. Zinc in plants. *New Phytologist,* 173**,** 677-702.

Burns, R. M. & Honkala, B. H. 1990. Silvics of North America. Volume 2: Hardwoods. USDA Forest Service. *Agriculture Handbook,* 654. 158 p.

Çalişkan, B. & Çalişkan, A. C. 2019. Potassium nutrition in plants and its interactions with other nutrients in hydroponic culture. *Improvement of Quality in Fruits and Vegetables Through Hydroponic Nutrient Management,* 9 p. doi: 10.5772/intechopen.71951.

Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M. & Husted, S. 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiology,* 177**,** 271-284.

Chen, G., Amoanimaa-Dede, H., Zeng, F., Deng, F., Xu, S. & Chen, Z.-H. 2022a. Chapter One - Stomatal regulation and adaptation to salinity in glycophytes and halophytes. *In:* Shabala, S. (ed.) *Advances in Botanical Research.* Academic Press, 103, 1-42.

Chen, L., Zhao, R., Yu, J., Gu, J., Li, Y., Chen, W. & Guo, W. 2022b. Functional analysis of plasma membrane H^+-ATPases in response to alkaline stress in blueberry. *Scientia Horticulturae,* 306**,** 111453.

Cnrl. 2017. 2017 stewardship report to stakeholders [Online]. Available: https://www.cnrl.com/sustainability/sustainability-reporting/stewardship-report-to-stakeholders/#none [Accessed 2021 July]. 19 p.

Cosgrove, D. J. 2022. Plant cell growth and cell wall enlargement. *Encyclopedia of Life Sciences.* 854 p. doi: https://doi.org/10.1002/9780470015902.a0029421.

Cosgrove, D. J. 1996. Plant cell enlargement and the action of expansins. *BioEssays,* 18**,** 533-540.

Cosgrove, D. J. 2005. Growth of the plant cell wall. *Nature Reviews. Molecular Cell Biology,* 6**,** 850-61.

Cramer, G. R., LäUchli, A. & Polito, V. S. 1985. Displacement of Ca^{2+} by Na^+ from the plasmalemma of root cells: a primary response to salt stress. *Plant Physiology,* 79**,** 207-211.

Dhar, A., Comeau, P. G., Karst, J., Pinno, B. D., Chang, S. X., Naeth, A. M., Vassov, R. & Bampfylde, C. 2018. Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. *Environmental Reviews,* 26**,** 286-298.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology,* 48**,** 223-250.

Düzyol, S. An evaluation of toxicity effect on flocculants usage in environmental aspects. 792 p.

E-Flora, E. a. O. T. F. O. B. C. 2023. *Puccinellia nuttalliana (Schult.) Hitchc. Nuttall's alkaligrass Poaceae (Grass family)* [Online]. Available: https://linnet.geog.ubc.ca/Atlas/Atlas.aspx?sciname=Puccinellia%20nuttalliana [Accessed 2023 May].

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C. & Rabhi, M. 2016. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum,* 38**,** 145-155. doi: 10.1007/s11738-016-2165-z.

Feis, F. E. I. S. 2023a. *Index of Species Information species: Betula papyrifera* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/betpap/all.html [Accessed 2021 July].

Feis, F. E. I. S. 2023b. Index of species information species: *Pinus banksiana*.

Feis, F. E. I. S. 2023c. Index of species information species*: Pinus banksiana* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/pinban/all.html [Accessed 2021 August].

Feis, F. E. I. S. 2023d. Index of species information species*: Poa pratensis* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/graminoid/poapra/all.html [Accessed 2022 August].

Feis, F. E. I. S. 2023e. Index of species information species*:* *Poa secunda* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/graminoid/poasec/all.html [Accessed 2022 August].

Feis, F. E. I. S. 2023f. Index of species information species*:* *Populus tremuloides* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/poptre/all.html [Accessed 2021 August].

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.

Fleurial, K. G., Zhang, W.-Q., Vassov, R. & Zwiazek, J. J. 2022. Root hypoxia aggravates the effects of saline tailings water on the growth and physiology of woody plants. *Plant and Soil***,** 1-18.

Flowers, T. J. & Colmer, T. D. 2008. Salinity tolerance in halophytes. *New Phytologist,* 179**,** 945-963.

Fung, M. Y. & Macyk, T. M. 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands,* 41**,** 755-774.

Geology. 2023. What are oil sands [Online]. Available: https://geology.com/articles/oil-sands/ [Accessed 2021 June].

Giesy, J. P., Anderson, J. C. & Wiseman, S. B. 2010. Alberta oil sands development. *Proceedings of the National Academy of Sciences,* 107**,** 951-952.

Goldberg, S. & Forster, H. 1991. Boron sorption on calcareous soils and reference calcites. *Soil Science,* 152**,** 304-310.

Grondin, A., Rodrigues, O., Verdoucq, L., Merlot, S., Leonhardt, N. & Maurel, C. 2015. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *The Plant Cell,* 27**,** 1945-1954.

Guo, R., Shi, L., Yan, C., Zhong, X., Gu, F., Liu, Q., Xia, X. & Li, H. 2017. Ionomic and metabolic responses to neutral salt or alkaline salt stresses in maize (*Zea mays L.*) seedlings. *BMC Plant Biology,* 17**,** 1-13.

Gupta, R. K. & Abrol, I. 1990. Salt-affected soils: their reclamation and management for crop production. *Advances in Soil Science: Soil Degradation Volume 11***,** 223-288.

Howat, D. 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Environmental Sciences Division Edmonton, AB, Canada. 8 p.

Howell, D. M. 2015. Influence of amendments and soil depth on available nutrients and microbial dynamics in contrasting topsoil materials used for oil sands reclamation. 8 p.

Islam, A., Edwards, D. & Asher, C. 1980. pH optima for crop growth: results of a flowing solution culture experiment with six species. *Plant and Soil,* 54**,** 339-357.

Jauregui, M. A. & Reisenauer, H. 1982. Dissolution of oxides of manganese and iron by root exudate components. *Soil Science Society of America Journal,* 46**,** 314-317.

Jones Jr, J. B. 2012. Plant nutrition and soil fertility manual. *CRC press*. 91 p.

Jouyban, Z. 2012a. The effects of salt stress on plant growth. *Tech J Engin & App Sci,* 2**,** 7-10.

Jouyban, Z. 2012b. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

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.

Kiani-Pouya, A., Rasouli, F., Bazihizina, N., Zhang, H., Hedrich, R. & Shabala, S. 2019. A large-scale screening of quinoa accessions reveals an important role of epidermal bladder cells and stomatal patterning in salinity tolerance. *Environmental and Experimental Botany,* 168**,** 103885.

Kopittke, P. M. & Menzies, N. W. 2005. Control of nutrient solutions for studies at high pH. *Plant and Soil,* 266**,** 343-354.

Kuboi, T. & Fujii, K. 1985a. Toxicity of cationic polymer flocculants to higher plants. *Soil Science and Plant Nutrition,* 31**,** 163-173.

Kuboi, T. & Fujii, K. 1985b. Toxicity of cationic polymer flocculants to higher plants: II. Hydroponic cultures. *Soil Science and Plant Nutrition,* 31**,** 163-173.

Läuchli, A. & Grattan, S. R. 2012. Soil pH extremes. *Plant stress physiology.* CABI Wallingford UK. 195 p.

Lewis, O. A. & Lewis, O. A. 1991. Plants and nitrogen. *Cambridge University Press*. 52 p.

Limited, H. B. 1989. Manual of plant species suitability for reclamation in Alberta: 2nd Edition [Online]. Available: https://www.cclmportal.ca/resource/manual-plant-species-suitability-reclamation-alberta-2nd-edition [Accessed 2021 July]. 178 p.

Lopes, L. D., Hao, J. & Schachtman, D. P. 2021. Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. *FEMS Microbiology Ecology,* 97**,** fiab028.

Lopes, L. D. S., Carvalho, H. H. D., Miranda, R. D. S., Gallão, M. I. & Gomes-Filho, E. 2020. The influence of dissolved oxygen around rice roots on salt tolerance during pre-tillering and tillering phases. *Environmental and Experimental Botany,* 178**,** 104169.

López-Bucio, J., Guevara-García, A., Ramírez-Rodríguez, V., Nieto, M. & Herrera-Estrella, L. Agriculture for marginal lands: transgenic plants towards the third millennium. In *Plant genetic engineering: towards the third millennium: Proceedings of the International Symposium on Plant Genetic Engineering, Havana, Cuba, 6-10 December, 1999.* (pp. 159-165). Elsevier Science Publishers.

Macdonald, S. E., Landhäusser, S. M., Skousen, J., Franklin, J., Frouz, J., Hall, S., Jacobs, D. F. & Quideau, S. 2015. Forest restoration following surface mining disturbance: challenges and solutions. *New Forests,* 46**,** 703-732.

Mackenzie, D. 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. *DesLibris*. 49 p.

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.

Malakar, P. & Chattopadhyay, D. 2021. Adaptation of plants to salt stress: the role of the ion transporters. *Journal of Plant Biochemistry and Biotechnology,* 30**,** 668-683.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. *Academic press*. 447 p.

Mcmillan, R., Quideau, S., Mackenzie, M. & Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. *Journal of Environmental Quality,* 36**,** 1470-1478.

Melton, J. R., Mahtab, S. K. & Swoboda, A. R. 1973. Diffusion of zinc in soils as a function of applied zinc, phosphorus, and soil pH. *Soil Science Society of America Journal,* 37**,** 379-381.

Mikula, R. J., Kasperski, K. L., Burns, R. D. & Mackinnon, M. D. 1996. Nature and fate of oil sands fine tailings. *ACS Publications*.

Mishra, A. & Tanna, B. 2017a. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8**,** 829-839. doi: 10.3389/fpls.2017.00829.

Mishra, A. & Tanna, B. 2017b. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8, 829-839. doi: 10.3389/fpls.2017.00829.

Moraghan, J. & Mascagni Jr, H. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxicities. *Micronutrients in Agriculture,* 4**,** 371-425.

Munns, R. & Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review Plant Biology,* 59**,** 651-681.

Murat, A., Turan, M., Awadelkarim, A., Khalifa, A. E., Taban, N. & Taban, S. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research,* 4**,** 893-897.

Naeth, M. A., Wilkinson, S., Mackenzie, D., Archibald, H. & Powter, C. B. 2013. Potential of LFH mineral soil mixes for reclamation of forested lands in Alberta. *Oil Sands Research and Information Network*. 3 p.

Neina, D. 2019. The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science,* 2019**,** 1-9.

O'neill, M. A., Ishii, T., Albersheim, P. & Darvill, A. G. 2004. Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review Plant Biology,* 55**,** 109-139.

Parent, C., Capelli, N., Berger, A., Crevècoeur, M. & Dat, J. 2008a. An overview of plant responses to soil waterlogging. *Plant Stress,* 2, 20-27.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M. & Dat, J. F. 2008b. An overview of plant responses to soil waterlogging. *Plant Stress,* 2**,** 20-27.

Passioura, J. B. 2010. Plant–water relations. *Encyclopedia of Life Sciences.* 60 p.

Penn, C. J. & Camberato, J. J. 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture,* 9**,** 120.

Producers, C. a. O. P. 2023. Canada's oil industry [Online]. Available: https://www.capp.ca/oil/ [Accessed 2023 April].

Purdy, B. G., Ellen Macdonald, S. & Lieffers, V. J. 2005. Naturally saline boreal communities as models for reclamation of saline oil sand tailings. *Restoration Ecology,* 13**,** 667-677.

Raghothama, K. G. 2005. Phosphorus and plant nutrition: an overview. *Phosphorus: Agriculture and the Environment.* 355 p.

Rawat, J., Pandey, N. & Saxena, J. 2022. Role of potassium in plant photosynthesis, transport, growth and yield. *Role of Potassium in Abiotic Stress***,** 1, 1-14.

Redfield, E., Croser, C., Zwiazek, J., Mackinnon, M. & Qualizza, C. 2003. Responses of red‐osier dogwood to oil sands tailings treated with gypsum or alum. *Journal of Environmental Quality,* 32**,** 1008-1014.

Redfield, E. B., Durnie, S. M. & Zwiazek, J. J. 2004. Effects of hypoxia on ion accumulation in wild raspberry (*Rubus idaeus*) exposed to water released from saline oil sands mine tailings. *Environmental and Experimental Botany,* 52**,** 1-9.

Regulator, A. E. 2023. Oil sands [Online]. Available: https://www.aer.ca/providing-information/by-topic/oil-sands [Accessed 2023 February].

Renault, S., Paton, E., Nilsson, G., Zwiazek, J. & Mackinnon, M. 1999. Responses of boreal plants to high salinity oil sands tailings water. (Vol. 28, No. 6, pp. 1957-1962). *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.*

Rengel, Z. 2002. Handbook of plant growth pH as the master variable. *CRC Press*. 88, 1-23.

Robin, A., Vansuyt, G., Hinsinger, P., Meyer, J. M., Briat, J.-F. & Lemanceau, P. 2008. Iron dynamics in the rhizosphere: consequences for plant health and nutrition. *Advances in Agronomy,* 99**,** 183-225.

Sajal, R. & Nasrin, C. 2020. Salt stress in plants and amelioration strategies: A critical review. *In:* Shah, F., Shah, S., Yajun, C., Chao, W. & Depeng, W. (eds.) *Abiotic Stress in Plants.* Rijeka: IntechOpen. 459 p. doi: 10.5772/intechopen.93552.

Sarath, N. G., Sruthi, P., Shackira, A. & Puthur, J. T. 2021a. Halophytes as effective tool for phytodesalination and land reclamation. *Frontiers in Plant-Soil Interaction.* Elsevier. 459-494. doi:10.1016/B978-0-323-90943-3.00020-1.

Sarath, N. G., Sruthi, P., Shackira, A. M. & Puthur, J. T. 2021b. Chapter 16 - Halophytes as effective tool for phytodesalination and land reclamation. *In:* Aftab, T. & Hakeem, K. R. (eds.) *Frontiers in Plant-Soil Interaction.* Academic Press. 459-494. doi:10.1016/B978-0-323-90943-3.00020-1.

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., Gómez, C., Mattson, N., Nasim, W. & Garcia-Sanchez, F. 2020. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy,* 10**,** 938-972.

Sheldon, A. R., Dalal, R. C., Kirchhof, G., Kopittke, P. M. & Menzies, N. W. 2017. The effect of salinity on plant-available water. *Plant and Soil,* 418**,** 477-491.

Sims, J. T. & Sharpley, A. N. 2005. Phosphorus: agriculture and the environment. *American Society of Agronomy*. 1-22 p. doi: 10.2134/agronmonogr46.

Steffens, D., Hutsch, B., Eschholz, T., Losak, T. & Schubert, S. 2005. Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment,* 51**,** 545-552.

Tan, X., Liu, M., Du, N. & Zwiazek, J. J. 2021. Ethylene enhances root water transport and aquaporin expression in trembling aspen (*Populus tremuloides*) exposed to root hypoxia. *BMC Plant Biology,* 21**,** 227.

Tan, X., Xu, H., Khan, S., Equiza, M. A., Lee, S. H., Vaziriyeganeh, M. & Zwiazek, J. J. 2018. Plant water transport and aquaporins in oxygen-deprived environments. *Journal of Plant Physiology,* 227**,** 20-30.

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. In:* Barrow, N. J. (ed.) *Plant Nutrition — from Genetic Engineering to Field Practice: Proceedings of the Twelfth International Plant Nutrition Colloquium,* 21–26 September 1993, Perth, Western Australia (pp. 763-765). Springer Netherlands.

Tarasoff, C., Mallory-Smith, C. & Ball, D. 2007. Comparative plant responses of *Puccinellia distans* and *Puccinellia nuttalliana* to sodic versus normal soil types. *Journal of Arid Environments,* 70**,** 403-417.

Tomar, R. S., Kataria, S. & Jajoo, A. 2021. Behind the scene: Critical role of reactive oxygen species and reactive nitrogen species in salt stress tolerance. *Journal of Agronomy and Crop Science,* 207**,** 577-588.

Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E. & Et Al. 2003a. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature,* 425**,** 393-397.

Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., Bligny, R. & Maurel, C. 2003b. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature,* 425**,** 393-397.

Turner, N. & Begg, J. 1981. Plant-water relations and adaption to stress. *Plant and Soil,* 58**,** 97-131.

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013a. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants*, 179-192. doi: 10.1007/978-81-322-0807-5_7.

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013b. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants***,** 179-192. doi: 10.1007/978-81-322-0807-5_7.

Usda, U. S. D. O. a. N. R. C. S. 2023. *Poa secunda J. Presl* Sandberg bluegrass [Online]. Available: https://plants.sc.egov.usda.gov/home/plantProfile?symbol=POSE [Accessed 2023 February].

Vaziriyeganeh, M., Lee, S. H. & Zwiazek, J. J. 2018. Water transport properties of root cells contribute to salt tolerance in halophytic grasses *Poa juncifolia* and *Puccinellia nuttalliana*. *Plant Science,* 276**,** 54-62.

Vedoy, D. R. & Soares, J. B. 2015. Water‐soluble polymers for oil sands tailing treatment: A Review. *Canadian Journal of Chemical Engineering,* 93**,** 888-904.

Wallace, A., Wallace, G. A. & Abouzamzam, A. 1986. Effects of excess levels of a polymer as a soil conditioner on yields and mineral nutrition of plants. *Soil Science,* 141**,** 377-380.

Wang, H., Ahan, J., Wu, Z., Shi, D., Liu, B. & Yang, C. 2012. Alteration of nitrogen metabolism in rice variety 'Nipponbare' induced by alkali stress. *Plant and Soil,* 355**,** 131.

Wendel, G. W. & Smith, H. C. 1990. Silvics of North America: 1. Conifers. *Pinus strobus L. - Eastern White Pine***,** 476-488.

Wennerberg, S. & Skinner, M. 2007. Plant Guide—Kentucky Bluegrass (*Poa pratensis*). *USDA-NRCS: Washington, DC, USA***,** 1-4.

Wilkinson, S. 1999. PH as a stress signal. *Plant Growth Regulation,* 29**,** 87-99.

Xiong, B., Loss, R. D., Shields, D., Pawlik, T., Hochreiter, R., Zydney, A. L. & Kumar, M. 2018. Polyacrylamide degradation and its implications in environmental systems. *NPJ Clean Water,* 1**,** 1-17.

Yang, C., Jianaer, A., Li, C., Shi, D. & Wang, D. 2008. Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. *Photosynthetica,* 46**,** 273-278.

Zhang, W.-Q., 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.

Zhu, J.-K. 2007. Plant Salt Stress. *Encyclopedia of Life Sciences.* 2 p.

Chapter 2 Effects of Enhanced Non-segregated Tailings on the Growth of Oil Sands Reclamation Tree Species

2.1 Introduction

Oil sands cover vast areas of northern Alberta, Canada. Most of the bitumen is extracted from the oil sands through open-pit mining after clearing the boreal forest. The process of bitumen extraction generates large amounts of tailings that are deposited in tailings ponds prior to reclamation. Although the tailings go through a process of partial consolidation using different additives, they will continue releasing tailings water for a prolonged period time after being deposited in mining pits (Matthews et al., 2002, Fleurial et al., 2022). According to the Environmental Protection and Enhancement Act (AER, 2022), oil sands companies must reclaim the disturbed land to natural equivalent land capability after mine closure (AER, 2021). When the mining activities cease, plants that are native to the area are used to revegetate the reclaimed land to reestablish the forest ecosystem and restore the land (CAPP, 2022). Prior to revegetation, oil sands tailings, overburden, and tailings sand are placed as the underlying substrates in the mining areas and are covered by the topsoil (either peat mineral mix or the forest floor mineral mix). (Fung and Macyk, 2000). The success indicator of land reclamation outcome is the biodiversity of the reclaimed sites, which must be the same as the local undisturbed boreal forest (Subgroup and Association, 2009).

To reclaim the tailings, this semi-liquid substance must first form a trafficable surface (Fung and Macyk, 2000). A natural settlement and consolidation of suspended particles in tailings would take about 100 years to reach this stage (Fung and Macyk, 2000). Therefore, different additives including gypsum, alum, and various polymers are used to speed up this process (MacKinnon et al., 2001, Gumfekar et al., 2019). However, the use of these chemicals may also further increase the salinity of tailings (MacKinnon et al., 2001). To address this problem, Canadian Natural Resources Limited (CNRL) has developed a novel enhanced non-segregated tailings (eNST) management technology, which uses cyclones and injects carbon dioxide as well as flocculants (anionic polyacrylamides) to fine tailings to make the solids clump together (CNRL, 2017). In the final stages, large metal plates press the tailings with filter materials and are placed in filter bags to consolidate and dewater, achieving 80% or greater fines capture (CNRL, 2017). However, the chemical and physical properties of eNST and its effects on plants have not been well documented. It was also demonstrated in previous studies that the polymers can decrease plant root dissolved organic carbon content and induce osmotic stress in plants (Búfalo et al., 2016, Wu et al., 2012).

Oil sands tailings are associated with phytotoxic factors including increased levels of salinity and high pH (pH 8-10) (Howat, 2000a). Furthermore, plants grown in oil sands reclamation sites can suffer from waterlogging if tailings release excessive amounts of water (Redfield et al., 2003, 2004). Soils with high pH are often characterized by reduced water permeability, which may exacerbate the waterlogging concern after rainfalls (Luna et al., 2017). Tailings can also release methane into the soil layer leading to a decrease in the root zone oxygen level (Redfield et al., 2004). Therefore, root hypoxia conditions should be considered a likely factor that may affect plants in the oil sands reclamation areas. Root hypoxia can lower the ability of plants to cope with salt stress and induce acidosis. Plants can exclude sodium ions through sodium-proton antiporters and ATPase from cytoplasm to prevent the sodium ions from reaching a toxic level, which is an energized process (Blumwald et al., 2000, Li et al., 2022). Therefore, the energy deficiency caused by hypoxia interferes with the salt exclusion process and, consequently, plant salt tolerance. Besides, the loss of energy restricts the activity of ATPase, resulting in the proton leakage to cytoplasm and cytosol acidosis, which can cause cell mortality (Drew, 1997).

Soil pH is one of the key soil variables affecting plant growth (Rengel, 2002). It affects nutrient availability and microorganism communities in soil (Brady, 1984, Marschner, 2011) . In oil sands reclamation sites, high soil pH is one of the main factors affecting establishment of plants (Renault et al., 2000). In addition to its effects on mineral nutrition, high soil pH upsets plant water balance and impairs a wide range of physiological parameters (Renault et al., 1998, Zhang et al., 2013, Zhang et al., 2015). High soil pH may also directly inhibit root cell growth by alkalizing the cell apoplast (Zhang et al., 2015, Geilfus, 2017, Xu et al., 2020, Hu et al., 2022).

Elevated soil salinity levels that are present in may oil sands reclamation areas (Lazorko and Van Rees, 2012, Zhang et al., 2020) may further hamper the revegetation efforts. In some of the sites, soil electrical conductivity (EC) values can reach 6 dS m^{-1}, or even higher (Lazorko and Van Rees, 2012). The main sources of salinity in these sites are the high-salinity overburden materials and sodium hydroxide that is added in the bitumen extraction process and becomes more concentrated each time the extraction water is recycled (Fung and Macyk, 2000). The high EC value of eNST release water mainly comes from the elevated concentration of Na ions (Fleurial et al., 2022), which can affect plant growth and survival through direct ion toxicity and osmotic effects combined with mineral nutrient deficiencies and oxidative stress (Ondrasek et al., 2022).

In the present study, the effects of eNST were examined in one-year-old paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*) seedlings. These tree species are native to northeastern Alberta, Canada, and have been widely used for oil sands reclamation (Watson, 1989). In earlier studies, composite tailings (CT, consolidated with gypsum) and non-segregated tailings (NST, tailings management technology previously used by CNRL), it was demonstrated that paper birch and jack pine were relatively sensitive , while trembling aspen and black spruce could relatively well tolerate the presence of these tailings in the growth substrates (Renault et al., 1999, Zhang and Zwiazek, 2016,Zhang et al., 2020, Fleurial et al., 2022).

The objectives of the present study were to: 1) examine the effects of eNST release water in the presence or absence of hypoxia on the growth and physiological parameters of tree seedlings that are commonly used for oil sands reclamation and to isolate the main factors in eNST tailings water that may contribute to these effects. I hypothesized that: eNST water impairs the growth and physiological processes of tailings-sensitive plants (paper birch and jack pine) more than the tailings-tolerant species (trembling aspen and black spruce) and that this effect would be aggravated by root hypoxia.).

2.2 Materials and methods

2.2.1 Plant material and growth conditions

One-year-old trembling aspen (*Populus tremuloides Michx.*.), paper birch (*Betula papyrifera Marshall*), jack pine (*Pinus banksiana lamb.*), and black spruce (*Picea mariana (Mill.) Britton, Sterns & Poggenb.*) seedlings were used in the study. The containerized dormant seedlings were purchased from the Boreal Horticulture Services Ltd. Plant Nursery (Bonnyville, Alberta, Canada). The seedlings were grown from seeds, which were collected from undisturbed boreal forest in the vicinity of the CNRL oil sands mining areas. All seedlings were stored at 4°C for two weeks before transferring them to hydroponics.

The experiment was conducted in a controlled-environment growth room with 22/18°C (day/night) temperature, 65±10% relative humidity, and 16-h photoperiod with 350 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provided by the full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

The hydroponic setup consisted of four 120 L drums filled with 50% modified Hoagland's mineral solution (control) (Epstein and Bloom, 1853) or treatment solutions. A water pump (Model 9.5 950GPH, Danner MFG Inc., New York, USA) was placed at the bottom of each drum and connected through the PVC tubing to three replicated 30 L plastic tubs (30 L) per treatment to circulate the solution between the tubs and the drum. A valve between the water pumps and the tubs was used to control the velocity of solution flow. After washing their roots in running water, five seedlings per species were fitted with foam plugs through the holes in a Styrofoam board of each tub (20 plants per tub) and floated on top of the solution. The treatments were: 1) aerated control (25% Hoagland's solution), 2) aerated eNST water (25% Hoagland's solution + 25% eNST water), 3) hypoxic control - (25% Hoagland's solution), and 4) hypoxic eNST water (25% Hoagland's solution). In the aerated treatments, air pump outlets were placed at the bottom of each tub to aerate the solutions and for the hypoxic treatments, there was no external air supply. The valves were kept at the minimum opening to maintain low water flow velocity between the drums and the tubs.

Every other day, the dissolved oxygen (DO) level, electrical conductivity (EC), and pH of solutions in the four treatments were measured with the a DO meter (Model 4320, Traceable Company, Waltham, Massachusetts, USA), an EC meter (Traceable Expanded Range Conductivity Meter, Model 4360, Traceable Company, Waltham, Massachusetts), and a pH meter (Thermo Scientific Orion Star A121 Portable pH Meter, Thermo Scientific Company, Waltham, Massachusetts, USA), respectively. The pH value in the aerated control group was maintained at pH 5 by adding 10% KOH solution three times a week.

2.2.2 Elemental composition of treatment solutions

Four 30-ml samples of treatment solutions were collected from each drum at the beginning of the treatment and combined. The Na, B, Fe, Zn, P, N, Mn, K, Ca, Cu, Mg, and S concentrations of the mixed solution sample from each bucket were determined by an inductively coupled plasma-optical emission spectroscopy (ICP-OES) instrument (Thermo iCAP 6000 series) at the Natural Resources Analytical Laboratory (NRAL) of the University of Alberta. The eNST water was filtered through a 0.45 μm Teflon membrane and the naphthenic acids were analyzed by the Fourier-transform infrared spectroscopy (FTIR) following their extraction with dichloromethane and sodium sulfate (Scott et al., 2008).

2.2.3 Relative shoot height growth (RSHG), and relative stem diameter (RSDG) growth rates.

At the beginning and end of the treatments, the shoot heights and stem diameters were measured in all seedlings from the root collar to the shoot tip. Stem diameters were measured twice above the root collar at the perpendicular direction and the mean value was taken for growth analysis. Differences between the final and initial values were divided by the initial values to calculate RSHG and RSDG.

2.2.4 Dry weights and leaf chlorophyll concentrations

After two months of treatments, the shoots and roots were separated in seedlings, placed in paper bags, and oven-dried at 70°C for four days to determine their dry weights.

At the end of the experiment, the upper leaves of six seedlings from each species in each treatment were taken for the measurements of leaf chlorophyll concentrations and elemental analysis. The leaves were freeze-dried (Labconco Freeze Dry System 4.5 Liter) for 4 days and ground into powder with a Thomas Wiley Mini-Mill (Thomas Scientific, Trenton, NJ, USA). Ten mg of dry leaf powder was collected from each seedling sample and mixed with 8 ml dimethyl sulfoxide (DMSO) in a 15 ml Falcon tube. After that, the chlorophyll was extracted at 65℃ for 22 h. Chlorophyll concentrations were measured with a spectrophotometer (Thermo Scientific Genesys 10S UV-Vis, Trenton, NJ, USA)at 648 nm and 665 nm for chlorophylls a and b, respectively (Sestak et al., 1971). The total chlorophyll concentration was calculated by combining chlorophylls a and b.

2.2.5 Gas exchange and chlorophyll fluorescence

Gas exchange parameters, including net photosynthesis and transpiration rates, were measured by a portable photosynthesis system (IL-6400 Portable Photosynthesis System, LI-COR Bioscience company, Lincoln, NE, USA). During the measurements, the CO_2 reference concentration was set to 400 μmol mol^{-1}, the flow rate in the leaf chamber to 200 μmol s^{-1}, the leaf chamber temperature was maintained at 20°C and the PPFD of the leaf chamber was 400 μmol m^{-2} s^{-1}. All data were collected from three to six hours following the onset of photoperiod. One fully expanded uppermost leaf was used from each of the eight seedlings per treatment per species to measure the gas exchange parameters. For jack pine and black spruce, the bundles of several needles were inserted into the leaf chamber to cover the entire opening of the chamber.

For chlorophyll fluorescence, the maximum efficiency of photosystem II (Fv'/Fm') and maximum quantum efficiency of photosystem II photochemistry (Fv/Fm) were measured by the IL-6400 Portable Photosynthesis System as well. The Fv'/Fm' was measured in the same seedlings as for the gas exchange parameters. Since the chlorophyll fluorescence parameters could not be measured for the needles of jack pine and black spruce, Fv/Fm was only measured in paper birch and trembling aspen. Before measuring the Fv/Fm, all light sources were turned off to make plant leaves adapt to the dark for 30 minutes.

2.2.6 Root hydraulic conductivity (Lpr)

Six seedlings from each plant species in each treatment were randomly taken to measure the root hydraulic conductivity. The shoots were excised about 3 cm above the root collar and the bark was peeled off from the stem. Plant roots were connected to a high-pressure flow meter (HPFM, Dynamax Inc., Houston, TX) with roots immersed in the respective treatment solution. The roots were gradually pressurized to 0.5 MPa with degassed distilled water to measure the hydraulic conductance (Kr) (Kamaluddin and Zwiazek, 2002a). Then, the roots were disconnected from the HPFM and fully immersed in water in a graduated cylinder to measure the volume of displaced water as a proxy for root volume (Kamaluddin and Zwiazek, 2002a). The Lpr was calculated by dividing Kr by the root volume.

2.2.7 Relative electrolyte leakage ratio (RELR)

After the two-months of treatments, six seedlings of each tree species were randomly taken from each treatment to measure the relative electrolyte leakage ratio from leaves (Saeed et al., 2014). For paper birch and trembling aspen, one 2-cm diameter leaf disk was collected from each seedling by a cork borer. After that, the leaf sample was immediately immersed in a collection tube filled with 10 ml milli-Q water. For jack pine and black spruce, six 1-cm long needles were excised with scissors from each seedling. After placing the leaf sample into the tube, an initial EC value (EC_0) was immediately measured. Then, the sample was incubated at room temperature for 1 h. After that, the EC value was measured again to obtain the EC_1 value. Finally, all collection tubes were placed in a water bath at 100°C for 20 minutes. After the tubes cooled to room temperature, the EC_{total} value was measured. The relative electrolyte leakage ratio was calculated as:

$$EC_{ratio} = \frac{(EC_1 - EC_0)}{(EC_{total} - EC_0)}$$

2.2.8 Elemental composition of dry leaves

The elemental compositions of freeze-dried leaf tissues were analyzed by ICP-OES and the dry combustion methods. The analyses were conducted at the Natural Resources Analytical Laboratory (NRAL) facility at the University of Alberta. Upper young leaf samples of six seedlings from each plant species in each treatment were randomly selected to measure the concentrations of Na, B, Fe, Zn, P, N, Mn, K, Ca, Cu, Mg, and S in the leaves. Briefly, 0.5g (dry weight) leaf samples were digested with 10 ml 70% HNO_3 in a heated microwave oven (MARS 5: Microwave Accelerated Reaction System (CEM Corporation, Matthews, NC, USA) for 10 min. Then, the extracts were diluted with 30 ml Milli-Q water. The samples were filtered through 0.25 µm PTFE lure-lok syringe filters before being analyzed by ICP-OES (Thermo iCAP6300 Duo). The N concentration of leaf tissues was determined by the dry combustion method using a Thermo FLASH 2000 HT Plus Organic Elemental Analyzer (Thermo Fisher Scientific Inc) (Schumacher, 2002).

2.2.9 Statistical analysis

To detect the statistically significant differences between the treatments within each species, all data were analyzed by the one-way analysis of variance (ANOVA) using the R software (version 4.1.1). The data that did not meet the assumptions of normal distribution and homogeneity of variance were transformed with sqrt or log10 functions. Pairwise comparisons between different treatments within each species were conducted using the Holm test.

2.3 Results

2.3.1 Physicochemical properties of eNST water and treatment solutions

The eNST release water was characterized by high pH and high EC levels compared with the control (Hoagland's) solution (Table 2.1). It also contained relatively high concentrations of NAs, Na, B, and S (Table 2.1). The concentrations of other elements (Fe, Zn, P, K, Mn, Ca, Cu, and Mg) in treatments containing eNST water were overall lower compared with the 25% Hoagland's solution (Table 2.1). The dissolved oxygen level of the aerated and hypoxic solutions were 5.3-5.8 mg/L and 1.0-1.3 mg/L, respectively (Table 2.1).

Reduced aeration increased the pH values in 25% Hoagland's solution (control) from 5 to 6, however and it decreased pH of the 25% eNST treatment solution from 8.5 to 7.5 (Table 2.1).

2.3.2 Relative shoot height growth (RSHG) and relative stem diameter growth (RSDG)

Both eNST water and root hypoxia treatments significantly reduced the RSHG of paper birch (Figure 2.1A). Compared with eNST water, root hypoxia had a stronger negative effect on RSHG in paper birch, resulting in lower RSHG values in the hypoxic Hoagland's group compared with the eNST water-treated group (Figure 2.1A). Root hypoxia aggravated the inhibitory effect of eNST water on the RSHG in paper birch (Figure 2.2A) The hypoxic eNST water treatment did not impact the RSHG in trembling aspen, jack pine, and black spruce (Figures 2.1B, C, and D).

The RSDG of paper birch was significantly decreased by the hypoxic eNST water treatment (Figure 2.1E). When applied separately, hypoxia and eNST water had similar inhibitory effects on the RSDG of paper birch seedlings, and the combination of eNST water and root hypoxia had the strongest negative effects on the RSDG of paper birch (Figure 2.1E). In contrast, the RSDG values of trembling aspen, jack pine, and black spruce did not show significant differences among treatments (Figures 2.1F, G, and H).

2.3.3 Plant dry weights

The total dry weights of paper birch sharply decreased in the treatments with eNST water with or without hypoxia, and the lowest total dry weight was observed in the hypoxic eNST water group (Figure 2.2A). In trembling aspen, there were no significant differences in total dry weights among treatments (Figure 2.2B). In jack pine, the total dry weights of the control group were lower compared to the other groups (Fig. 2.2C). In black spruce, the hypoxia and eNST water treatments did not have significant effects on the total dry weights (Figure 2.2D).

In paper birch, the shoot to root dry weight ratios of seedlings exposed to the eNST water and hypoxia were significantly lower compared with the control group, with the hypoxic group having the lowest ratio (Figure 2.2E). In trembling aspen and black spruce, there were no significant differences in the shoot to root dry weight ratios among the treatments (Figure 2.2F, H). In jack pine, the control group had a significantly higher shoot to root dry weight ratios compared with the other three treatments (Figure 2.2G).

2.3.4 Chlorophyll concentrations

Both eNST water and hypoxia significantly reduced the total chlorophyll concentrations in paper birch, with the lowest total chlorophyll concentrations observed in the aerated eNST water-treated group (Figure 2.3A). Chlorophyll concentrations in the hypoxic treatment were higher than the hypoxic eNST water group (Figure 2.3A). In trembling aspen, leaf chlorophyll concentrations were the highest in the aerated control group, lower in the hypoxic group, and the lowest in the aerated and hypoxic eNST water groups (Figure 2.3B). In jack pine, the needle chlorophyll concentrations of seedlings grown in in aerated eNST and hypoxic eNST water were significantly lower compared with the control group (Figure 2.3C). In black spruce, seedlings treated with both eNST water and hypoxia had significantly lower needle chlorophyll concentrations compared with the control group, with the lowest chlorophyll concentrations measured in the hypoxic eNST water group. (Figure 2.3D).

In paper birch, the chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios were the lowest in the aerated eNST and hypoxic eNST water groups and were also reduced by the hypoxia treatment (Figure 2.3E). In trembling aspen, seedlings grown in control and hypoxic Hoagland's solution showed significantly higher Chl a to Chl b ratios compared with the aerated and hypoxic eNST water groups (Figure 2.3F). In jack pine, both eNST water and hypoxia treatments significantly reduced Chl a to Chl b ratios compared with the control group, and the lowest Chl a to Chl b ratios were observed in the hypoxic eNST water group (Figure 2.3G). In black spruce, the Chl a to Chl b ratio was significantly lower in the hypoxic eNST water treatment compared with the other experimental groups (Figure 2.3H).

2.3.5 Gas exchange

In paper birch, the net photosynthesis rate significantly decreased by the eNST water and hypoxia treatments, and the lowest (negative) net photosynthesis rate was measured in plants treated with the hypoxic eNST water (Figure 2.4A). In trembling aspen, the eNST water treatment significantly inhibited net photosynthesis compared with the control and hypoxic Hoagland's groups (Figure 2.4B). In jack pine, the eNST water treatment alone did not have a significant impact on the net photosynthesis rate, while the net photosynthesis rates of seedlings in hypoxic Hoagland's and eNST water groups were lower compared with the aerated groups (Figure 2.4C). In black spruce, hypoxia significantly reduced the net photosynthesis rates compared with the aerated groups, while eNST water alone did not affect net photosynthesis (Figure 2.4D).

In paper birch, the transpiration rates were not affected by the eNST water, however, the transpiration rates decreased in both the hypoxic and hypoxic eNST groups (Figure 2.4E). In trembling aspen, the patterns of transpiration rates in the four groups were similar to the net photosynthesis rates, and the transpiration rates were significantly reduced only by the eNST water treatment (Figure 2.4F). In jack pine, the eNST water and hypoxia did not significantly affected the transpiration rates (Figure 2.4G). In black spruce, the transpiration rates were significantly reduced by the hypoxia treatment, while the effect of eNST water was not significant (Figure 2.4H).

2.3.6 Chlorophyll fluorescence

The PSII maximum efficiency (Fv'/Fm') in paper birch was significantly reduced by both the eNST water and hypoxia treatments, and the combination of these stresses caused the lowest Fv'/Fm' ratio (Figure 2.5A). In trembling aspen, only the eNST water significantly inhibited the Fv'/Fm' (Figure 2.5B). In jack pine, the Fv'/Fm' values in seedlings treated with the hypoxic eNST water were significantly lower compared with the other three treatments (Figure 2.5C). In black spruce, no significant differences were observed for the Fv'/Fm' values among treatments (Figure 2.5D).

In paper birch, a significantly lower maximum quantum efficiency of PSII photochemistry (Fv/Fm) was observed only in the aerated eNST water-treated group (Figure 2.5E). In trembling aspen, only the Fv/Fm values of seedlings treated with the hypoxic eNST water were significantly lower than the seedlings of the hypoxic control group (Figure 2.5F).

2.3.7 Root hydraulic conductivity (Lpr)

In paper birch, the Lpr values of seedlings treated with the aerated eNST water were significantly higher than the other three treatments (Figure 2.6A). In trembling aspen and jack pine, the Lpr was not significantly affected by either the eNST water or the hypoxia treatments (Figures 2.6B and C).

2.3.8 Relative electrolyte leakage ratio (RELR)

In paper birch, the RELR of seedlings in the control group was significantly higher than the other three groups (Figure 2.7A). In trembling aspen, the RELR of seedlings in the hypoxic group was significantly lower compared with the eNST water treated groups (Figure 2.7B). In jack pine, there were no significant differences in RELR among treatments (Figure 2.7C). In black spruce, seedlings of the hypoxic Hoagland's group had significantly lower RELR values than in the control group, while the eNST water did not have a significant effect on RELR (Figure 2.7D).

2.3.9 Foliar elements

The composition of leaf tissue elements was significantly affected by the eNST water and root hypoxia. All four tree species showed similar patterns in leaf Na and B concentrations, as the Na and B concentrations significantly increased in both aerated eNST and hypoxic eNST water-treated groups (Figures 2.8A and C). However, in paper birch, trembling aspen, and black spruce seedlings treated with the hypoxic eNST water, leaf B concentrations were significantly lower compared with the aerated eNST water-treated group (Figure 2.8C).

The leaf Na/K ratios significantly increased in paper birch, jack pine, and black spruce by the eNST water treatment (Figure 2.8B). The leaf Na/K ratio of trembling aspen treated with the hypoxic eNST water was similar to the values measured in the aerated control plants (Figure 2.8B). Additionally, in paper birch, the leaf Na/K ratio significantly increased in the hypoxic eNST water group compared with the aerated eNST water group (Figure 2.8B). In jack pine and black spruce, there were no significant differences in the leaf Na/K ratios between the seedlings treated with hypoxic eNST water and aerated eNST water (Figure 2.8B).

The Na/Ca ratios of all species in eNST water-treated groups were significantly higher compared with the control group, while the differences in trembling aspen were much smaller compared with the other three species. In paper birch, seedlings in the hypoxic eNST water group had significantly higher Na/Ca ratios compared with the control group. Hypoxic Hoagland's treatment did not significantly affect the foliar Na/Ca ratios in paper birch, trembling aspen, and jack pine (Figure 2.8D).

Both eNST water and root hypoxia affected the leaf concentrations of other measured elements (Table 2.2). In paper birch, eNST water significantly reduced the leaf Zn, P, Fe, and N concentrations. Among these four elements, the concentrations of N and P were the lowest in the hypoxic eNST water group. The leaf K concentrations of seedlings in aerated eNST water were the highest of all treatment groups. The aerated eNST water significantly increased leaf Ca and Mg concentrations, resulting in the highest values compared with the other groups. However, the combination of eNST water and root hypoxia significantly decreased Ca, Mg, N, and P concentrations, resulting in the lowest values among all treatment groups. The leaf S concentrations were significantly reduced in plants treated with eNST water (Table 2.2).

In trembling aspen, leaf N, Zn, and Cu contents were significantly reduced by the eNST water and hypoxia (Table 2.2). The leaf N concentration reached the lowest values in plants treated with the hypoxic eNST water. The leaf P and S concentrations in trembling aspen were more sensitive to eNST water than hypoxia (Table 2.2). The eNST water and hypoxia did not have any significant impact on the leaf Fe and Mn concentrations (Table 2.2). The leaf Ca concentrations in seedlings treated with hypoxic eNST water were significantly lower compared with the control group (Table 2.2). The leaf Mg concentrations were significantly reduced in both hypoxic groups (Table 2.2).

In jack pine, the leaf Zn, P, Ca and Mg concentrations were significantly reduced by the hypoxic eNST treatment, while Zn and Ca concentrations were also significantly lower in the aerated eNST water treatment compared with the control group (Table 2.2). Leaf Fe concentrations were lower in seedlings subjected to hypoxia compared with the well aerated plants (Table 2.2). On the contrary, both hypoxic treatments significantly increased the leaf N concentrations (Table 2.2). Leaf K concentrations were significantly reduced by eNST water (Table 2.2). Neither eNST water nor hypoxia significantly impacted the leaf concentrations of Mn, Cu and S (Table 2.2).

In black spruce, the leaf P, N, K, Ca, Mg. and S concentrations were significantly lower in the hypoxic eNST water treatment compared with the control plants (Table 2.2). There were no significant differences in the leaf Fe concentrations (Table 2.2). The leaf K concentrations were significantly reduced in plants treated with the aerated eNST water. The leaf N concentrations of seedlings treated with eNST water were significantly lower compared with the plants treated with the Hoagland's solution and reached the lowest level in plants treated with the hypoxic eNST water (Table 2.2).

2.4 Discussion

2.4.1 The pH and EC of the eNST water

In the present study, the pH and EC of the eNST water were 9.1 and 4.49 mS cm^{-1}, respectively (Table 1), both of which are significantly higher than the values found for both CT tailings water (pH 7.42 and EC 2.27 mS cm^{-1}) (Renault et al., 1998) and the NST (pH 8.8 and EC 0.8 mS cm^{-1}) (Zhang et al., 2020). However, in the treatment solution (25% eNST water in 25% Hoagland's solution), the pH and EC were 8.5 and 2.8 mS cm^{-1}, similar to the previously reported values in the study involving CT water (pH 8.46 and EC 2.52 mS cm^{-1}) (Redfield et al., 2004). The solution pH values in control group decreased before being adjusted with KOH solution. The decline of pH in the control group can be attributed to the release of protons from roots due to ammonium (NH_4^+) absorption (Kieko and Masahiko, 2012). Since the proton pump consumes energy produced from respiration, the root hypoxia may disrupt the function of proton pumps in root cells, leading to a lower pH value of the well-aerated Hoagland's solution (pH 5) compared with the hypoxic Hoagland's solution (pH 6). When plants take up nitrate (NO_3^-), the pH of the growth solution may increase over the time (Redfield et al., 2004), which may also explain the pH differences between the aerated and hypoxic eNST water groups. Furthermore, the dissolved oxygen content can affect the chemical forms in the growth solution, which may affect its pH. For example, the increase of dissolved oxygen significantly decreases the NH_4^+ concentration (Khalid et al., 1978).

2.4.2 Leaf elemental composition

High soil pH is one of the biggest challenges for the oil sands tailings reclamation since it can significantly reduce the availability of essential elements to plants, including Fe, Mn, Zn, N, and P. Similarly to the present study, earlier reports also implicated high pH as a major factor in oil sands tailings that was responsible for decreases in plant foliar concentrations of several essential nutrients (Redfield et al., 2004, Zhang et al., 2013). In the present study, the leaf tissues of seedlings treated with the eNST water had significantly lower concentrations of Fe, Mn, Zn, P, and total N compared with the respective controls, which can be attributed to the high pH of eNST water (pH 9.1). Other factors that also likely affected plant nutrient uptake and other physiological processes in plants treated with eNST water include elevated concentrations of NaCl and naphthenic acids (Apostol et al., 2004, Arif et al., 2020).

2.4.3 Naphthenic acids

The eNST water contained 65 mg/L naphthenic acids, which is in a similar range as the concentrations reported for the CT water (Renault et al., 1998, Renault et al., 2001). Naphthenic acids were reported to reduce the gas exchange, root hydraulic conductivity, and chlorophyll concentrations in aspen seedlings (Kamaluddin and Zwiazek, 2002b, Fleurial et al., 2022). In jack pine, the presence of naphthenic acids in the growth medium aggravated the negative effects of NaCl on stomatal conductance, root hydraulic conductivity, and needle electrolyte leakage (Apostol et al., 2004). The effects of naphthenic acids and the mechanisms of their action in plants have not been thoroughly studied. However, it has been reported that they can also be toxic to fish. Naphthenic acids extracted from oil sands tailings water resulted in 100% mortality of yellow perch after four days of treatment (Nero et al., 2006).

2.4.4 Leaf Na, K and Ca concentrations

In the present study, the eNST water significantly increased leaf Na concentrations in all four plant species. Trembling aspen and black spruce had lower leaf Na concentrations compared with paper birch and jack pine. This may partly help explain the relatively higher tailings tolerance of trembling aspen and black spruce reported in short-term studies for compared with the other studied plants (Renault et al., 1999, Zhang and Zwiazek, 2016). However, the low leaf Na concentrations could be due to the lower Na uptake and slow growth, or even dormancy that could be triggered by high salinity as reported in other studies (Yokoi et al., 2002, Ahmad et al., 2013).

The high Na concentration of eNST water can also affect K homeostasis in plants. Since Na and K have similar chemical nature (Zhu, 2001), they compete for the uptake sites, which may reduce the tissue K concentration, resulting in increased Na/K ratios. K is essential for maintaining plant growth and physiological processes (Zhao et al., 2001, Çalişkan and Çalişkan, 2019). Maintenance of high tissue K concentrations and low Na/K ratios is considered to be associated with higher salt tolerance in plants (Renault et al., 1999, Yi et al., 2008). In the present study, the Na/K ratios of paper birch and trembling aspen treated with eNST water were lower compared with jack pine and black spruce, which have been reported to be more sensitive to salinity than the deciduous tree species (Renault et al., 1998). The leaf K concentrations in paper birch treated with the aerated eNST water were higher by about 45% compared with the control group and those in trembling aspen were similar to the control. These results suggest that paper birch and trembling aspen can tolerate salt stress by increasing or maintaining their shoot K concentration, which was also observed in previous NaCl-treated plants (Yi et al., 2008, Tozlu et al., 2000, Fleurial et al., 2022).

Maintaining high tissue Ca concentration could also contribute to relatively higher salt tolerance of paper birch and trembling aspen compared with the two conifer species. Ca is essential to maintain cell membrane permeability and integrity and can alleviate K efflux (Liang et al., 1996a, Renault et al., 1999, Fleurial et al., 2022). The high Na concentration in the eNST water could likely displace the Ca in cell membranes, resulting in a high Na/Ca ratio (Redfield et al., 2004), which could potentially impair membrane integrity and result in membrane leakiness (Cramer et al., 1985, Liang et al., 1996a). In the present study, both paper birch and trembling aspen maintained high leaf Ca concentrations in the aerated eNST water treatment. However, in plants treated with the hypoxic eNST water, the leaf Ca concentrations in paper birch seedlings were significantly lower compared with plants treated with the aerated eNST water, resulting in a significant increase in the leaf Na/Ca ratios. The high Na/Ca ratio could aggravate K leakage which would explain the decrease in leaf K concentrations in paper birch subjected to the hypoxic NST water. The combination of salinity and hypoxia could also increase K leakage from cells by activating nonselective cation channels (NSCCs) (Barrett-Lennard and Shabala, 2013). In jack pine and black spruce, the high Na concentration in eNST water was likely responsible for the reduced leaf K and Ca concentrations, which could also be a factor contributing to the reduced leaf chlorophyll concentrations (Zhao et al., 2001).

2.4.5 Leaf boron concentrations

Apart from the elevated Na levels, relatively high B levels in the eNST water could contribute to its phytotoxic effects. Although B is an essential micronutrient, there is a narrow tissue concentration difference between B deficiency and phytotoxicity (Brdar-Jokanović, 2020). In the present study, B concentrations in the eNST water were slightly higher than the values reported for the CT water (1.85 mg/L, 3.5 mg/L, 2.7 mg/L) (Renault et al., 1998, Renault et al., 1999 , Renault et al., 2001,Redfield et al., 2004). Due to the differences between experimental conditions and plant species, the specific optimal and phytotoxic B concentrations are still uncertain (Brdar-Jokanović, 2020). Nevertheless, the common symptoms of B toxicity are leaf chlorosis and necrosis, which start from the leaf edge and spread to the whole leaf (Nable et al., 1997, Princi et al., 2016, Brdar-Jokanović, 2020). In the present study, this visible injury was widely observed in the foliage of all four tree species. However, similar symptoms may also be caused by the Na toxicity (Ahmad et al., 2013). Also, the leaf B concentrations in paper birch, trembling aspen, and black spruce treated with the aerated eNST water were significantly higher compared with the plants treated with the hypoxic eNST water. This could possibly be attributed to the reductions in transpiration rates caused by root hypoxia, since transpiration plays a crucial role in B transport from roots to shoots (Raven, 1980, Mengel and Kirkby, 2012, Princi et al., 2016, Brdar-Jokanović, 2020).

2.4.6 Total dry weights, RSHG, and RSDG

In the present study, the growth of paper birch was more sensitive to the eNST water compared with the other three species. The aerated eNST water significantly reduced total dry weights, RSHG, and RSDG in paper birch. Root hypoxia aggravated the negative effects of eNST water on paper birch. The combination of root hypoxia and eNST water significantly reduced the leaf K concentrations in paper birch compared with the aerated eNST water. Since K plays a crucial role in cell elongation and other plant physiological processes (Çalişkan and Çalişkan, 2019, Tozlu et al., 2000), paper birch had the lowest total dry weight and RSDG in the hypoxic eNST water group. Additionally, paper birch has low tolerance of high pH (Zhang et al., 2023) that is among the contributory factors to phytotoxicity of the eNST water.

Dry weights, RSHG, and RSDG in trembling aspen and black spruce did not show significant differences between the different treatments. Shoot growth of black spruce and jack pine is performed with the needle primordia set in the bud in the previous growing season. Therefore, it may take another year to see the full impact of experimental treatments on shoot growth. Also, harsh environmental conditions can trigger a premature dormancy set and completely inhibit shoot growth (Ahmad et al., 2013). The two-month treatment duration may not be long enough for a slow growing species such as black spruce to see the full effects on growth. This can also lead to incorrect interpretation of the study results, especially if the growth effects during the experimental timeframe are small and the sample size is limited. This is evidenced in the present study for jack pine seedlings, which had significantly higher total dry weights when treated with the eNST water compared with control plants. This can be attributed to the high size variation of jack pine seedlings, which masked the treatment effects at the measurement time.

2.4.7 Chlorophyll concentration, chlorophyll fluorescence, and gas exchange

In this study, the eNST water significantly reduced the net photosynthesis of paper birch and trembling aspen and these decreases were associated with the decreased chlorophyll concentrations, Fv'/Fm' ratios, and leaf concentrations of several essential elements (Fe, Zn, Mn, N, P, K, and Mg). The high salinity of eNST water can disrupt plant-water relations through direct ion toxicity and osmotic stress and result in water deficiency and stomatal closure (Bradford and Hsiao, 1982, Turan et al., 2009). Reduced stomatal conductance decreases the CO_2 uptake, which can reduce photosynthesis and affect the energy balance and growth (Turan et al., 2009). Additionally, salt stress reduces leaf chlorophyll concentrations (Turan et al., 2009) and blocks electron transport in the PS II (Mehta et al., 2010), leading to the reduction of Fv'/Fm' and photosynthesis. Moreover, high salinity reduces the plant uptake of Fe, Zn, Mg, Mn, N, P, and K by disrupting plant water balance, ion allocation, and cell membrane permeability (Chrysargyris et al., 2019, Arif et al., 2020). In addition to salt, the high pH of eNST water reduces the availability of Fe, Mn, Zn, N, P, and Mg in the growth medium (Marschner, 2011). The deficiencies of these nutrient elements severely affected the photosynthetic activity in paper birch and trembling aspen treated with the eNST water. Fe starvation can disrupt the synthesis of chlorophyll, damaging the function of PS I in the chloroplasts (Ana Flor et al., 2016, Therby-Vale et al., 2022). The deficiency of Mn in chloroplasts can also inhibit the photosynthetic processes and Fv'/Fm' by damaging the functions of PS I and PS II (Ana Flor et al., 2016, Zhang et al., 2018). The, deficiencies of Zn and N can also block the synthesis of chlorophylls, leading to the reduction of photosynthetic activity (Hu and Sparks, 1991, Wang and Jin, 2005, Mu and Chen, 2021). The Mg deficiency inhibits plant photosynthesis and growth by reducing chlorophyll synthesis (Farhat et al., 2016). Of the other essential elements affected by the eNST water and hypoxia, K deficiency can affect osmotic balance, stomatal conductance, and inhibit the activity of the Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) enzyme, leading to the reduction of net photosynthesis (Rawat et al., 2022). Phosphorus deficiency can also affect photosynthesis by reducing the electron transport to PS I (Carstensen et al., 2018).

Apart from high salinity and high pH of the eNST water, the results demonstrated that root hypoxia aggravated the negative effects of eNST water on the net photosynthesis in paper birch and reduced the concentrations of leaf Zn, P, N, Mn, and K. Root hypoxia induces stomatal closure and reduces transpiration rates, which can limit the photosynthetic process (Bradford and Hsiao, 1982). Root hypoxia can directly reduce the energy production and activities of the Rubisco enzyme and PSII (Yordanova and Popova, 2007). The ATP deficiency caused by root hypoxia inhibits the activity of root plasma membrane H^+ATPase, which facilitates the uptake of N, P, K, Mn, and Zn (Steffens et al., 2005). This can further reduce leaf chlorophyll concentrations and the photosynthesis (Hu and Sparks, 1991, Wang and Jin, 2005, Yruela, 2013, Carstensen et al., 2018, Mu and Chen, 2021, Rawat et al., 2022). Moreover, in the present study, the combination of root hypoxia and eNST water significantly decreased the leaf Ca and Mg concentrations of paper birch. The reduction of leaf Ca concentrations contributes to K leakage of from the leaf cells (Liang et al., 1996a), which reduces plant photosynthesis and growth (Rawat et al., 2022). The Mg deficiency inhibits chlorophyll synthesis and deactivates the Rubisco enzyme (Farhat et al., 2016). These mechanisms can explain the significant decline of net photosynthesis rate of paper birch treated with the hypoxic eNST water.

Compared with the eNST water alone, the combination of eNST water and root hypoxia significantly reduced chlorophyll concentrations and net photosynthesis rates in jack pine and black spruce and this was likely partly due to the reduced leaf Mg, Ca, P, and K concentrations. The deficiencies of these elements inhibit chlorophyll synthesis, Rubisco activities, and photosystem functions, leading to the reduction of photosynthesis (Liang et al., 1996b, Farhat et al., 2016, Carstensen et al., 2018, Rawat et al., 2022). Apart from disruptions of leaf elemental composition, jack pine and black spruce in the hypoxic eNST water were affected by the energy depletion, which may also affect the activity of Rubisco and the function of photosystems (Bradford and Hsiao, 1982, Yordanova and Popova, 2007, Marschner, 2011).

2.4.8 Root hydraulic conductivity (Lpr)

The Lpr of paper birch treated with the aerated eNST water showed the highest values among all treatments. This was probably due to the injury of roots since the damaged roots open the least resistance entry of water to roots (Apostol and Zwiazek, 2003). The eNST water and root hypoxia did not affect the Lpr in trembling aspen and jack pine, possibly because the treatment duration was not sufficiently long since the plant species under salt stress can establish new water relations and osmotic adjustments after a period of time (López-Berenguer et al., 2006). These results are consistent with the previous study, which demonstrated less extent of Lpr reduction under salt stress in the later compared with the early stages of salt treatment(López-Berenguer et al., 2006).

2.4.9 Relative electrolyte leakage ratio

The increase of electrolyte leakage from tissues is related to cell membrane damage (Redfield et al., 2004, Hniličková et al., 2019), which commonly occurs in plants exposed to salinity and root hypoxia (Bai et al., 2009, Khan et al., 2013). In the present study, the RELR of trembling aspen in the hypoxic group was significantly lower than in the eNST water-treated groups. This can be due to the lower leaf Na concentrations and leaf Na/Ca ratio in trembling aspen seedlings exposed to root hypoxia compared with the plants exposed to the eNST water. Ca is capable of maintaining the stability of cell membranes and reducing the leaf RELR (Khayyat et al., 2009, Saeed et al., 2014). The high concentration of Na in eNST water can displace the Ca in the cell membrane, increasing the leaf Na/Ca ratio and membrane permeability (Liang et al., 1996a). Also, Na efflux from the leaf tissues can directly contribute to RELR in plants with high Na tissue concentrations.

2.5 Conclusions

In conclusion, the experimental results demonstrated that eNST water significantly reduced the chlorophyll concentrations, net photosynthesis, and transpiration rates in paper birch and trembling aspen. Similar to the composite tailings (CT), the eNST water contains high Na, B, and naphthenic acid concentrations. The EC and pH levels of eNST water are also very high and can affect the survival and growth of plants affected by the tailings release water. The high salinity and high B levels of eNST water resulted in high leaf Na, B concentrations, Na/K, and Na/Ca ratios in all four species. The high pH of eNST water was likely the main factor responsible for decreased leaf concentrations of Zn, P, N, K, and Ca in all four species. When root hypoxia was combined with eNST water, it significantly aggravated the detrimental effects of eNST water, especially in paper birch, and led to the lower leaf concentrations of essential elements, reduced growth parameters, Fv'/Fm', and net photosynthesis. In this study, the net photosynthesis rates of jack pine and black spruce were more sensitive to hypoxia than to eNST water. Additionally, the combination of root hypoxia and eNST water reduced the chlorophyll concentrations, most severely in jack pine and black spruce. Furthermore, root hypoxia reduced the transpiration rates in paper birch, trembling aspen, and black spruce, resulting in significant reductions of leaf B concentrations in these tree species. The present study demonstrated that eNST water impaired the growth and physiological processes in the four studied plant species that are commonly used for oil sands reclamation. Future studies should explore the reclamation potential of plants that exhibit high tolerance of salinity and high soil pH levels.

2.6 References

Aer. 2021. Reclamation process and criteria for oil and gas sites [Online]. *Alberta Energy Regulator*. Available: https://www.aer.ca/regulating-development/projectclosure/reclamation/oil-and-gas-site-reclamation-requirements/reclamation-process-and-criteria-for-oil-and-gas-sites [Accessed 2021 July].

Aer. 2023. Environmental protection and enhancement act [Online]. *Alberta Energy Regulator*. Available: https://open.alberta.ca/publications/e12#summary [Accessed 2022 February]. 134 p.

Ahmad, P., Azooz, M. M. & Prasad, M. N. V. 2013. Ecophysiology and responses of plants under salt stress. *Springer*. 32-33 p. doi: 10.1007/978-1-4614-4747-4

Ana Flor, E.-M., Daniela, E. & Katrin, E. 2016. Chloroplast iron transport proteins - function and impact on plant physiology. *Frontiers in Plant Science,* 7, 1-12. doi: 10.3389/fpls.2016.00178

Andrews, J. A., Johnson, J. E., Torbert, J. L., Burger, J. A. & Kelting, D. L. 1998. Minesoil and site properties associated with early height growth of eastern white pine. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Vol. 27, No. 1, pp. 192-199.

Apostol, K. & Zwiazek, J. J. 2003. Hypoxia affects root sodium and chloride concentrations and alters water conductance in salt-treated jack pine (*Pinus banksiana*) seedlings. *Trees,* 17**,** 251-257.

Apostol, K. G., Zwiazek, J. J. & Mackinnon, M. D. 2004. Naphthenic acids affect plant water conductance but do not alter shoot Na^+ and Cl^- concentrations in jack pine (*Pinus banksiana*) seedlings. *Plant and Soil,* 263**,** 183-190.

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. & Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156**,** 64-77.

Bai, T., Li, C., Ma, F., Shu, H. & Han, M. 2009. Exogenous salicylic acid alleviates growth inhibition and oxidative stress induced by hypoxia stress in *Malus robusta Rehd*. *Journal of Plant Growth Regulation,* 28**,** 358-366.

Bansal, R. & Srivastava, J. 2015. Effect of waterlogging on photosynthetic and biochemical parameters in *pigeon pea*. *Russian Journal of Plant Physiology,* 62**,** 322-327.

Barrett-Lennard, E. G. & Shabala, S. N. 2013. The waterlogging/salinity interaction in higher plants revisited–focusing on the hypoxia-induced disturbance to K^+ homeostasis. *Functional Plant Biology,* 40**,** 872-882.

Blumwald, E., Aharon, G. S. & Apse, M. P. 2000. Sodium transport in plant cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes,* 1465**,** 140-151.

Bradford, K. J. & Hsiao, T. C. 1982. Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiology,* 70**,** 1508-1513.

Brady, N. 1984. The nature and properties of soils (ninth edition). *New York: Macmillan*.

Brdar-Jokanović, M. 2020. Boron toxicity and deficiency in agricultural plants. *International Journal of Molecular Sciences,* 21**,** 1424-1444.

Búfalo, J., Rodrigues, T. M., De Almeida, L. F. R., Dos Santos Tozin, L. R., Marques, M. O. M. & Boaro, C. S. F. 2016. PEG-induced osmotic stress in *Mentha x piperita L.*: structural features and metabolic responses. *Plant Physiology and Biochemistry,* 105**,** 174-184.

Çalişkan, B. & Çalişkan, A. C. 2019. Potassium nutrition in plants and its interactions with other nutrients in hydroponic culture. *Improvement of Quality in Fruits and Vegetables Through Hydroponic Nutrient Management*. doi: 10.5772/intechopen.71951.

Capp. 2022. Land Reclamation [Online]. *Canadian Association of Petroleum Producers*. Available: https://www.capp.ca/explore/land-reclamation/ [Accessed 2022 September].

Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M. & Husted, S. 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiology,* 177**,** 271-284.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S. A. & Tzortzakis, N. 2019. The combined and single effect of salinity and copper stress on growth and quality of *Mentha spicata* plants. *Journal of Hazardous Materials,* 368**,** 584-593.

Cnrl. 2017. CNRL 2017 Stewardship report to stakeholders [Online]. *Canadian Natural Resources Limited*. Available: https://www.cnrl.com/upload/report/112/05/2017-stewardship-report-to-stakeholders.pdf [Accessed 2021 June]. 19 p.

Cramer, G. R., LäUchli, A. & Polito, V. S. 1985. Displacement of Ca_2^+ by Na^+ from the plasmalemma of root cells: a primary response to salt stress. *Plant Physiology,* 79**,** 207-211.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Biology,* 48**,** 223-250.

Epstein, E. & Bloom, A. J. 1853. Mineral nutrition of plants: principles and perspectives. *Sinauer*.

Evans, G. & Hughes, A. 1961. Plant growth and the aerial environment: I. Effect of artificial shading on *Impatiens parviflora*. *New Phytologist,* 60**,** 150-180.

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C. & Rabhi, M. 2016. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum,* 38**,** 145.

Fleurial, K. G., Zhang, W.-Q., Vassov, R. & Zwiazek, J. J. 2022. Root hypoxia aggravates the effects of saline tailings water on the growth and physiology of woody plants. *Plant and Soil***,** 481, 1-18.

Franklin, J. A., Renault, S., Croser, C., Zwiazek, J. J. & Mackinnon, M. 2002. Jack pine growth and elemental composition are affected by saline tailings water. *Journal of Environmental Quality,* 31**,** 648-653.

Fung, M. Y. & Macyk, T. M. 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands,* 41**,** 755-774.

Geilfus, C.-M. 2017. The pH of the apoplast: dynamic factor with functional impact under stress. *Molecular plant,* 10**,** 1371-1386.

Gumfekar, S. P., Vajihinejad, V. & Soares, J. B. 2019. Advanced polymer flocculants for solid–liquid separation in oil sands tailings. *Macromolecular Rapid Communications,* 40**,** 1800644.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K. & Bohnert, H. J. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology,* 51**,** 463-499.

Hniličková, H., Hnilička, F., Hejnák, V. & Orsák, M. 2019. Effect of salt stress on growth, electrolyte leakage, Na^+ and k^+ content in selected plant species. *Plant, Soil and Environment,* 65**,** 90-96-96.

Howat, D. 2000a. Acceptable salinity, sodicity and pH values for boreal forest reclamation. *Environmental Sciences Division Edmonton, AB, Canada*, p. 191. 8 p.

Howat, D. 2000b. Acceptable salinity, sodicity and pH values for boreal forest reclamation. *Alberta Environment, Environmental Service, Environmental Sciences Division*, p. 191. 8 p.

Hu, H. & Sparks, D. 1991. Zinc deficiency inhibits chlorophyll synthesis and gas exchange in *Stuart Pecan*. *HortScience,* 26**,** 267-268.

Hu, X., Wang, D., Ren, S., Feng, S., Zhang, H., Zhang, J., Qiao, K. & Zhou, A. 2022. Inhibition of root growth by alkaline salts due to disturbed ion transport and accumulation in *Leymus chinensis*. *Environmental and Experimental Botany,* 200**,** 104907.

Jouyban, Z. 2012. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

Kamaluddin, M. & Zwiazek, J. J. 2002a. Ethylene enhances water transport in hypoxic aspen. *Plant Physiology,* 128**,** 962-969.

Kamaluddin, M. & Zwiazek, J. J. 2002b. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. *Tree Physiology,* 22**,** 1265-1270.

Khalid, R., Patrick Jr, W. & Gambrell, R. 1978. Effect of dissolved oxygen on chemical transformations of heavy metals, phosphorus, and nitrogen in an estuarine sediment. *Estuarine and Coastal Marine Science,* 6**,** 21-35.

Khan, M. M., Al-Mas'oudi, R. S., Al-Said, F. & Khan, I. Salinity effects on growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (*Cucumis sativus L.*). *International Conference on Food and Agricultural Sciences Malaysia: IACSIT Press*, 2013. 28-32.

Khayyat, M., Tehranifar, A., Akbarian, A., Shayesteh Nia, S. & Khabari, S. 2009. Effects of calcium forms on electrolyte leakage, total nitrogen, yield and biomass production by strawberry plants under NaCl salinity. *Journal of Central European Agriculture,* 10**,** 297-302.

Kieko, S. & Masahiko, I. 2012. The function of roots of tea plant (*Camellia sinensis*) cultured by a novel form of hydroponics and soil acidification. *American Journal of Plant Sciences,* 4, 1-4.

Lazorko, H. & Van Rees, K. C. 2012. Root distributions of planted boreal mixedwood species on reclaimed saline–sodic overburden. *Water, Air, & Soil Pollution,* 223**,** 215-231.

Li, Y., Zeng, H., Xu, F., Yan, F. & Xu, W. 2022. H^+-ATPases in plant growth and stress responses. *Annual Review of Plant Biology,* 73**,** 495-521.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996a. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183-183.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996b. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183.

López-Berenguer, C., García-Viguera, C. & Carvajal, M. 2006. Are root hydraulic conductivity responses to salinity controlled by aquaporins in broccoli plants. *Plant and Soil,* 279**,** 13-23.

Luna, D. F., Aguirre, A., Pittaro, G., Bustos, D., Ciacci, B. & Taleisnik, E. 2017. Nutrient deficiency and hypoxia as constraints to *Panicum coloratum* growth in alkaline soils. *Grass and Forage Science,* 72**,** 640-653.

Lyubimova, I., Pankova, Y. & Pestov, L. 2009. Amelioration of alkali (sodic/solonetz) soils. *Agricultural Land Improvement'Amelioration and Reclamation,* 2**,** 241-266.

Mackinnon, M., Matthews, J., Shaw, W. & Cuddy, R. 2001. Water quality issues associated with composite tailings (CT) technology for managing oil sands tailings. *International Journal of Surface Mining, Reclamation and Environment,* 15**,** 235-256.

Malakar, P. & Chattopadhyay, D. 2021. Adaptation of plants to salt stress: the role of the ion transporters. *Journal of Plant Biochemistry and Biotechnology,* 30**,** 668-683.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. *Academic press*. 447 p.

Matthews, J., Shaw, W., Mackinnon, M. & Cuddy, R. 2002. Development of composite tailings technology at Syncrude. *International Journal of Surface Mining, Reclamation and Environment,* 16**,** 24-39.

Mehta, P., Jajoo, A., Mathur, S. & Bharti, S. 2010. Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. *Plant Physiology and Biochemistry,* 48**,** 16-20.

Mengel, K. & Kirkby, E. A. 2012. Principles of plant nutrition. *Springer Science & Business Media*, 93, 479-480.

Mu, X. & Chen, Y. 2021. The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry*, *158*,76-82.

Munns, R. & Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59**,** 651.

Nable, R. O., Bañuelos, G. S. & Paull, J. G. 1997. Boron toxicity. *Plant and soil,* 193**,** 181-198.

Nero, V., Farwell, A., Lee, L., Van Meer, T., Mackinnon, M. & Dixon, D. 2006. The effects of salinity on naphthenic acid toxicity to yellow perch: Gill and liver histopathology. *Ecotoxicology and Environmental Safety,* 65**,** 252-264.

Ondrasek, G., Rathod, S., Manohara, K. K., Gireesh, C., Anantha, M. S., Sakhare, A. S., Parmar, B., Yadav, B. K., Bandumula, N. & Raihan, F. 2022. Salt stress in plants and mitigation approaches. *Plants,* 11**,** 717.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M. & Dat, J. F. 2008. An overview of plant responses to soil waterlogging. *Plant stress,* 2**,** 20-27.

Perala, D., Burns, R. & Honkala, B. 1990. *Populus tremuloicies Michx*.-Quaking Aspen. *Silvics of North America: Hardwoods; Burns, RM, Honkala, BH, Eds***,** 555-569.

Princi, M. P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F. & Abenavoli, M. R. 2016. Boron toxicity and tolerance in plants: Recent advances and future perspectives. *Plant Metal Interaction***,** 115-147. doi: 10.1007/978-3-030-20732-8.

Raven, J. 1980. Short-and long-distance transport of boric acid in plants. *New Phytologist,* 84**,** 231-249.

Rawat, J., Pandey, N. & Saxena, J. 2022. Role of potassium in plant photosynthesis, transport, growth and yield. *Role of Potassium in Abiotic Stress***,** 1-14. doi: 10.1007/978-981-16-4461-0_1.

Redfield, E., Croser, C., Zwiazek, J., Mackinnon, M. & Qualizza, C. 2003. Responses of red‐osier dogwood to oil sands tailings treated with gypsum or alum. *Journal of Environmental Quality,* 32**,** 1008-1014.

Redfield, E. B., Durnie, S. M. & Zwiazek, J. J. 2004. Effects of hypoxia on ion accumulation in wild raspberry (*Rubus idaeus*) exposed to water released from saline oil sands mine tailings. *Environmental and Experimental Botany,* 52**,** 1-9.

Renault, S., Croser, C., Franklin, J., Zwiazek, J. & Mackinnon, M. 2001. Effects of consolidated tailings water on red-osier dogwood (*Cornus stolonifera Michx*) seedlings. *Environmental Pollution,* 113**,** 27-33.

Renault, S., Lait, C., Zwiazek, 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. & Mackinnon, M. 1999. Responses of boreal plants to high salinity oil sands tailings water. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Vol. 28, No. 6, pp. 1957-1962.

Renault, S., Zwiazek, J., Fung, M. & Tuttle, S. 2000. Germination, growth and gas exchange of selected boreal forest seedlings in soil containing oil sands tailings. *Environmental Pollution,* 107**,** 357-365.

Rengel, Z. 2002. Handbook of plant growth pH as the master variable. *CRC Press*, Vol. 88. 17 p.

Saeed, R., Mirza, S. & Ahmad, R. 2014. Electrolyte leakage and relative water content as affected by organic mulch in okra plant (*Abelmoschus esculentus (L.) Moench*) grown under salinity. *FUUAST Journal of Biology,* 4**,** 221-227.

Schneider, R., Fortin, M., Berninger, F., Ung, C.-H., Swift, D. E. & Zhang, S. 2011. Modeling jack pine (*Pinus banksiana*) foliage density distribution. *Forest Science,* 57**,** 180-188.

Schumacher, B. A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. *Ecological Risk Assessment Support Center Office of Research and Development*.

Scott, A. C., Young, R. F. & Fedorak, P. M. 2008. Comparison of GC–MS and FTIR methods for quantifying naphthenic acids in water samples. *Chemosphere,* 73**,** 1258-1264.

Sestak, Z., Catský, J. & Jarvis, P. G. 1971. Plant photosynthetic production. Manual of methods. *Plant Photosynthetic Production. Manual of methods.*

Steffens, D., Hutsch, B., Eschholz, T., Losak, T. & Schubert, S. 2005. Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment,* 51**,** 545-552.

Subgroup, T. & Association, C. E. M. 2009. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region. *Alberta Environment*. 43 p.

Tan, X., Liu, M., Du, N. & Zwiazek, J. J. 2021. Ethylene enhances root water transport and aquaporin expression in trembling aspen (*Populus tremuloides*) exposed to root hypoxia. *BMC Plant Biology,* 21, 1-8.

Tan, X., Xu, H., Khan, S., Equiza, M. A., Lee, S. H., Vaziriyeganeh, M. & Zwiazek, J. J. 2018. Plant water transport and aquaporins in oxygen-deprived environments. *Journal of Plant Physiology,* 227**,** 20-30.

Therby-Vale, R., Lacombe, B., Rhee, S. Y., Nussaume, L. & Rouached, H. 2022. Mineral nutrient signaling controls photosynthesis: focus on iron deficiency-induced chlorosis. *Trends in Plant Science,* 27**,** 502-509.

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.

Tozlu, I., Moore, G. A. & Guy, C. L. 2000. Effects of increasing NaCl concentration on stem elongation, dry mass production, and macro-and micro-nutrient accumulation in *Poncirus trifoliata*. *Functional Plant Biology,* 27**,** 35-42.

Turan, M. A., Elkarim, A. H. A., Taban, N. & Taban, S. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research,* 4**,** 893-897.

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants***,** 179-192. doi: 10.1007/978-81-322-0807-5_7.

Viereck, L. A. & Johnston, W. F. 1990. *Picea mariana (Mill.)* BSP black spruce. *Silvics of North America,* 1**,** 227-237.

Wang, H. & Jin, J. Y. 2005. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. *Photosynthetica: International Journal for Photosynthesis Research,* 43**,** 591-596.

Watson, L. 1989. Manual of plant species suitability for reclamation in Alberta. *Alberta Land Conservation and Reclamation Council, Reclamation Research Technical Advisory Committee.* 178 p. doi: 10.5962/bhl.title.104055

Wu, L., Ok, Y. S., Xu, X. L. & Kuzyakov, Y. 2012. Effects of anionic polyacrylamide on maize growth: a short term 14 C labeling study. *Plant and soil,* 350**,** 311-322.

Xu, F. 2017. Effects of mineral nutrition and iron supply on growth and physiological responses of selected boreal plant species to root zone pH. *Thesis, University of Alberta. Department of Renewable Resources.* 66 p. doi: 10.7939/R3CR5NT49

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.

Yi, H., Polanco, M. C., Mackinnon, M. D. & Zwiazek, J. J. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany,* 62**,** 357-363.

Yokoi, S., Bressan, R. A. & Hasegawa, P. M. 2002. Salt stress tolerance of plants. *JIRCAS Working Report,* 23**,** 25-33.

Yordanova, R. Y. & Popova, L. P. 2007. Flooding-induced changes in photosynthesis and oxidative status in maize plants. *Acta Physiologiae Plantarum,* 29**,** 535-541.

Yruela, I. 2013. Transition metals in plant photosynthesis. *Metallomics,* 5**,** 1090-1109.

Zhang, B., Zhang, C., Liu, C., Jing, Y., Wang, Y., Jin, L., Yang, L., Zhao, F., Lan, W., Fu, A., Shi, J. & Luan, S. 2018. Inner envelope CHLOROPLAST MANGANESE TRANSPORTER 1 supports manganese homeostasis and phototrophic growth in Arabidopsis. *Molecular Plant,* 11**,** 943-954-954.

Zhang, W.-Q., 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**,** 113945-113953.

Zhang, W., Calvo-Polanco, M., Chen, Z. & 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. Q., Fleurial, K., Moawad, M., Vassov, R., Macdonald, S. E. & Zwiazek, J. J. Growth responses of 20 boreal forest species to oil sands non-segregating tailings: significance for reclamation. *Restoration Ecology*, e13874. doi: 10.1111/rec.13874

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, D., Oosterhuis, D. & Bednarz, C. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. *Photosynthetica,* 39, 103-109.

Zhu, J.-K. 2001. Plant salt tolerance. *Trends in Plant Science,* 6, 66-71.

2.6 Tables

Table 2. 1: pH, electrical conductivity (EC, mS cm^{-1}), dissolved oxygen (DO, mg L^{-1}), naphthenic acids (NAs, mg L^{-1}), and elemental concentrations (mg L^{-1}) of treatment solutions at the beginning of the experiment. 25% Hoagland's + 25% eNST treatment is the mixture of 50% Hoagland's solution and 50% eNST water. NM -not measured.

	pH	EC	DO	NAs	Na	B	Fe	Zn	P	K	Mn	Ca	Cu	Mg	S
Aerated 25% Hoagland's	5	1.8	5.8	NM	0.721	0.143	0.385	0.036	42.3	121.9	0.014	76.873	0.015	12.43	18.15
Aerated 25% Hoagland's +25% eNST	8.5	2.8	5.3	NM	271.5	1.589	0.004	0.012	18.7	68.43	0	31.628	0	7.523	23.53
Hypoxic 25% Hoagland's	6	1.4	1	NM	0.878	0.137	0.363	0.059	40.3	115.4	0.042	72.611	0.009	12.04	17.5
Hypoxic 25% Hoagland's +25% eNST	7.5	2.3	1.3	NM	282.8	1.665	0.004	0.015	19.5	71.18	0	34.521	0	7.875	22.58
100% eNST	9.1	4.5	NM	65	1032	5.632	0.005	0	0.15	28	0.013	5.258	0	6.344	59.59

Table 2. 2: Leaf element concentrations (mg kg^{-1}, unit for N is %) of paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), jack pine (*Pinus banksiana*), and black spruce (*Picea mariana*) seedlings treated with eNST water and/or hypoxia. Different letters indicate significant differences between treatments within each species (P<0.05).

		Fe	Zn	P	N	Mn	K	Ca	Cu	Mg	S
Paper Birch	Aerated	92.3 ±17.3 a	92.9 ±11.8 a	6789.8 ±622.1 a	3.9 ±0.1 a	63.2 ±11.8 a	28659.7 ±1678.7 b	7521.6 ±894 b	1.8 ±0.64 ab	2439 ±203.7 b	2584.4 ±134 a
	Aerated eNST	11.8 ±0.69 c	20.3 ±2.9 bc	4834 ±319.4 b	2.2 ±0.15 b	19.1 ±4.1 b	41665.8 ±3566.2 a	10396 ±1156.6 a	1.1 ±0.53 b	4412.6 ±565.7 a	1449.2 ±155.4 b
	Hypoxic	36 ±2.4 b	13.4 ±1.2 b	2328.4 ±403 c	2 ±0.09 b	49.8 ±6.4 a	28634.8 ±3260.1 b	4849.1 ±402.6 c	3.3 ±0.45 a	1407.1 ±134.5 c	2165.2 ±230.1 a
	Hypoxic eNST	29.4 ±1.3 b	24.3 ±2 c	1472.3 ±122.8 d	1.5 ±0.07 c	29.9 ±3.4 ab	14739 ±752.4 c	2407.3 ±110 d	3.3 ±0.4 a	911.5 ±44.8 d	1132.1 ±82.6 b
Trembling Aspen	Aerated	64 ±3.4 a	49.8 ±6.2 a	4824.9 ±590.2 a	3.4 ±0.2 a	21.6 ±5.8 a	26990.1 ±2440.2 a	12961.4 ±1749.8 a	7.1 ±1.1 a	2266.3 ±116.7 a	2699.1 ±206.1 a
	Aerated eNST	58.5 ±14.4 a	12.5 ±4.5 b	2425.6 ±278.6 b	2.2 ±0.06 b	18.9 ±4.8 a	26140.3 ±2276.2 a	12170.3 ±1361.6 ab	1.7 ±0.7 b	2455 ±207.1 a	1490.6 ±78.6 b
	Hypoxic	53.3 ±7.7 a	16.9 ±2.6 b	3577.7 ±338.3 a	3 ±0.16 c	25.7 ±3.7 a	17761.3 ±898.2 b	12121.4 ±754.2 ab	2.7 ±0.49 b	1559.4 ±92 b	2338.6 ±168.3 a
	Hypoxic eNST	39.8 ±7.3 a	12.8 ±2.7 b	1969.7 ±119 b	1.8 ±0.08 d	21.3 ±6.6 a	23728.5 ±1061 a	7619.3 ±1095.1 b	2.8 ±0.52 b	1168.1 ±167.6 b	1642.3 ±193.6 b
Jack Pine	Aerated	117.9 ±13.6 ab	86.7 ±5.1 a	2789.1 ±231.2 a	1.9 ±0.09 b	218.4 ±22 a	12338.8 ±991.2 a	4642.4 ±523.8 a	5.3 ±0.58 a	1854.7 ±71 a	2354.6 ±209.7 a
	Aerated eNST	150.8 ±30.5 a	57.7 ±10.1 b	2521.3 ±312.7 ab	2 ±0.17 b	208.9 ±19.9 a	6910.2 ±518.2 b	3112.7 ±271 b	5.3 ±0.59 a	1605.6 ±72.4 ab	2535.2 ±197.7 a
	Hypoxic	61.5 ±7.4 c	47.4 ±5.5 bc	2050.5 ±232.2 ab	2.5 ±0.13 a	171.9 ±17.8 a	12177.3 ±1162.3 a	3408 ±281.1 ab	6.1 ±0.5 a	1562.1 ±79.3 ab	2017.8 ±196.8 a
	Hypoxic eNST	73.6 ±5.3 bc	35.4 ±2.2 c	1768 ±100.8 b	2.4 ±0.12 a	161.3 ±16.7 a	5367.3 ±677.9 b	2643 ±227.6 b	5.6 ±0.43 a	1491.3 ±99 b	1960.4 ±154.6 a
Black Spruce	Aerated	72 ±11.4 a	55.5 ±4.9 b	3133.7 ±135.5 a	2.2 ±0.1 a	170.1 ±8 ab	11044.5 ±467.9 b	4567.7 ±445 ab	7.8 ±0.73 a	1777.2 ±95.6 ab	1369.2 ±55.3 ab
	Aerated eNST	48.9 ±4.8 a	52.9 ±6 b	2792.9 ±171.9 ab	1.8 ±0.1 b	147.9 ±13.7 b	7333.1 ±506.3 c	3319 ±292.5 bc	6.4 ±0.91 a	1399.1 ±117 bc	1246.4 ±106.2 bc
	Hypoxic	78.3 ±6.9 a	88.2 ±8.4 a	3020.6 ±173.4 a	2.3 ±0.14 a	227.3 ±22.3 a	15558.6 ±461.7 a	5961.8 ±583.7 a	8.4 ±0.4 a	1948.9 ±169.6 a	1669.2 ±74 a
	Hypoxic eNST	55.7 ±9 a	51.7 ±7.7 b	2353.2 ±153.2 b	1.3 ±0.07 c	142.4 ±23.9 b	6684.9 ±783.5 c	2994.4 ±381.5 c	7.1 ±0.75 a	1149.3 ±104.7 c	1004 ±91.9 c

2.7 Figures

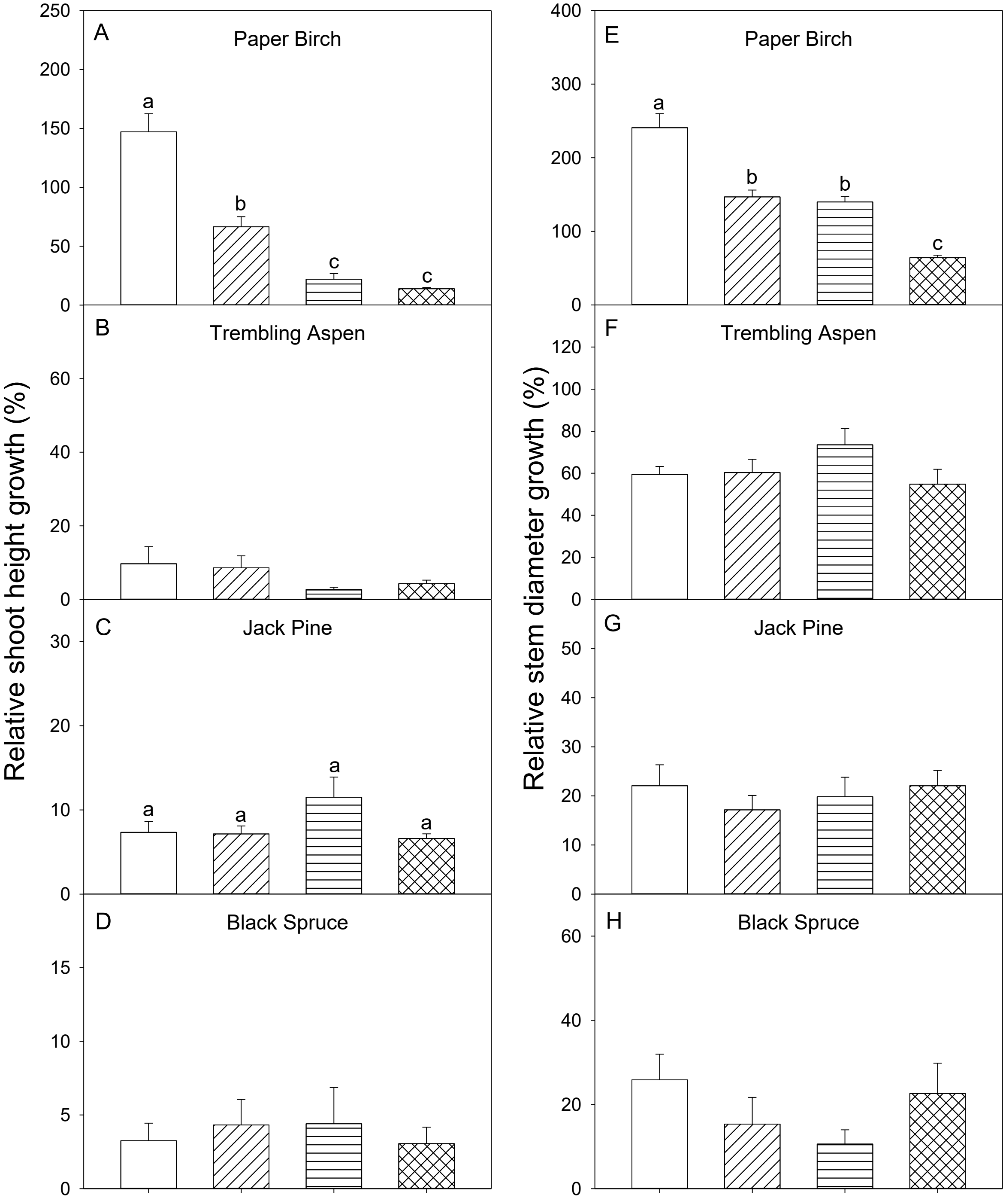

Figure 2. 1: Relative shoot height growth (RSHG) and relative diameter growth (RSDG) of paper birch (A, E), trembling aspen (B, F), jack pine (C, G), and black spruce (D, H) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=15). Different letters indicate significant differences between treatments within each species ($P<0.05$).

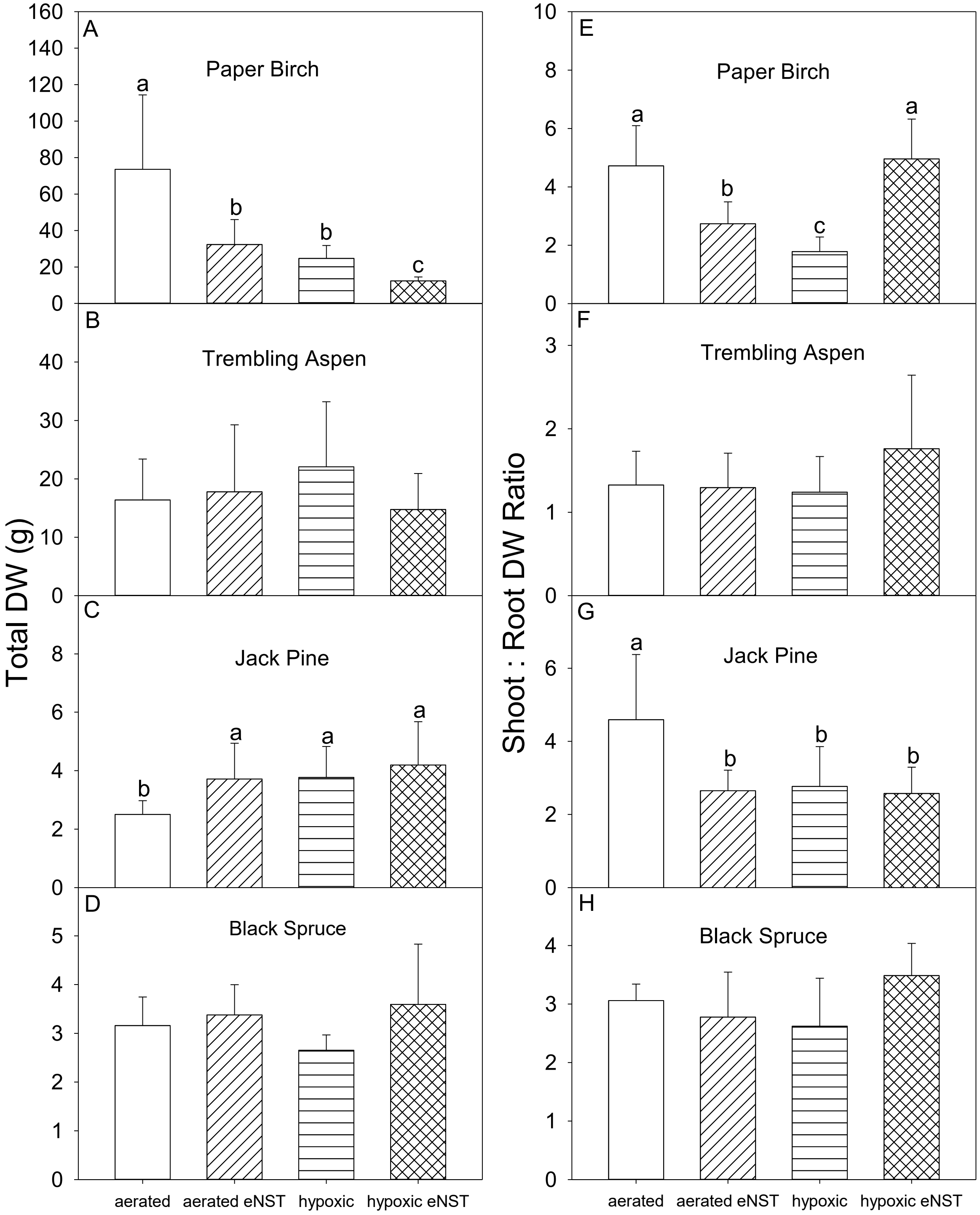

Figure 2. 2: Total dry weight (DW) and shoot to root dry weight ratio of paper birch (A, E), trembling aspen (B, F), jack pine (C, G), and black spruce (D, H) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=15). Different letters indicate significant differences between treatments within each species (*P*<0.05).

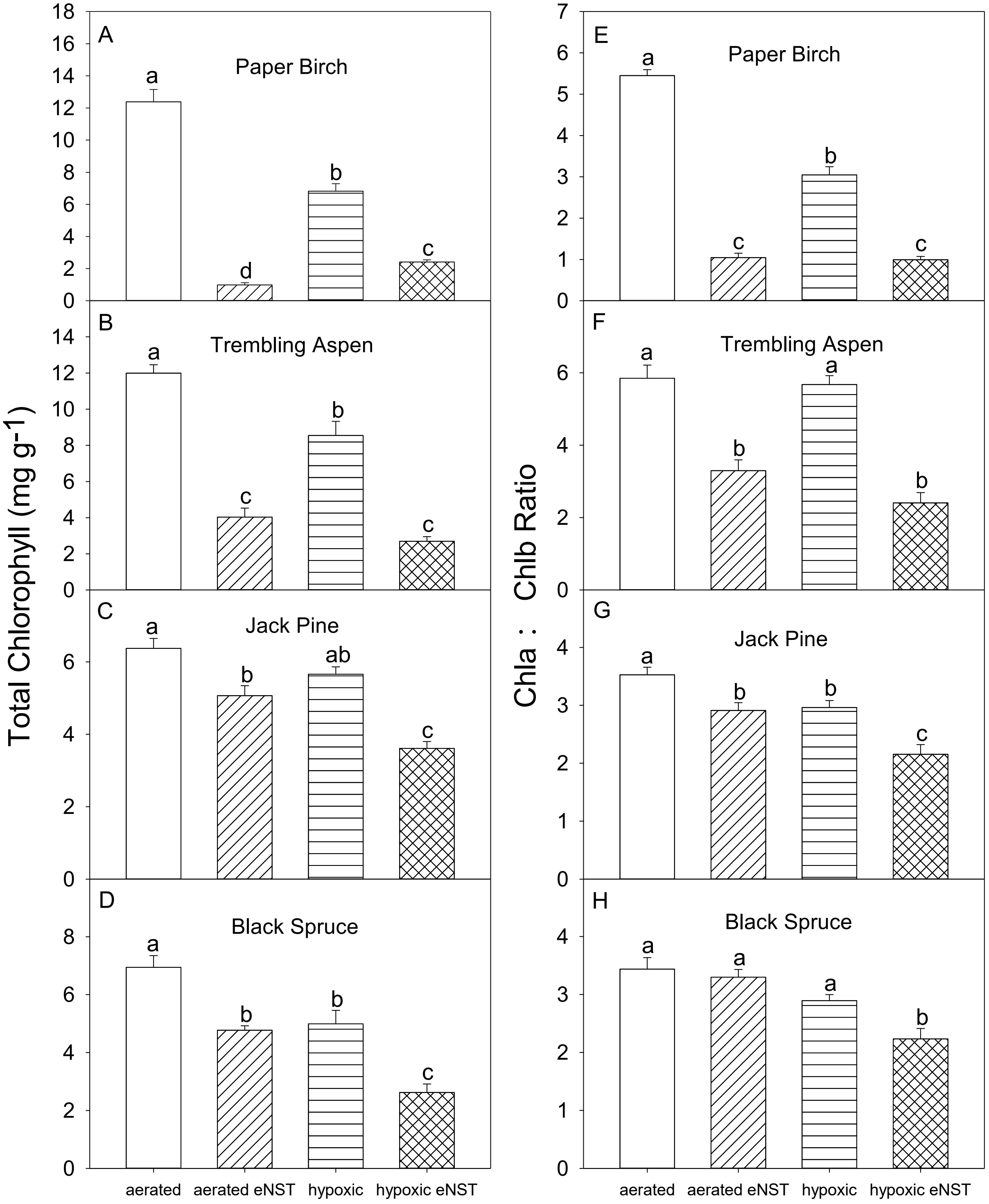

Figure 2. 3: Total chlorophyll concentration and chlorophyll a (Chl a) to chlorophyll b (Chl b) ratio of paper birch (A, E), trembling aspen (B, F), jack pine (C, G), and black spruce (D, H) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=6). Different letters indicate significant differences between treatments within each species (P<0.05).

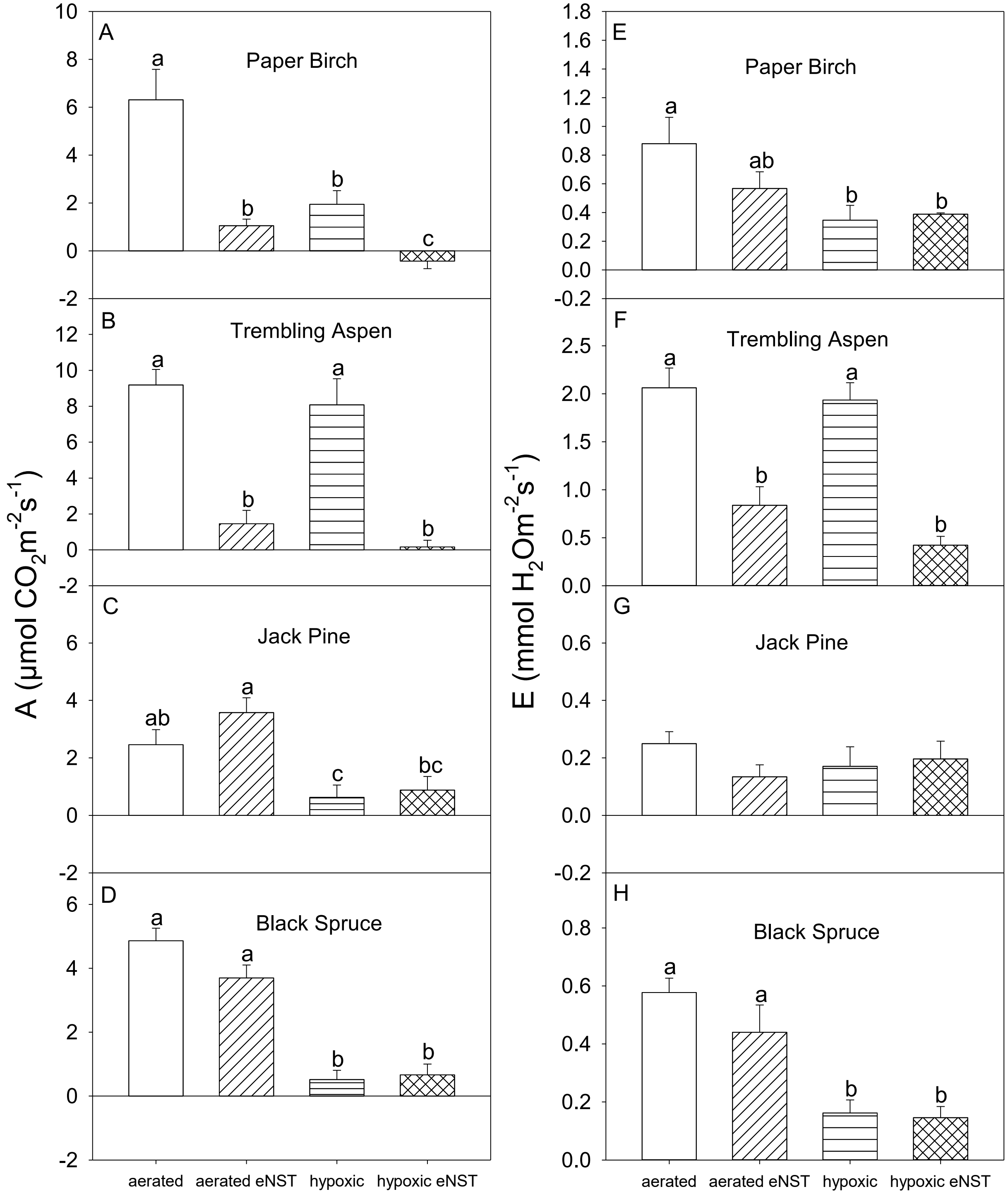

Figure 2. 4: Net photosynthesis and transpiration rate of paper birch (A, E), trembling aspen (B, F), jack pine (C, G), and black spruce (D, H) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=6). Different letters indicate significant differences between treatments within each species (P<0.05).

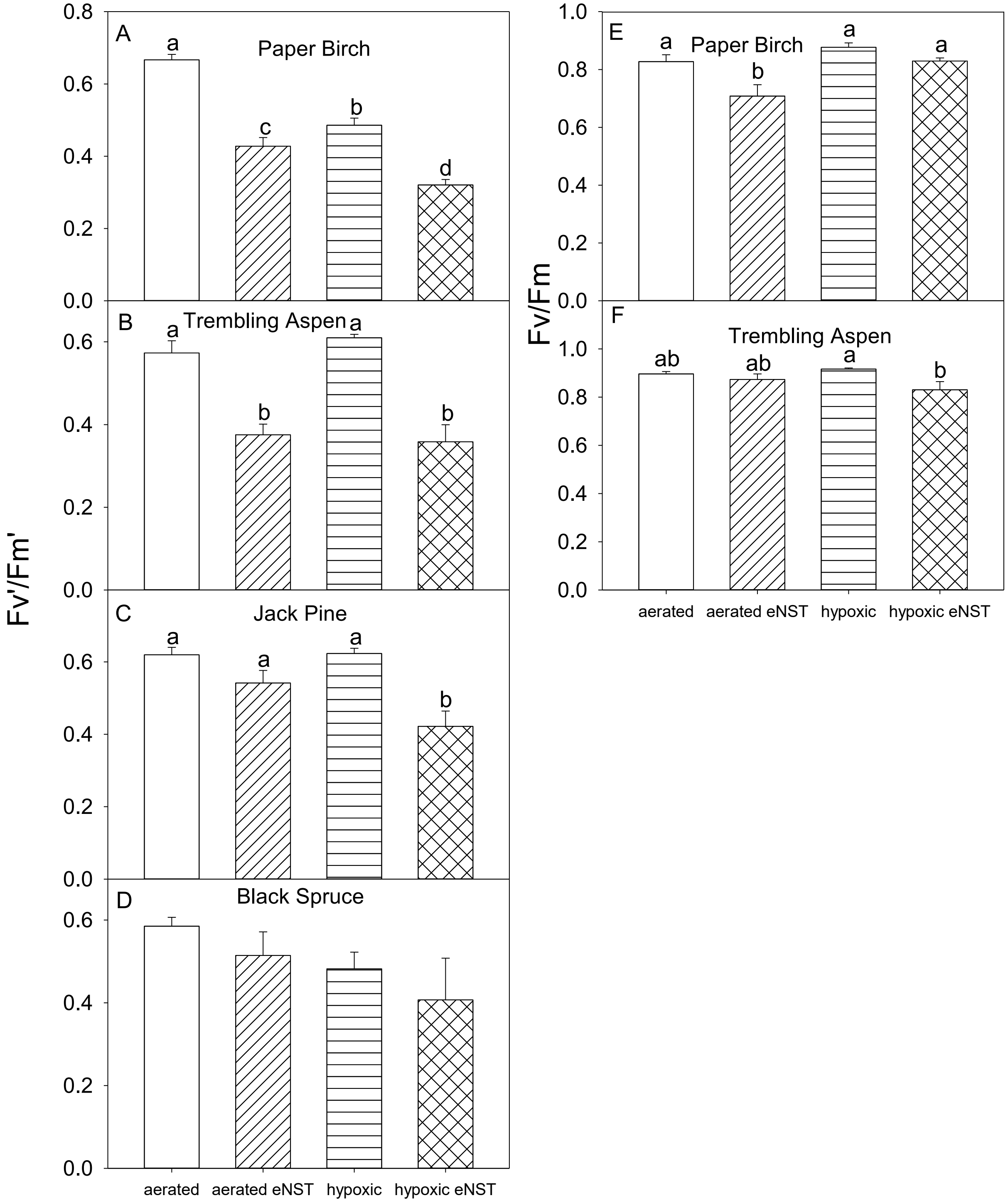

Figure 2. 5: Photosystem II (PS II) maximum efficiency (Fv'/Fm') of paper birch (A), trembling aspen (B,), jack pine (C), and black spruce (D) and maximum quantum efficiency of PS II photochemistry (Fv/Fm) of paper birch (E) and trembling aspen (F) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=6). Different letters indicate significant differences between treatments within each species ($P<0.05$).

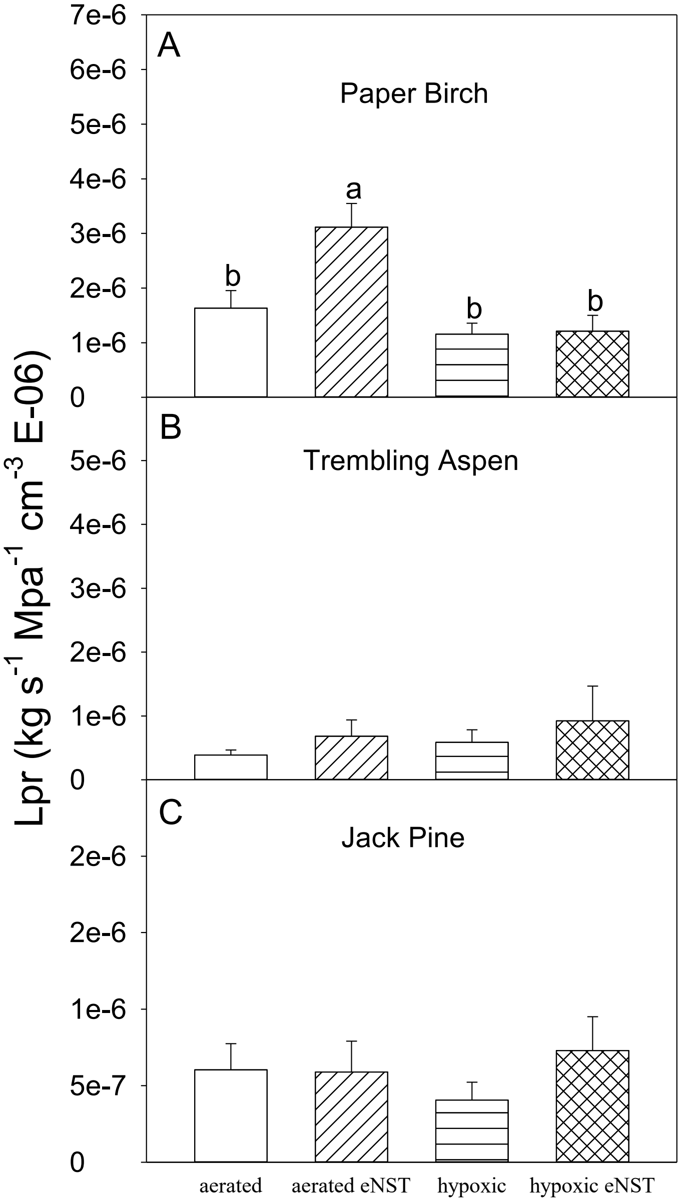

Figure 2. 6: Root hydraulic conductivity (Lpr) of paper birch (A), trembling aspen (B), and jack pine (C) seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=15). Different letters indicate significant differences between treatments within each species (*P*<0.05).

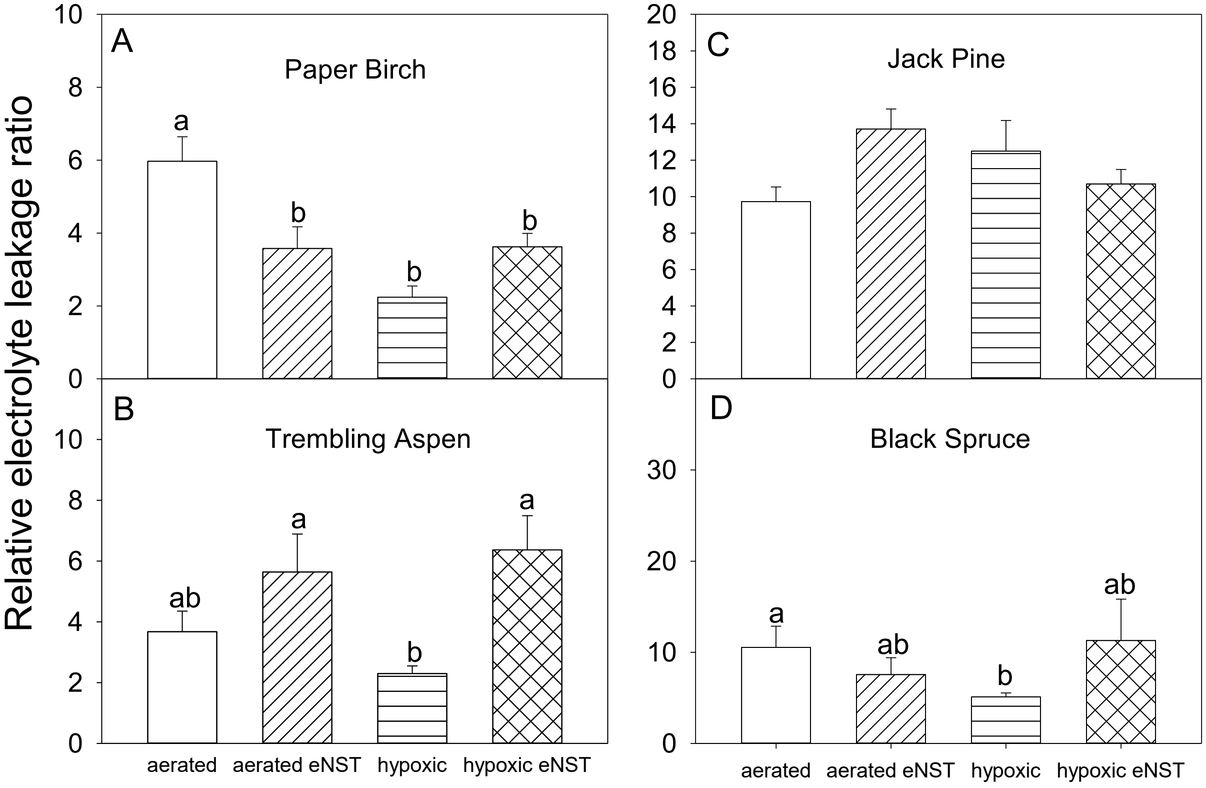

Figure 2. 7: Relative electrolyte leakage ratio of paper birch (A), trembling aspen (B), jack pine (C), and black spruce (D) seedlings treated with eNST water and/or root hypoxias (Mean ± SE, n=6). Different letters indicate significant differences between treatments within each species (*P*<0.05).

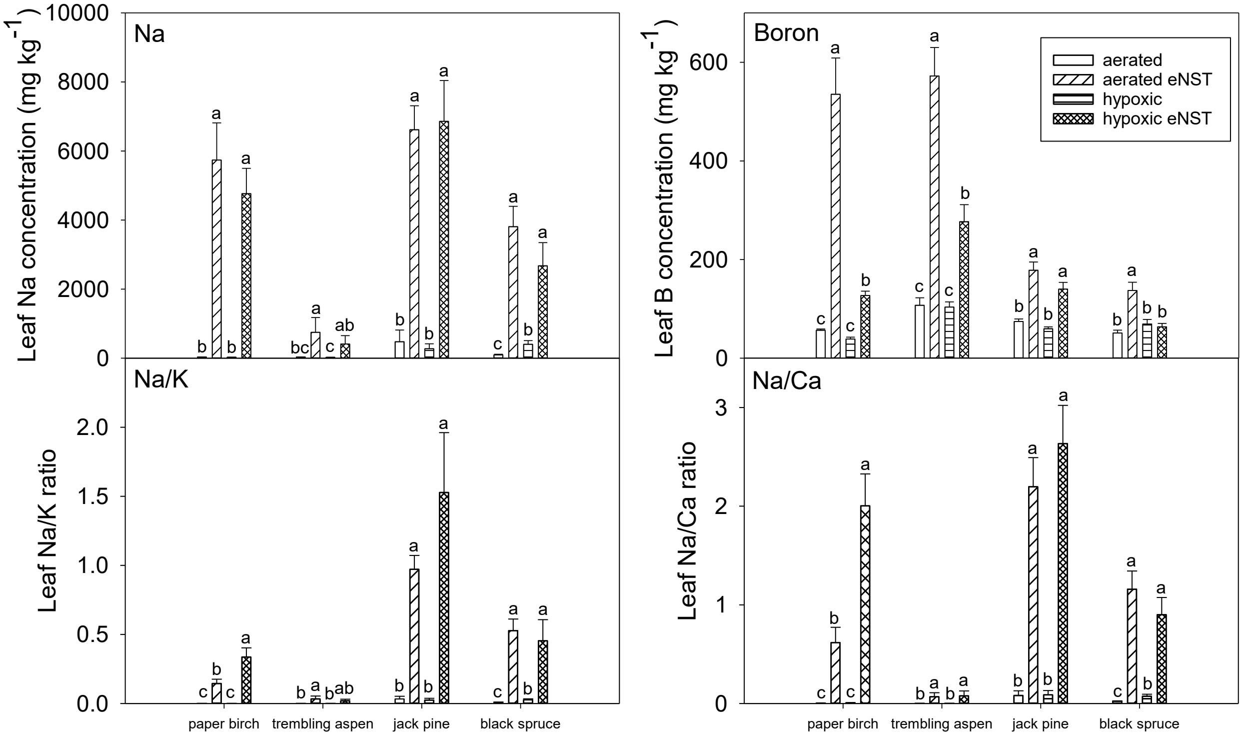

Figure 2. 8: Leaf concentrations of Na, Na/K ratio, Na/Ca ratio, and leaf B of paper birch, trembling aspen, jack pine and black spruce seedlings treated with eNST water and/or root hypoxia (Mean ± SE, n=6). Different letters indicate significant differences between treatments within each species (*P*<0.05).

Chapter 3 Effects of Enhanced Non-Segregated Tailings and Salinity on the Growth of Three Northern Grasses

3.1 Introduction

Elevated salinity levels are of concern in the oil sands reclamation areas that are affected by the oil sands tailings. In the mine tailings produced by the hot water extraction process, salt levels can reach 4.5 mS cm^{-1}. As a result, the revegetation of sites affected by the oil sands tailings may be challenging. Salt stress inhibits plant growth and physiological processes (Marschner, 2011) by inducing osmotic stress and disrupting plant ion balance (Hasegawa et al., 2000, Lambers et al., 2008). In addition to a direct inhibition of root hydraulic conductivity through the effects on aquaporins (Lee and Zwiazek, 2015, Vaziriyeganeh et al., 2018), salts accumulated in the soil decrease soil osmotic potential, which leads to reduced water uptake and water deficit stress (Zhu, 2001, Munns and Tester, 2008). Salt stress also induces the synthesis of abscisic acid, leading to a stomatal closure and reduced transpiration rates (Zhu, 2001, Munns and Tester, 2008). Since Na also inhibits the plant uptake of K and other essential elements, Na salts also affect plant nutrition and ion balance (Zhu, 2001, Jouyban, 2012,). High Na concentration also displaces Ca from the plant cell membranes, resulting in increased membrane permeability and K leakage (Liang et al., 1996).

In addition to elevated salt levels, oil sands tailings are characterized by the pH values that may be as high as 9.0 (Renault et al., 2001, Zhang et al., 2020, Fleurial et al., 2022) High soil pH affects plant uptake of the essential elements such as Fe, Zn, Mn, and P, leading to nutrient deficiencies (Howat, 2000, George et al., 2012, Zhang et al., 2020)). Moreover, high root zone pH induces stomatal closure and upsets plant water relations (Zhang et al., 2013, 2015). High pH also disrupts the H^+ gradient in plant roots. Since the H^+ gradient is essential for nutrient uptake, high root zone pH induces nutrient deficiencies and limits plant growth (Hu et al., 2022).

Most oil sands land reclamation studies have focused on the effects of tailings on woody plants. However, grass species are also important for improving the success of land reclamation. Grasses can grow rapidly to establish vegetation cover. Prior to planting tree seedlings, grasses are often planted to improve the soil environment and reduce water erosion for subsequent revegetation of woody plants (Fung and Macyk, 2000, Renault et al., 2003, Renault et al., 2004, Dhar et al., 2018). Therefore, elucidating growth performance of grasses in soils affected by oil sands tailings provides valuable information for oil sands reclamation.

Halophytes account for about one percent of the world's plant species. Halophytic plants can tolerate soil concentrations of 200 mM NaCl or higher, which is detrimental for most other plants (Flowers and Colmer, 2008a). The majority of plants are glycophytes and can only survive in soils with low salt levels (Cheeseman, 2015). Halophytes can tolerate high salinity through several mechanisms including osmolyte accumulation and succulence, which help plants lower osmotic potential and maintain pressure under salt stress (Van Zelm et al., 2020). Besides, halophytes have stronger abilities than glycophytes to remove sodium from cytoplasm, sequester it into vacuoles, and retain potassium (Van Zelm et al., 2020, Barros et al., 2021). Furthermore, halophytes may develop special structures, including salt glands and epidermal bladder cells, to secret salts (Van Zelm et al., 2020).

Since some of the oil sands reclamation sites are affected by elevated salinity levels, their revegetation is especially challenging. In the pure mature fine oil sands tailings, the grass species that are adapted to saline soils had significantly higher aboveground biomass compared with the salt-sensitive grass species (Luna Wolter and Naeth, 2014). The plant species from natural saline areas are considered valuable to convert saline land to agricultural land and should be considered for revegetation of oil sands reclamation sited affected by salinity (Purdy et al., 2005, Sarath et al., 2021). Moreover, since halophytes can accumulate salts in their tissues, harvesting and removing halophytes from the land on which they grow can significantly contribute to land desalination (Sarath et al., 2021). Thus, the halophytic grasses could prove useful for improving the success of oil sands land reclamation.

To enhance the environmental sustainability of oil sands production, Canadian Natural Resources Limited (CNRL) developed the enhanced non-segregating tailings (eNST) technology, as an improvement of the earlier developed non-segregating tailings (NST) (CNRL, 2017). To produce eNST, a flocculant (A-PAM) is added in the tailings streamline prior to deposition. However, the effects of eNST on the growth of both halophytic and glycophytic plants are little understood.

The present study aimed to elucidate the effects of eNST water released during tailings consolidation on three grass species varying in salt tolerance levels: Nuttall's alkaligrass (*Puccinellia nuttalliana*), alkali bluegrass (*Poa juncifolia*), and Kentucky bluegrass (*Poa pratensis*). Among these three grass species, *Puccinellia nuttalliana* is one of the most salt-tolerant halophytic grass species in western North America (Macke and Ungar, 1971), *Poa juncifolia* is considered a relatively salt-tolerant grass species (Vaziriyeganeh et al., 2018, Vaziriyeganeh et al., 2022), and *Poa pratensis* is a glycophytic grass species, and is relatively sensitive to salt (Bushman et al., 2016, Vaziriyeganeh et al., 2022). All three grass species are considered native to northern Alberta. According to the previous studies, *Puccinellia nuttalliana* is adapted to the boreal high salinity landscape in Alberta (Purdy et al., 2005, Phillips et al., 2016). The Athabasca oil sands land reclamation guideline categorizes *Puccinellia nuttalliana* as a high salinity tolerant grass species in the Athabasca region wet meadow plant community (Government of Alberta, 2010).

The objectives of this study were to: 1) examine the effects of eNST water on the growth of grasses varying in salt tolerance and 2) examine the role of salinity in growth responses of plants affected by the eNST tailings water. I tested the hypothesis that salinity of eNST water is the main factor affecting the growth and physiological processes in the studied plants and, hence, *Puccinellia nuttalliana* would exhibit better growth performance compared with *Poa juncifolia* and *Poa pratensis* when affected by the eNST water.

3.2 Materials and methods

3.2.1 Plant materials and growth conditions

Nuttall's alkaligrass (*Puccinellia nuttalliana (Schult.) Hitchc.*), alkali bluegrass (*Poa juncifolia Scribn.*), and Kentucky bluegrass (*Poa pratensis L.*) were grown from seeds collected in central Alberta, Canada. The seeds were surface sterilized with 70% ethanol for two minutes and rinsed with distilled water before being placed in pots in the autoclaved commercial growing mix (sunshine professional growing mix 2.8 CU FT SS LA4, Sun Gro Horticulture, Seba Beach, AB, Canada), which is made of Canadian sphagnum peat moss, coarse perlite, dolomitic limestone, and long-lasting wetting agent. Following germination, the plants were grown for 8 weeks in a controlled-environment growth room set to 16 hours (6:00 - 22:00 h) photoperiod with 300 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). The growth room temperature was 22/18°C (day/night) and the relative humidity was 65±10%. After 8 weeks, the roots were gently washed with water and the seedlings were transferred to the hydroponic system with 25% Hoagland's solution (Epstein and Bloom, 1853).

The hydroponic setup consisted of 30 plastic 10-L containers filled with nutrient solution. The containers were covered by plastic lids in which 9 holes were drilled to pass seedling roots through the lid into the nutrient solution. A foam plug was wrapped around the root collar to hold the seedlings in place. Each treatment had six replicated plastic tubs. There were three seedlings of each species in each of the six replicated containers per treatment for the total of 18 seedlings per species per treatment (n=18).

The dissolved oxygen level in the nutrient solution in each container was maintained at about 6 mg L^{-1} by air pumps, which were immersed in the mineral solution. The dissolved oxygen level was measured by a dissolved oxygen meter (Traceable Dissolved Oxygen meter, Model 4320, Traceable company, USA). The electrical conductivity (George et al., 2012) and pH of the mineral solution in each container were measured by the electrical conductivity meter (Traceable Expanded Range Conductivity Meter, Model 4360, Traceable company, USA), and a pH meter (Thermo Scientific Orion Star A121 Portable pH Meter, Thermo Scientific Company, USA) twice a week.

After two weeks in Hoagland's solution, the following treatments were applied by mixing equal volumes of distilled water and pure eNST water, which were adjusted to 50% concentration of Hoagland's solution by adding the by adding the concentrated nutrient solution: 1) 50% Hoagland's solution (control) 2) 50% Hoagland's solution + 50% eNST water, 3) 50% Hoagland's solution + 100% eNST water; 4) 50% Hoagland solution + 50% eNST water + 60 mM NaCl; 5) 50% Hoagland solution + 50% eNST water + 120 mM NaCl.

3.2.2 Elemental composition of hydroponic growth solution

The Na, B, Fe, Zn, P, N, Mn, K, Ca, Cu, Mg, and S concentrations of the treatment solutions were analyzed by the Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Thermo iCAP6300 Duo) in the Natural Resource Analytical Laboratory (NRAL) at the University of Alberta. Four containers were selected from each treatment group. A 30 ml treatment solution sample was collected from each of the four containers in each treatment. Prior to the analysis, all samples were filtered with a 0.45 µm Polyvinyl Difluoride (PVDF) syringe-driven filter unit (EMD Millipore Corporation, Billerica, MA, USA).

3.2.3 Mortality rates, dry weights, leaf chlorophyll concentration

At the end of the experiment (six-weeks of treatments), the mortality rate for each species in each treatment was calculated. All plants were harvested, and their stems and roots separated and placed in separate paper bags. To determine the dry weights, 18 samples per species per treatment were dried in an oven at 70°C for 96 hours.

For the leaf chlorophyll concentrations and elemental analysis, six seedlings of each species in each treatment were randomly selected and the fully expanded leaves were excised and freeze dried (Labconco Freeze Dry System 4.5 Liter) for 96 h. After that, the leaf samples were ground into powder with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). For chlorophyll extraction, 10 mg samples of leaf powder were mixed with 8 ml dimethyl sulfoxide (DMSO) and placed in a water bath at 65°C for 22 h. After filtering, the chlorophyll a and b concentrations were measured with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden) at 648 nm and 665 nm. The total chlorophyll concentration was calculated by the Arnon's equation (Sestak et al., 1971).

3.2.5 Gas exchange and chlorophyll fluorescence

Eight seedlings of each species from each treatment were randomly taken to measure the net photosynthesis rates (Pn), transpiration rates (E), and the maximum efficiency of photosystem II (Fv'/Fm') values. The measurements of Pn, E, and Fv'/Fm' were conducted from three to six hours following the onset of photoperiod after 3 and 6 weeks of treatments using the LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NB, USA). For the measurements, the CO_2 reference concentration was set to 400 μmol mol^{-1}, the flow rate in the leaf chamber was 200 μmol s^{-1}, the leaf chamber temperature was at 20 °C, and the PPFD of the leaf chamber was 400 μmol m^{-2} s^{-1}.

3.2.6 Leaf elemental concentrations

The leaf Na, B, Fe, Zn, P, Mn, K, Ca, Cu, Mg, and S concentrations were analyzed by the Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Thermo iCAP6300 Duo) in the NRAL laboratory at the University of Alberta. The freeze-dried leaf tissue samples of 0.2 g were digested by 10 ml 70% HNO_3 in a microwave oven (MARS 5: Microwave Accelerated Reaction System – CEM Corporation, USA) for 10 minutes. The extracts were diluted with 40 ml milli-Q water. The samples were then filtered before being analyzed by the ICP-OES. The total nitrogen (N) concentrations of leaf samples were determined by the dry combustion method by using a Thermo FLASH 2000 HT Plus Organic Elemental Analyzer (Thermo Fisher Scientific Inc) (Schumacher, 2002).

3.2.7 Statistical analysis

All the data were analyzed by one-way analysis of variance (ANOVA) in the R software (version 4.1.1) to determine the significant differences ($P \leq 0.05$) between treatments within each species. The data that did not meet the ANOVA assumptions of normal distribution and homogeneity of variance were transformed with sqrt or log10 functions. The pairwise comparison was performed using the Holm test.

3.3 Results

3.3.1 Chemical properties of treatment solutions

The treatment solutions in the control group had the lowest pH (6.7) and electrical conductivity (1.5 mS cm^{-1}) values among all groups (Table 3.1). The highest pH value (9.5) of the treatment solution was observed in the 100% eNST water-treated group. The highest EC value (23133 µS cm^{-1}) was measured in the 50% eNST+120 mM NaCl-treated group (Table 3.1). Treatment solutions in the 100% eNST water-treated group had the highest B concentration (22 mg L^{-1}), while the highest Na concentration (4501.8 mg kg^{-1}) was measured in the 50% eNST+120 mM NaCl-treated group (Table 3.1). Compared with the control group, the eNST water and NaCl-added eNST groups had lower Ca and Fe concentrations. Besides, treatment solutions in 50% eNST+60 mM NaCl and 50% eNST+120 mM NaCl groups had significantly higher Ca concentrations than eNST water-treated groups without NaCl (Table 3.1). Treatment solutions containing eNST water had significantly higher S concentrations compared with control group (Table 3.1).

3.3.2 Mortality rate

Compared with the control group, eNST water and eNST + NaCl did not have significant effects on the mortality rate (lower than 10%) in *Puccinellia nuttalliana* among all treatment groups (Figure 3.1). Also, *Puccinellia nuttalliana* had lower mortality rates than *Poa juncifolia* and *Poa pratensis* in all treatment groups (Figure 3.1). In *Poa juncifolia*, mortality rate was the highest (35%) in 50% eNST+120 mM NaCl group (Figure 3.1). In *Poa pratensis*, the 100% eNST water caused a significantly higher mortality rate (45%) than the control (5%) (Figure 3.1).

3.3.3 Plant dry weights

The total dry weights of *Puccinellia nuttalliana* were not significantly affected by the treatments (Figure 3.2A). In *Poa juncifolia*, the 50% eNST water did not have significant effects on the total dry weights, while the 100% eNST water, 50% eNST+60 mM NaCl and 120 mM NaCl significantly reduced the total dry weights of *Poa juncifolia* (Figure 3.2B). The total dry weight of *Poa pratensis* was significantly reduced by 50% eNST water (Figure 3.2C). In *Poa pratensis*, the 100% eNST water plus 60 mM and 120 mM NaCl further reduced the total dry weights (Figure 3.2C).

In *Puccinellia nuttalliana* and *Poa juncifolia*, the shoot to root dry weight ratios were significantly reduced by the eNST water and NaCl treatments (Figures 3.2D, E). In *Poa pratensis*, the shoot to root ratios of plants treated with 50% eNST+60 mM NaCl were significantly higher compared with the 50% eNST+120 mM treatment (Figure 3.2F).

3.3.4 Leaf chlorophyll concentrations

In *Puccinellia nuttalliana*, the leaf chlorophyll concentrations were not significantly affected by the treatments (Figure 3.3A). In *Poa juncifolia*, the leaf chlorophyll concentrations were significantly lower in plants treated with 100% eNST water and 50% eNST+60 mM NaCl, and the lowest chlorophyll concentrations were measured in the 50% eNST+120 mM NaCl-treated group (Figure 3.3B). In *Poa pratensis*, all eNST water and NaCl treatments significantly reduced the leaf chlorophyll concentration (Figure 3.3C). The 100% eNST water and 50% eNST+120 mM NaCl treatments significantly lowered the chlorophyll concentrations compared with the 50% eNST and 50% eNST+60 mM NaCl treatments (Figure 3.3C).

In *Puccinellia nuttalliana* and *Poa juncifolia*, the patterns of chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios were similar to those of chlorophyll concentrations: the Chl a to Chl b ratios of *Puccinellia nuttalliana* were not significantly different in all treatments; the Chl a to Chl b ratios of *Poa juncifolia* were significantly reduced by 100% eNST water and 50% eNST water plus NaCl (Figures 3.3D, E). In *Poa pratensis*, the 100% eNST water and 50% eNST+120 mM NaCl treatments significantly reduced the Chl a to Chl b ratios, while the lowest Chl a to Chl b ratios were measured in the 100% eNST water treatment (Figure 3.3F).

3.3.5 Gas exchange

In *Puccinellia nuttalliana*, the net photosynthesis rates were not significantly affected by any of the applied treatments (Figure 3.4A). In *Poa juncifolia*, the 100% eNST water, 50% eNST+60 mM and 120 mM NaCl significantly decreased the net photosynthesis rates, while the lowest net photosynthesis rate was w measured in the 50% eNST+120 mM NaCl treatment (Figure 3.4B). In *Poa pratensis*, all eNST water and NaCl treatments significantly reduced the net photosynthesis rates, while the 100% eNST water, 50% eNST+60 mM and 120 mM NaCl treatments reduced the net photosynthesis rates more compared with the 50% eNST water treatment alone (Figure 3.4C).

The transpiration rates of *Puccinellia nuttalliana* were not significantly affected by the treatments (Figure 3.4D). However, in *Poa juncifolia*, the eNST water and eNST + NaCl treatments significantly reduced the transpiration rates compared with control. The lowest transpiration rate values were measured in the 50% eNST+120 mM NaCl treatment (Figure 3.4E). In *Poa pratensis*, the trends of transpiration rate in all treatment groups were similar to net photosynthesis, as the 100% eNST water, 50% eNST+60 mM and 120 mM treatments impaired the transpiration rates more compared with the 50% eNST water treatment (Figure 3.4F).

3.3.6 Chlorophyll fluorescence

The PSII maximum efficiency (Fv'/Fm') of *Puccinellia nuttalliana* did not show significant differences among treatments (Figure 3.6A). In *Poa juncifolia*, the 50% eNST water did not significantly affect the Fv'/Fm'; however, the 100% eNST, 50% eNST+60 mM and 120 mM NaCl treatments significantly decreased the Fv'/Fm' ratios. The lowest Fv'/Fm' was in the 50% eNST+120 mM NaCl treatment (Figure 3.6B). In *Poa pratensis*, both eNST water and NaCl treatments significantly reduced the Fv'/Fm' (Figure 3.6C).

3.3.7 Foliar elements

The eNST water significantly increased the leaf Na and B concentrations in all three grass species (Figure 3.7A, B). All three grass species in 50% eNST+60 mM and 120 mM NaCl groups had significantly lower leaf B concentrations than the 50% or 100% eNST water treatments (Figure 3.7A). In *Poa juncifolia*, seedlings in the 50% eNST+120 mM NaCl treatment had the highest leaf Na concentration (Figure 3.7B). In *Poa pratensis*, leaf Na concentrations in 100% eNST water and 50% eNST+120 mM NaCl treatments were significantly higher compared with the other treatments (Figure 3.7B).

The eNST water treatments significantly increased the leaf Na/K and Na/Ca ratios in all three species (Figure 3.7C, D). The leaf Na/K and Na/Ca ratios of *Puccinellia nuttalliana* in 100% eNST water, 50% eNST+60 mM and 120 mM NaCl treatments were lower compared with *Poa juncifolia* and *Poa pratensis* (Figure 3.7C, D). In *Poa juncifolia* and *Poa pratensis*, the highest Na/K ratios were observed in the 50% eNST+120 mM NaCl treatment (Figure 3.7C). *Poa juncifolia* in the 50% eNST+120 mM NaCl treatment also had the highest Na/Ca ratios (Figure 3.7D). In *Poa pratensis*, the leaf Na/Ca ratios in the 100% eNST water and 50% eNST+120mM NaCl groups were significantly higher than in the other treatments (Figure 3.7D).

The eNST water significantly affected the concentrations of other leaf elements including Ca, Cu, K, Mg, Mn, P, S, and N (Table 3.2). In *Puccinellia nuttalliana*, the 100% eNST water and 50% eNST+120 mM NaCl treatments significantly reduced the leaf Ca, K, Mg, P, S, and N concentrations compared with of the control plants. However, leaf Fe and Zn concentrations were not significantly affected by the eNST water and NaCl treatments (Table 3.2). Compared with control, the 50% eNST+120 mM NaCl treatment significantly increased the leaf Cu and Mn concentrations (Table 3.2).

In *Poa juncifolia*, compared with control, the 100% eNST water+120 mM NaCl treatment significantly reduced the leaf Ca, K, P, and N concentrations. The leaf Cu and Zn concentrations were not significantly affected by the treatments. Moreover, the 100% eNST water significantly increased the leaf Fe, Mg, Mn, and S concentrations compared with the control plants. Plants treated with 50% eNST+120 mM NaCl also had significantly higher leaf Mn and S concentrations compared with control (Table 3.2).

In *Poa pratensis*, the leaf Ca, K, Mg, P, and N concentrations were significantly lower in plants treated with 100% eNST water and 50% eNST+120 mM NaCl compared with control. The 50% eNST water and 50% eNST+60 mM NaCl treatments significantly reduced the leaf Fe, Zn, and S concentrations than the plants grown in the control solution (Table 3.2).

3.4 Discussion

The eNST water had elevated levels of pH, Na, B, electrical conductivity, and naphthenic acids. *Puccinellia nuttalliana* is more salt tolerant than *Poa juncifolia* and *Poa pratensis*, since the growth and physiological processes of *Puccinellia nuttalliana* were not affected in any of the eNST water and NaCl treatments, suggesting that NaCl was a major factor affecting plants in the eNST water.

Puccinellia nuttalliana and *Poa juncifolia* are salt-tolerant plants and *Puccinellia nuttalliana* is considered a true halophyte (Mishra and Tanna, 2017, Vaziriyeganeh et al., 2018) while *Poa pratensis* is relatively sensitive to salt stress (Vaziriyeganeh et al., 2018, Chen et al., 2022). In the previous study, the growth of *Poa juncifolia* was significantly inhibited by the 150 mM and 300 mM NaCl, while *Puccinellia nuttalliana* was not affected (Vaziriyeganeh et al., 2018). When exposed to salinity, halophytes and other salt-tolerant plants may exclude Na into the vacuoles more effectively than salt-sensitive glycophytes, resulting in a higher cytosolic K/Na ratio (Mishra and Tanna, 2017, Sarath et al., 2021). This helps maintain osmotic potential and vital plant processes such as photosynthesis, enzyme activities, and growth (Tozlu et al., 2000, Ali and Yun, 2017, Çalişkan and Çalişkan, 2019). Halophytes, including *Puccinellia nuttalliana*, and some other salt-tolerant plants, also secrete salts through the salt glands and epidermal bladder cells on the leaf and shoot surfaces (Vaziriyeganeh et al., 2018, Chen et al., 2022).

The elevated NaCl levels in oil sands tailings inhibit the growth and physiological processes of most plants in reclamation areas (Renault et al., 1998, Renault et al., 1999, Fleurial et al., 2022). The high Na concentration can decrease the water potential of the growth medium, which directly restricts the plant water uptake, resulting in physiological drought (Acosta-Motos et al., 2017). In the present study, the high Na concentration (1000 mg L^{-1}) in eNST water significantly increased the leaf Na concentrations, leaf Na/K, and Na/Ca ratios in all three species. Sodium ions can compete with K for root uptake sites and inhibit the plant K uptake (Jouyban, 2012), resulting in a decrease in K uptake and a high Na/K ratio (Cramer et al., 1985, Renault et al., 1999). The deficiency of K affects stomatal conductance, cell turgor, availability of CO_2, chlorophyll concentration, and the activity of ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) (Zhao et al., 2001, Çalişkan and Çalişkan, 2019, Rawat et al., 2022), which impairs photosynthesis, transpiration, and plant growth (Çalişkan and Çalişkan, 2019, Rawat et al., 2022). Sodium can also can displace the Ca in the cell membranes, leading to a high Na/Ca ratios (Cramer et al., 1985, Liang et al., 1996). The displacement of membrane Ca increases membrane permeability and induces cytosolic K leakage (Cramer et al., 1985, Liang et al., 1996). Meanwhile, the impaired water uptake caused by salt stress also inhibits the Ca uptake and transport (Arif et al., 2020).

Puccinellia nuttalliana is more salt tolerant compared with *Poa juncifolia* and *Poa pratensis* (Vaziriyeganeh et al., 2018, 2021, 2022, 2023). *Puccinellia nuttalliana* maintained leaf chlorophyll concentration, net photosynthesis rate, and total dry weight by maintaining low leaf Na concentration, low leaf Na/K, and low leaf Na/Ca ratios in all treatment groups. In *Poa juncifolia*, the 50% eNST did not have significant effects on the leaf chlorophyll concentrations, net photosynthesis rate, and total dry weights. Since *Poa juncifolia* is a salt tolerant species that is also sometimes considered a facultative halophyte (Vaziriyeganeh, 2018, 2021), moderate salt it can tolerate moderate salt concentrations. Both *Puccinellia nuttalliana* and *Poa juncifolia* had significantly lower shoot to root dry weight ratios when treated with the eNST water and NaCl compared with the control plants. The lower shoot to root dry weight ratio is a common response of plants exposed to salinity, since relatively larger roots help plants absorb more water and nutrients (Yang et al., 2022). However, the salinity levels in 100% eNST, 50% eNST+60 mM and 120 mM NaCl treatments exceed the salt tolerance threshold of *Poa juncifolia* and reduced the chlorophyll concentrations and net photosynthesis in plants, resulting in lower total dry weights. As a salt sensitive species, *Poa pratensis* suffered from reductions in leaf chlorophyll concentration, net photosynthesis rate, and total dry weight in all applied treatments.

The decreases in growth and physiological processes of *Poa juncifolia* and *Poa pratensis* can be attributed to the high salinity and high pH (9.1) of the eNST water, which can result in ion toxicity, osmotic stress, and reduce nutrient availability (Munns and Tester, 2008, Marschner, 2011). When Na enters the cytoplasm, it reduces chlorophyll concentrations and upsets the activities of many enzymes, such as RuBisCO and antioxidative enzymes, leading to the disruption of photosynthesis and impairing the ability to scavenge reactive oxygen species (De Azevedo Neto et al., 2006, Jouyban, 2012). The elevated Na/K ratio can inhibit RuBisCO activities, reduce chlorophyll concentrations, decrease net photosynthesis, and alter plant water potentials (Jouyban, 2012, Çalişkan and Çalişkan, 2019, Rawat et al., 2022). High salinity also directly reduces plant leaf K, N, and Zn concentrations by disrupting water relations and cell permeability barriers (Arif et al., 2020).

High pH of the eNST water can expected to cause the precipitation of essential nutrients (Fe, Mn, Zn, and P) in growth solution and reduce their availability for root uptake (Marschner, 2011, Hu et al., 2022). Moreover, high pH in the root zone inhibits plant nutrient uptake by disrupting the H^+ gradient across the plasma membrane, which is the driving force for nutrient transporters and channels (Zhao et al., 2016, Hu et al., 2022). High pH also aggravates the detrimental effects of salt stress, as the membrane H^+ gradient is vital for plant Na exclusion (Hu et al., 2022). In this study, *Poa juncifolia* and *Poa pratensis* suffered from decreases in leaf Ca, K, P, and N concentrations in 100% eNST, 50% eNST+60 mM and 120 mM NaCl treatments. The reduction of P can affect the electron transport in the photosystem I and the regeneration of ribulose-1,5-bisphosphate (RuBP) in the Calvin cycle (Carstensen et al., 2018). The decrease in leaf nitrogen reduces chlorophyll concentrations, affects photosystem efficiency, and decreases the activities of photosynthetic enzymes and proteins (Mu and Chen, 2021, Aleksandrov, 2022).

Potassium plays a crucial role in CO_2 absorption, stomatal regulation, and maintaining photosynthetic enzyme activities (Aleksandrov, 2022, Rawat et al., 2022). Calcium is also involved in photosynthetic processes by maintaining the photosystem II efficiency (Tozlu et al., 2000, Aleksandrov, 2022,). Therefore, the decreases of K and Ca concentrations induced by the elevated Na levels in eNST water inhibit plant photosynthetic processes and impair growth. Furthermore, *Poa juncifolia* seedlings in the 50% eNST+120 mM NaCl group suffered from the decrease of leaf Mg concentrations, which could inhibit the chlorophyll synthesis and as well as the activity of the photosynthetic enzymes and result in the inhibition of photosynthesis (Farhat et al., 2016). In *Poa pratensis*, the decrease of net photosynthesis rates in the 100% eNST and 50% eNST+120 mM NaCl treatments can also be partially attributed to the reduction of leaf Mg concentrations (Farhat et al., 2016). Moreover, *Poa pratensis* in the 50% eNST+60 mM NaCl treatment also had significantly higher leaf Ca concentrations, which can activate Na^+/H^+ antiport, contribute to Na exclusion, and help maintain leaf chlorophyll concentrations (Guo et al., 2017).

High B concentrations in eNST water may also be detrimental to plants (Princi et al., 2016). The eNST water significantly increased the leaf B concentrations in all three species and in all treatments. However, the leaf B concentrations in *Puccinellia nuttalliana* and *Poa juncifolia* were lower compared with *Poa pratensis* in all treatment groups. These results are consistent with the frequently reported responses of halophytes to B, as the halophytes can tolerate high B concentrations by restricting B accumulation and transport from roots to shoots (Stiles et al., 2010, Centofanti and Bañuelos, 2015, Rámila et al., 2016). The mechanism of B phytotoxicity is still unclear (Princi et al., 2016). However, common visible B toxicity symptom include the yellowing and drying of leaf tips and edges (Princi et al., 2016, Brdar-Jokanović, 2020). In the present study, the yellowing and drying of leaves were prominent in *Poa juncifolia* and *Poa pratensis*. Moreover, all three species in 50% eNST+NaCl treatments had significantly lower leaf B concentrations than in the eNST water treatments without NaCl. In *Puccinellia nuttalliana*, the decrease in leaf B concentration can be explained by the B exclusion ability of halophytes (Lamdan et al., 2012). In *Poa juncifolia* and *Poa pratensis*, the lower leaf B concentrations can be attributed to the decrease in transpiration rates caused by the high salinity in 50% eNST+60 mM and 120 mM NaCl treatments, since B is largely transported by the transpiration stream in plants (Grieve and Poss, 2000, Brdar-Jokanović, 2020).

The high-level naphthenic acids (65 mg L^{-1}) were also observed in the eNST water in this study. Naphthenic acids upset plant water balance, reduce chlorophyll concentration and inhibit gas exchange parameters (Kamaluddin and Zwiazek, 2002). However, further investigations are needed to reveal the detailed mechanisms of how naphthenic acids impair plant physiological processes.

The present study demonstrated that the growth and physiological processes of *Puccinellia nuttalliana* were unaffected even in the highest salinity treatment (100% eNST water+120mM NaCl). The oil sands reclamation sites are often associated with combined high pH and high salinity stresses for reclamation plants (Fleurial et al., 2022). The alkali salts is more harmful to plants than neutral salts due to the combination of ion toxicity, osmotic stress, and high pH stress (Zhao et al., 2016). Therefore, the high alkali salt stress tolerance of *Puccinellia nuttalliana* makes it a promising plant species for oil sands land reclamation.

3.5 Conclusions

In this study, *Puccinellia nuttalliana* was more tolerant to the eNST water compared with *Poa juncifolia* and *Poa pratensis*. *Poa pratensis* was the most sensitive of the three studied grass species to all applied treatments. This is consistent with the ranking of salt tolerance of these species of plants, confirming that salinity is the most detrimental factor to plants in the eNST oil sands tailings water and, therefore, that the plants with higher salt tolerance levels should be considered for the revegetation of sites affected by these tailings. The results demonstrated that the eNST water severely reduced the growth, chlorophyll concentrations, and gas exchange in *Poa pratensis*. The addition of NaCl aggravated the negative effects of eNST water on *Poa pratensis*. The 50% eNST water did not affect the growth of *Poa juncifolia*. The 100% eNST water and 50% eNST water + NaCl treatments exceeded the salt tolerance threshold of *Poa juncifolia*, leading to severe decreases in the growth and physiological parameters. The high eNST water tolerance of *Puccinellia nuttalliana* can be likely partly attributed to its salt exclusion ability, which contributed to maintainance of lower leaf Na concentration, leaf Na/K, and leaf Na/Ca ratios compared with *Poa juncifolia* and *Poa pratensis*. Future field studies are needed to investigate the field growth performance and soil desalination potential of *Puccinellia nuttalliana*.

3.6 References

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J. & Hernandez, J. A. 2017. Plant responses to salt stress: adaptive mechanisms. *Agronomy,* 7**,** 18. doi: 10.3390/agronomy7010018.

Aleksandrov, V. 2022. Identification of nutrient deficiency in plants by artificial intelligence. *Acta Physiologiae Plantarum,* 44**,** 29. doi: 10.1007/s11738-022-03363-0.

Ali, A. & Yun, D.-J. 2017. Salt stress tolerance; what do we learn from halophytes. *Journal of Plant Biology,* 60**,** 431-439.

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. & Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: an omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156**,** 64-77.

Barros, N. L. F., Marques, D. N., Tadaiesky, L. B. A. & De Souza, C. R. B. 2021. Halophytes and other molecular strategies for the generation of salt-tolerant crops. *Plant Physiology and*

Biochemistry, 162**,** 581-591.

Brdar-Jokanović, M. 2020. Boron toxicity and deficiency in agricultural plants. *International Journal of Molecular Sciences,* 21**,** 1424-1444.

Bushman, B. S., Amundsen, K. L., Warnke, S. E., Robins, J. G. & Johnson, P. G. 2016. Transcriptome profiling of kentucky bluegrass (*Poa pratensis L.*) accessions in response to salt stress. *BMC Genomics,* 17**,** 1-12.

Çalişkan, B. & Çalişkan, A. C. 2019. Potassium nutrition in plants and its interactions with other nutrients in hydroponic culture. *Improvement of Quality in Fruits and Vegetables Through Hydroponic Nutrient Managemen*. doi: 10.5772/intechopen.71951.

Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M. & Husted, S. 2018. The impacts of pphosphorus deficiency on the pphotosynthetic electron transport chain. *Plant Physiology,* 177**,** 271-284.

Centofanti, T. & Bañuelos, G. 2015. Evaluation of the halophyte *Salsola soda* as an alternative crop for saline soils high in selenium and boron. *Journal of Environmental Management,* 157**,** 96-102.

Cheeseman, J. M. 2015. The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist,* 206**,** 557-570.

Chen, G., Amoanimaa-Dede, H., Zeng, F., Deng, F., Xu, S. & Chen, Z.-H. 2022. Chapter One - Stomatal regulation and adaptation to salinity in glycophytes and halophytes. *In:* SHABALA, S. (ed.) *Advances in Botanical Research.* Academic Press, 103, 1-42.

CNRL. 2017. Stewardship report to stakeholders [Online]. Available: https://www.cnrl.com/content/uploads/2023/01/2017-stewardship-report-to-stakeholders.pdf [Accessed 2022 July]. 19 p.

Context Energy Examined. 2023. Myth: The oil sands are destroying Canada's boreal forests [Online]. Available: https://context.capp.ca/energy-matters/2019/mythbuster-oil-sands-extraction-and-canadas-boreal-forest/ [Accessed 2023 May].

Cramer, G. R., LäUchli, A. & Polito, V. S. 1985. Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells: a primary response to salt stress. *Plant Physiology,* 79**,** 207-211.

Dai, Q. & Chung, K. H. 1996. Hot water extraction process mechanism using model oil sands. *Fuel,* 75**,** 220-226.

Dassanayake, M. & Larkin, J. C. 2017. Making plants break a sweat: the structure, function, and evolution of plant salt glands. *Frontiers in Plant Science,* 8, 406-426.

De Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., De Abreu, C. E. B. & Gomes-Filho, E. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany,* 56**,** 87-94.

Dhar, A., Comeau, P. G., Karst, J., Pinno, B. D., Chang, S. X., Naeth, A. M., Vassov, R. & Bampfylde, C. 2018. Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. *Environmental Reviews,* 26**,** 286-298.

Duarte, B., Santos, D., Marques, J. C. & Caçador, I. 2013. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback – Implications for resilience in climate change. *Plant Physiology and Biochemistry,* 67**,** 178-188.

Epstein, E. & Bloom, A. J. 1853. Mineral nutrition of plants: principles and perspectives. *Sinauer*.

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C. & Rabhi, M. 2016. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum,* 38**,** 145-155.

Fleurial, K. G., Zhang, W.-Q., Vassov, R. & Zwiazek, J. J. 2022. Root hypoxia aggravates the effects of saline tailings water on the growth and physiology of woody plants. *Plant and Soil***,** 481, 1-18.

Flowers, T. J. & Colmer, T. D. 2008a. Salinity tolerance in halophytes. *New phytologist***,** 179, 945-963.

Flowers, T. J. & Colmer, T. D. 2008b. Salinity tolerance in halophytes. *New Phytologist,* 179**,** 945-963.

Flowers, T. J. & Colmer, T. D. 2015. Plant salt tolerance: adaptations in halophytes. *Annals of Botany,* 115**,** 327-331.

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. In *Marschner's mineral nutrition of higher plants* (pp. 409-472). Academic press. *Elsevier*.

Government of Alberta. 2010. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region [Online]. Available: https://open.alberta.ca/publications/9780778588252 [Accessed 2021 August]. 43 p.

Government of Alberta. 2023. Oil sands – Overview [Online]. Available: https://www.alberta.ca/oil-sands-overview.aspx [Accessed 2021 July].

Grieve, C. M. & Poss, J. A. 2000. Wheat response to interactive effects of boron and salinity. *Journal of Plant Nutrition,* 23**,** 1217-1226.

Guo, R., Shi, L., Yan, C., Zhong, X., Gu, F., Liu, Q., Xia, X. & Li, H. 2017. Ionomic and metabolic responses to neutral salt or alkaline salt stresses in maize (*Zea mays L.*) seedlings. *BMC Plant Biology,* 17**,** 1-13.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K. & Bohnert, H. J. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology,* 51**,** 463-499.

Howat, D. 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. Environmental Sciences Division Edmonton, AB, Canada. 8 p.

Hu, X., Wang, D., Ren, S., Feng, S., Zhang, H., Zhang, J., Qiao, K. & Zhou, A. 2022. Inhibition of root growth by alkaline salts due to disturbed ion transport and accumulation in Leymus chinensis. *Environmental and Experimental Botany,* 200**,** 104907-104918.

Jouyban, Z. 2012. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

Kamaluddin, M. & Zwiazek, J. J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. *Tree Physiology,* 22**,** 1265-1270.

Lambers, H., Chapin, F. S. & Pons, T. L. 2008. Plant physiological ecology (Vol. 2, pp. 11-99). *New York: Springer*. 122 p.

Lamdan, N. L., Attia, Z., Moran, N. & Moshelion, M. 2012. The *Arabidopsis*-related halophyte *Thellungiella halophila*: boron tolerance via boron complexation with metabolites. *Plant, Cell & Environment,* 35**,** 735-746.

Lee, S. H. & Zwiazek, J. J. 2015. Regulation of Aquaporin-Mediated Water Transport in *Arabidopsis* Roots Exposed to NaCl. *Plant and Cell Physiology,* 56**,** 750-758.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183.

Luna Wolter, G. L., Dhar, A. & Naeth, M. A. 2021. Response of three native grass species on dry tailings reclamation substrate amended with petroleum coke. *Wiley Online Library*, (Vol. 50, No. 2, pp. 384-395).

Luna Wolter, G. L. & Naeth, M. A. 2014. Dry mature fine tailings as oil sands reclamation substrates for three native grasses. *Journal of Environmental Quality,* 43**,** 1510-1516.

Macke, A. J. & Ungar, I. A. 1971. The effects of salinity on germination and early growth of *Puccinellia nuttalliana*. *Canadian Journal of Botany,* 49**,** 515-520.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. *Academic Press.* London, UK 447 p.

Mishra, A. & Tanna, B. 2017. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8, 829-839. doi: 10.3389/fpls.2017.00829.

Mu, X. & Chen, Y. 2021. The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry,* 158**,** 76-82.

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

Phillips, T., Petrone, R., Wells, C. & Price, J. 2016. Characterizing dominant controls governing evapotranspiration within a natural saline fen in the Athabasca oil sands of Alberta, Canada. *Ecohydrology,* 9**,** 817-829.

Princi, M. P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F. & Abenavoli, M. R. 2016. Chapter 5 - Boron toxicity and tolerance in plants: recent advances and future perspectives, pp.115-147.

Purdy, B. G., Ellen Macdonald, S. & Lieffers, V. J. 2005. Naturally saline boreal communities as models for reclamation of saline oil sand tailings. *Restoration Ecology,* 13**,** 667-677.

Rámila, C. D. P., Contreras, S. A., Di Domenico, C., Molina-Montenegro, M. A., Vega, A., Handford, M., Bonilla, C. A. & Pizarro, G. E. 2016. Boron stress response and accumulation potential of the extremely tolerant species *Puccinellia frigida*. *Journal of Hazardous Materials,* 317**,** 476-484.

Rawat, J., Pandey, N. & Saxena, J. 2022. Role of potassium in plant photosynthesis, transport, growth and yield. *Role of Potassium in Abiotic Stress.* pp.1-14. doi: 10.1007/978-981-16-4461-0_1.

Redfield, E., Croser, C., Zwiazek, J., Mackinnon, M. & Qualizza, C. 2003. Responses of red-osier dogwood to oil sands tailings treated with gypsum or alum. *Journal of Environmental Quality,* 32**,** 1008-1014.

Renault, S., Croser, C., Franklin, J., Zwiazek, J. & Mackinnon, M. 2001. Effects of consolidated tailings water on red-osier dogwood (*Cornus stolonifera Michx*) seedlings. *Environmental Pollution,* 113**,** 27-33.

Renault, S., Lait, C., Zwiazek, 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., Mackinnon, M. & Qualizza, C. 2003. Barley, a potential species for initial reclamation of saline composite tailings of oil sands. *Journal of Environmental Quality,* 32**,** 2245-2253.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J. & Mackinnon, M. 1999. Responses of boreal plants to high salinity oil sands tailings water. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Vol. 28, No. 6, pp. 1957-1962.

Renault, S., Qualizza, C. & Mackinnon, M. 2004. Suitability of altai wildrye (*Elymus angustus*) and slender wheatgrass (*Agropyron trachycaulum*) for initial reclamation of saline composite tailings of oil sands. *Environmental Pollution,* 128**,** 339-349.

Sarath, N. G., Sruthi, P., Shackira, A. & Puthur, J. T. 2021. Halophytes as effective tool for phytodesalination and land reclamation. *Frontiers in Plant-Soil Interaction,* pp. 459-494.

Sestak, Z., Catský, J. & Jarvis, P. G. 1971. Plant photosynthetic production. Manual of methods. The Hague, Netherlands.

Stiles, A. R., Bautista, D., Terry, N., Atalay, E. & Babaoğlu, M. 2010. Mechanisms of boron tolerance and accumulation in plants: A physiological comparison of the extremely boron-tolerant plant species, *Puccinellia distans*, with the moderately boron-tolerant *Gypsophila arrostil*. *Environmental Science and Technology,* 44**,** 7089-7095.

Tarasoff, C., Mallory-Smith, C. & Ball, D. 2007. Comparative plant responses of *Puccinellia distans* and *Puccinellia nuttalliana* to sodic versus normal soil types. *Journal of Arid Environments,* 70**,** 403-417.

Tozlu, I., Moore, G. A. & Guy, C. L. 2000. Effects of increasing NaCl concentration on stem elongation, dry mass production, and macro- and micro-nutrient accumulation in *Poncirus trifoliata*. *Functional Plant Biology,* 27**,** 35-42.

Vaziriyeganeh, M., Carvajal, M., Du, N. & Zwiazek, J. J. 2022. Salinity tolerance of halophytic grass *Puccinellia nuttalliana* is associated with enhancement of aquaporin-mediated water transport by sodium. *International Journal of Molecular Sciences,* 23**,** 5732-5750.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2021. Transcriptome and metabolome analyses reveal potential salt tolerance mechanisms contributing to maintenance of water balance by the halophytic grass Puccinellia nuttalliana. *Frontiers in Plant Science,* 12**,** 760863-760887.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2023. Analysis of aquaporins in northern grasses reveal functional importance of Puccinellia nuttalliana PIP2; 2 in salt tolerance. *Plant, Cell & Environment*, 46, 1033-1418.

Vaziriyeganeh, M., Lee, S. H. & Zwiazek, J. J. 2018. Water transport properties of root cells contribute to salt tolerance in halophytic grasses *Poa juncifolia* and *Puccinellia nuttalliana*. *Plant Science,* 276**,** 54-62.

Yang, Y., Wassie, M., Liu, N.-F., Deng, H., Zeng, Y.-B., Xu, Q. & Hu, L.-X. 2022. Genotypic-specific hormonal reprogramming and crosstalk are crucial for root growth and salt tolerance in bermudagrass (*Cynodon dactylon*). *Frontiers in Plant Science,* 13, 956410-956426.

Yi, H., Calvo Polanco, M., Mackinnon, M. D. & Zwiazek, J. J. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany,* 62**,** 357-363.

Yuan, F., Leng, B. & Wang, B. 2016. Progress in studying salt secretion from the salt glands in recretohalophytes: how do plants secrete salt. *Frontiers in Plant Science,* 7, 977-989.

Zhang, W.-Q., 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**,** 113945-113953.

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, D., Oosterhuis, D. M. & Bednarz, C. W. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. *Photosynthetica,* 39**,** 103-109.

Zhao, Q., Suo, J., Chen, S., Jin, Y., Ma, X., Yin, Z., Zhang, Y., Wang, T., Luo, J., Jin, W., Zhang, X., Zhou, Z. & Dai, S. 2016. Na_2CO_3-responsive mechanisms in halophyte *Puccinellia tenuiflora* roots revealed by physiological and proteomic analyses. *Scientific Reports,* 6**,** 32717-32740.

Zhu, J.-K. 2001. Plant salt tolerance. *Trends in Plant Science,* 6**,** 66-71.

3.7 Table

Table 3. 1: pH, electrical conductivity (EC, mS cm^{-1}), and elemental concentrations (mg L^{-1}) of the treatment solutions at the beginning of the experiment. 50% Hoagland + 50% eNST is the mixture of 100% Hoagland solution and eNST water (1:1, v/v). Different letters indicate significant differences between treatments determined by Holm's test (P <0 .05). (NM-Not measured). Means (n = 4) ± SE are shown.

	pH	EC	NAs	B	Na	Ca	Fe	K	Mg	P	S
50% Hoagland's (Control)	6.7 ±0.11 d	1456.8 ±98.6 e	NM	0.37 ±0.04 d	2.3 ±0.28 d	142.27 ±19.9 a	1.2 ±0.19 a	195.1 ±22.8 a	22.7 ±3.8 a	29.7 ±15.1 a	23.9 ±3.2 d
50% Hoagland's +50% eNST	9.3 ±0.05 ab	6900 ±838.2 d	NM	11.3 ±1.6 b	1698.5 ±241 c	29.09 ±8.15 c	0.04 ±0.01 bc	232.9 ±54.4 a	21 ±4.8 a	15 ±4.1 a	147.8 ±23.7 b
50% Hoagland's +100% eNST	9.5 ±0.02 a	11051 ±1402.2 c	NM	22 ±1.6 a	3003.6 ±247 b	25.28 ±4.95 c	0.12 ±0.05 b	261.8 ±9.03 a	33.8 ±2 a	27.3 ±3.1 a	245 ±20.8 a
50% Hoagland's +50% eNST+60mM NaCl	9.1 ±0.04 bc	14511 ±556.3 b	NM	8.6 ±0.89 bc	3215.7 ±161.9 b	59.95 ±6.17 b	0.02 ±0.001 c	235.4 ±3.1 a	24 ±2.3 a	13 ±2.2 a	109.4 ±10.8 bc
50% Hoagland +50% eNST+120mM NaCl	9.0 ±0.05 c	23133 ±418.1 a	NM	7.4 ±0.92 c	4501.8 ±153.9 a	72.91 ±6.29 b	0.09 ±0.05 bc	225.7 ±9.2 a	22.4 ±1.2 a	16.7 ±0.6 a	91.4 ±6.2 c
100%eNST	9.1	4500	65	6.7	869.2	7.9	0.005	30.4	7.9	0.07	63

Table 3. 2: Leaf elemental concentrations (mg kg^{-1}, % for N) of *Puccinellia nuttalliana, Poa juncifolia, and Poa pratensis* seedlings treated with eNST water with or without NaCl. Different letters indicate significant differences between treatments within each species determined by Holm's test ($P <$ 0.05). Means (n = 6) ± SE are shown. (Hoa - Hoagland's solution).

		Ca	Cu	Fe	K	Mg	Mn	P	S	Zn	N
Puccinellia nuttalliana	50%Hoa	11528.5 ±2307.9 a	7.6 ±1.1 c	72.9 ±4.3 a	37103.7 ±3207.2 a	4238 ±542.5 a	28.5 ±4.4 b	5994 ±179.9 a	4037 ±165.8 a	41.6 ±5.4 a	4 ±0.11 a
	50%Hoa +50%eNST	7522 ±437.8 ab	9.2 ±0.66 bc	65.7 ±3.6 a	22844.4 ±1412.3 b	2692.8 ±264.2 ab	52.1 ±11.4 ab	2447 ±165.5 cd	3150.7 ±286.1 b	33.1 ±3 a	3.7 ±0.11 ab
	50%Hoa +100%eNST	5085.7 ±686.1 b	11.7 ±1.2 abc	67 ±3.7 a	24823.4 ±2167.2 b	2492.2 ±365.7 b	120.3 ±32.7 a	2064.8 ±120.5 d	2805.3 ±180.3 b	51.6 ±7.3 a	3.3 ±0.1 b
	50%Hoa +50%eNST+60mM NaCl	5248.9 ±479 b	15.2 ±1.8 a	71.3 ±4.9 a	30335.5 ±1967.3 ab	2469.8 ±357.8 b	69.7 ±10 ab	3273.9 ±276.5 b	2745.7 ±111.2 b	47.2 ±5.8 a	3.6 ±0.11 ab
	50%Hoa +50%eNST+120mM NaCl	5767.1 ±841.1 b	13.7 ±1.4 ab	64.1 ±3 a	26169.1 ±2997.9 b	2326.3 ±263.7 b	100.9 ±24.4 a	2653.4 ±147.5 bc	2622.1 ±166.8 b	51.4 ±9.4 a	3.4 ±0.15 b
Poa juncifolia	50%Hoa	5053 ±287.7 a	6.4 ±0.48 a	47.5 ±1.7 b	21585.8 ±632.4 a	2126 ±156.6 ab	28.2 ±5 c	3597.4 ±131.5 a	1838.7 ±33.4 c	22.8 ±1.2 a	2.7 ±0.1 a
	50%Hoa +50%eNST	2878 ±100 b	7.8 ±0.96 a	48.5 ±3.9 b	13650.7 ±618.2 b	2145 ±121.9 ab	53.5 ±5 b	2043.7 ±205.7 b	1689.4 ±82.9 c	25.2 ±4.1 a	2.5 ±0.1 ab
	50%Hoa +100%eNST	2983.2 ±431.3 b	8.6 ±0.54 a	126.9 ±26.5 a	9968.5 ±592.6 d	2360.4 ±196.3 a	112.8 ±14.1 a	1709.2 ±24.1 b	2502.2 ±215.5 ab	28.6 ±2 a	2 ±0.1 c
	50%Hoa +50%eNST+60mM NaCl	2692.2 ±294.3 b	8.5 ±0.55 a	54.4 ±6.1 b	11426.1 ±501.6 cd	1649.7 ±194.1 bc	72.4 ±10.7 ab	1820.8 ±73 b	1916.4 ±115.3 bc	22.7 ±1.5 a	2.1 ±0.06 bc
	50%Hoa +50%eNST+120mM NaCl	2670.5 ±76 b	8 ±0.59 a	62.2 ±4.6 b	12201.8 ±646.5 bc	1307.4 ±61.4 c	76.5 ±7.1 ab	2010.5 ±69.9 b	2565.2 ±212.2 a	20.5 ±1.5 a	1.8 ±0.19 c
Poa pratensis	50%Hoa	10904.6 ±1050.3 a	9 ±0.7 a	56.6 ±3 a	28542.9 ±1061.9 a	5550.1 ±561.7 a	56.9 ±13.2 ab	4646.3 ±309.2 a	3804 ±593.3 a	26.1 ±3.3 a	3.5 ±0.08 a
	50%Hoa +50%eNST	3957.33 ±347.8 b	5 ±0.49 b	31 ±2 b	17444.7 ±1833.2 bc	5678.7 ±449.8 a	62.7 ±5.3 ab	2422.4 ±283.5 b	1940.2 ±163.4 b	15.4 ±2.00 c	2.2 ±0.08 b
	50%Hoa +100%eNST	1856.4 ±195.4 d	7 ±0.57 ab	62.2 ±9.9 a	19374.3 ±1057 b	3218.9 ±180.7 b	42.3 ±7.6 b	2127.8 ±78.2 b	4534.4 ±515.1 a	17.1 ±1.8 bc	2.2 ±0.2 b
	50%Hoa +50%eNST+60mM NaCl	2494.6 ±289.8 c	6.6 ±0.84 ab	30.5 ±2.4 b	13025 ±640.9 d	4768.2 ±532.1 a	75.3 ±11.6 ab	2347.5 ±113.7 b	1857.6 ±66.4 b	16.5 ±1.2 c	2 ±0.1 b
	50%Hoa +50%eNST+120mM NaCl	2469.2 ±122 c	9.2 ±0.89 a	46.9 ±3.5 a	13566.3 ±1100.3 cd	2991.4 ±154.1 b	91.6 ±8.9 a	2706.7 ±148 b	2458.5 ±119 c	25.1 ±1.8 ab	2.2 ±0.07 b

3.8 Figures

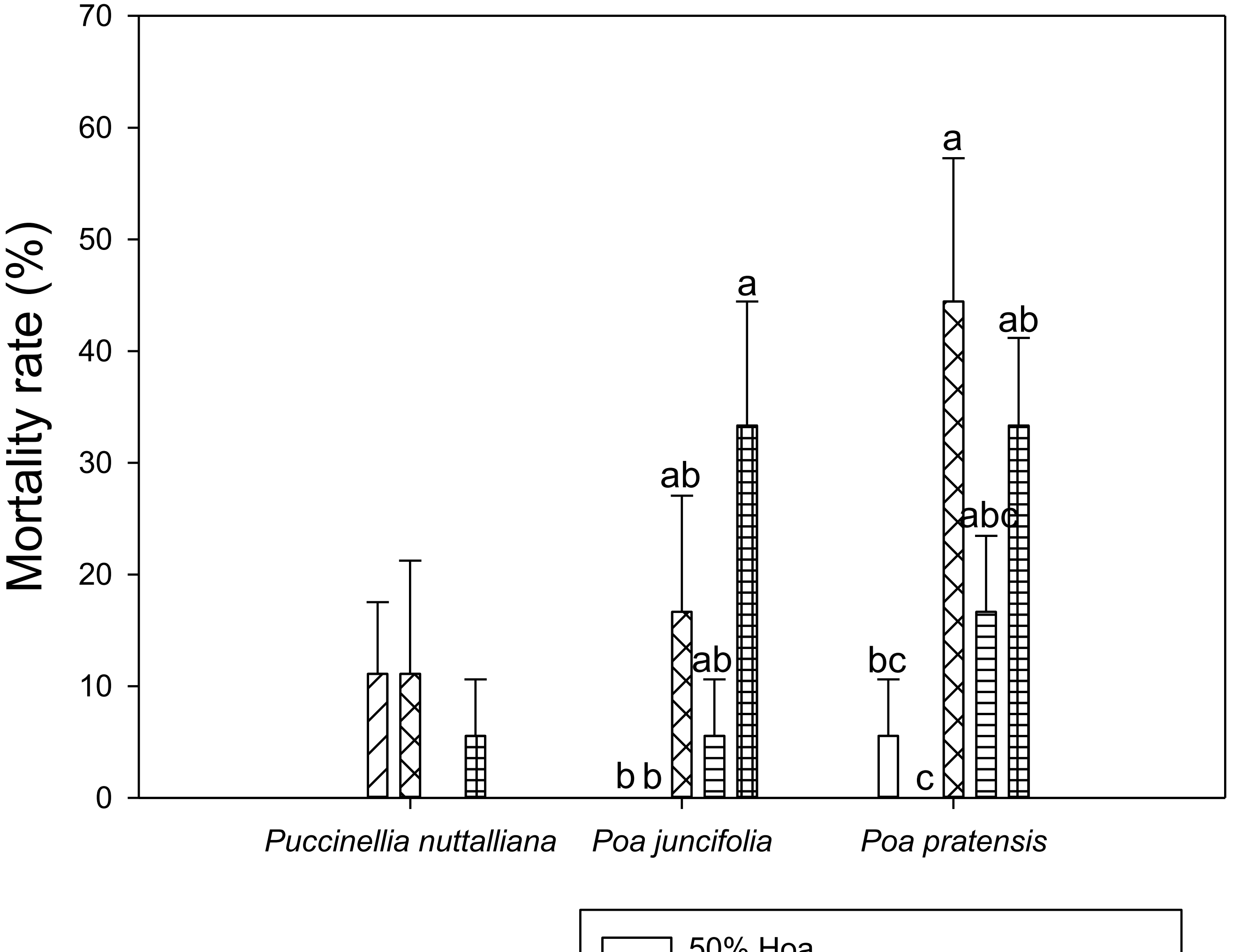

Figure 3. 1: The mortality rates of *Puccinellia nuttalliana*, *Poa juncifolia*, and *Poa pratensis* seedlings treated with eNST water with or without NaCl. Means (n=18) ± SE are shown. Different letters indicate significant differences between treatments within each species determined by Holm's test ($P<0.05$). (Hoa - Hoagland's solution).

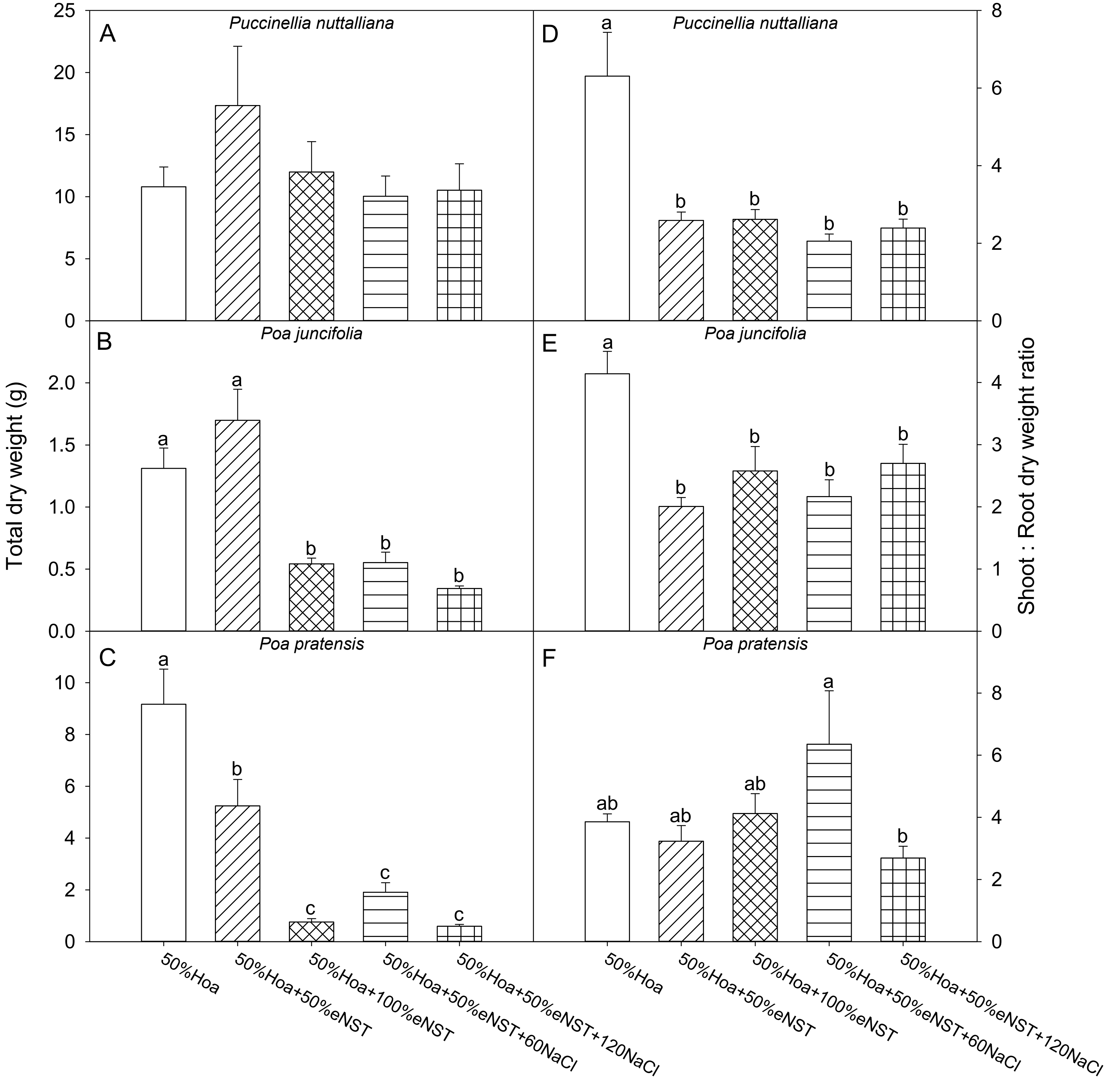

Figure 3. 2: Total dry weights (DW) and shoot to root dry weight ratios of *Puccinellia nuttalliana* (A, D), *Poa juncifolia* (B, E), and *Poa pratensis* (C, F) seedlings treated with eNST water with or without NaCl. Means (n=18) + SE are shown. Different letters indicate significant differences between treatments within each species ($P<0.05$) as determined by Holm's test. (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

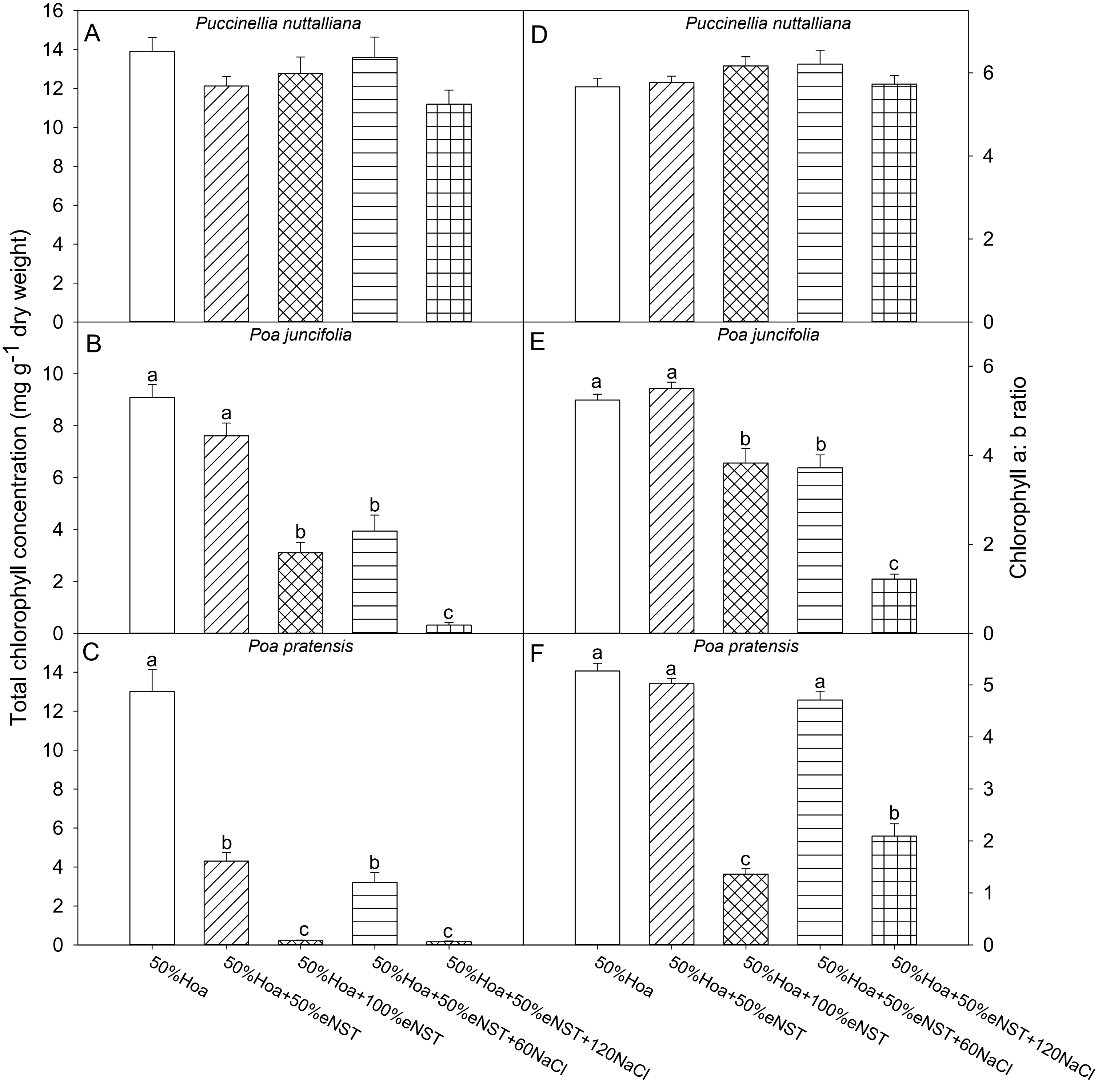

Figure 3. 3: Total leaf chlorophyll concentrations and chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios of *Puccinellia nuttalliana* (A, D), *Poa juncifolia* (B, E), and *Poa pratensis* (C, F) seedlings treated with eNST water with/without NaCl. Means (n=6) ± SE are shown. Different letters indicate significant differences between treatments within each species determined by Holm's test (*P*<0.05). (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

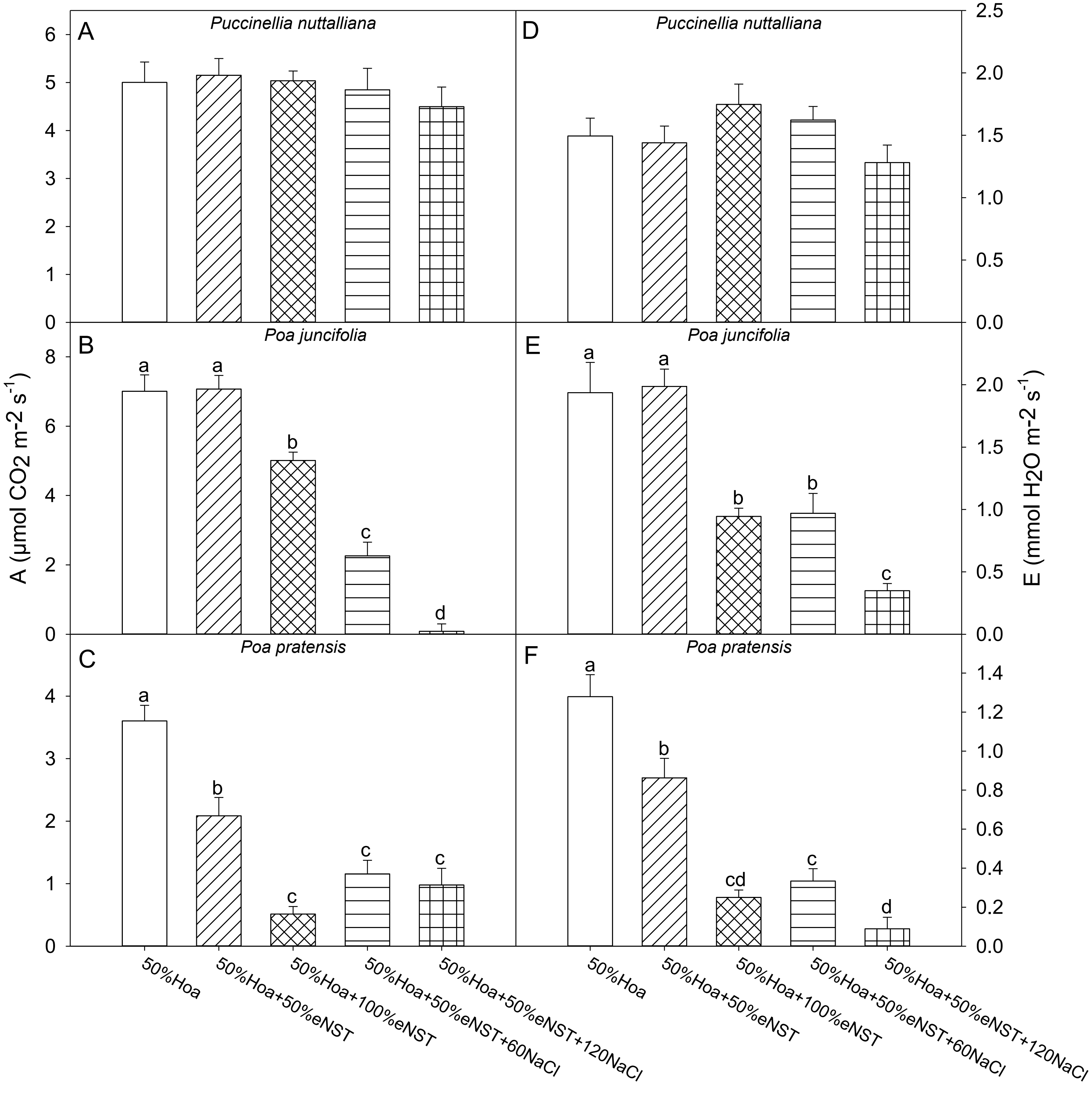

Figure 3. 4: Net photosynthesis and transpiration rate of *Puccinellia nuttalliana* (A, D), *Poa juncifolia* (B, E), and *Poa pratensis* (C, F) seedlings treated with eNST water with or without NaCl. Means (n=8-10) ± SE are shown. Different letters indicate significant differences between treatments within each species determined by Holm's test ($P<0.05$). (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

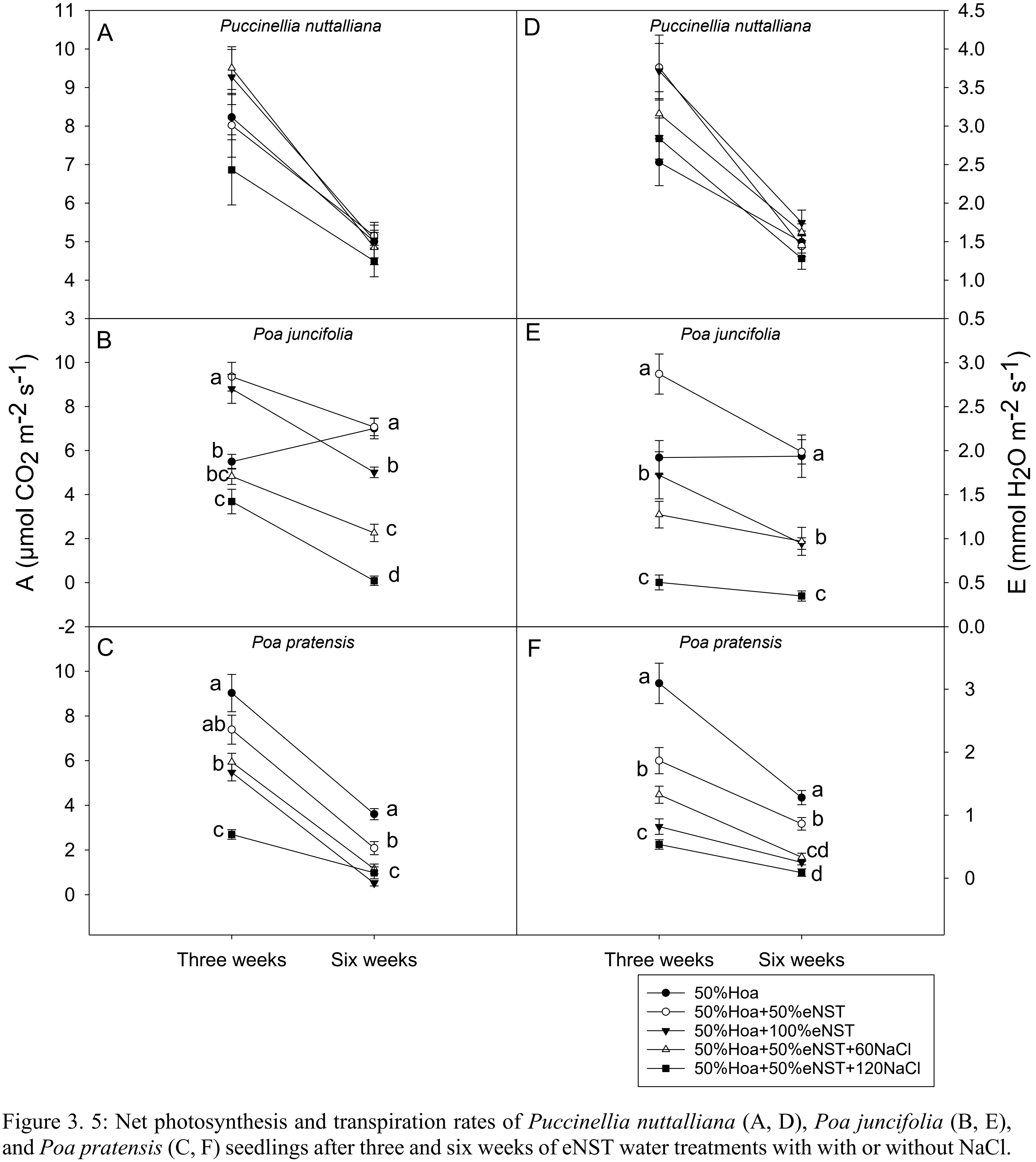

Figure 3. 5: Net photosynthesis and transpiration rates of *Puccinellia nuttalliana* (A, D), *Poa juncifolia* (B, E), and *Poa pratensis* (C, F) seedlings after three and six weeks of eNST water treatments with with or without NaCl. Means (n=8-10) ± SE are shown. Different letters indicate significant differences between treatments at the same time period within each species determined by Holm's test ($P<0.05$). (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

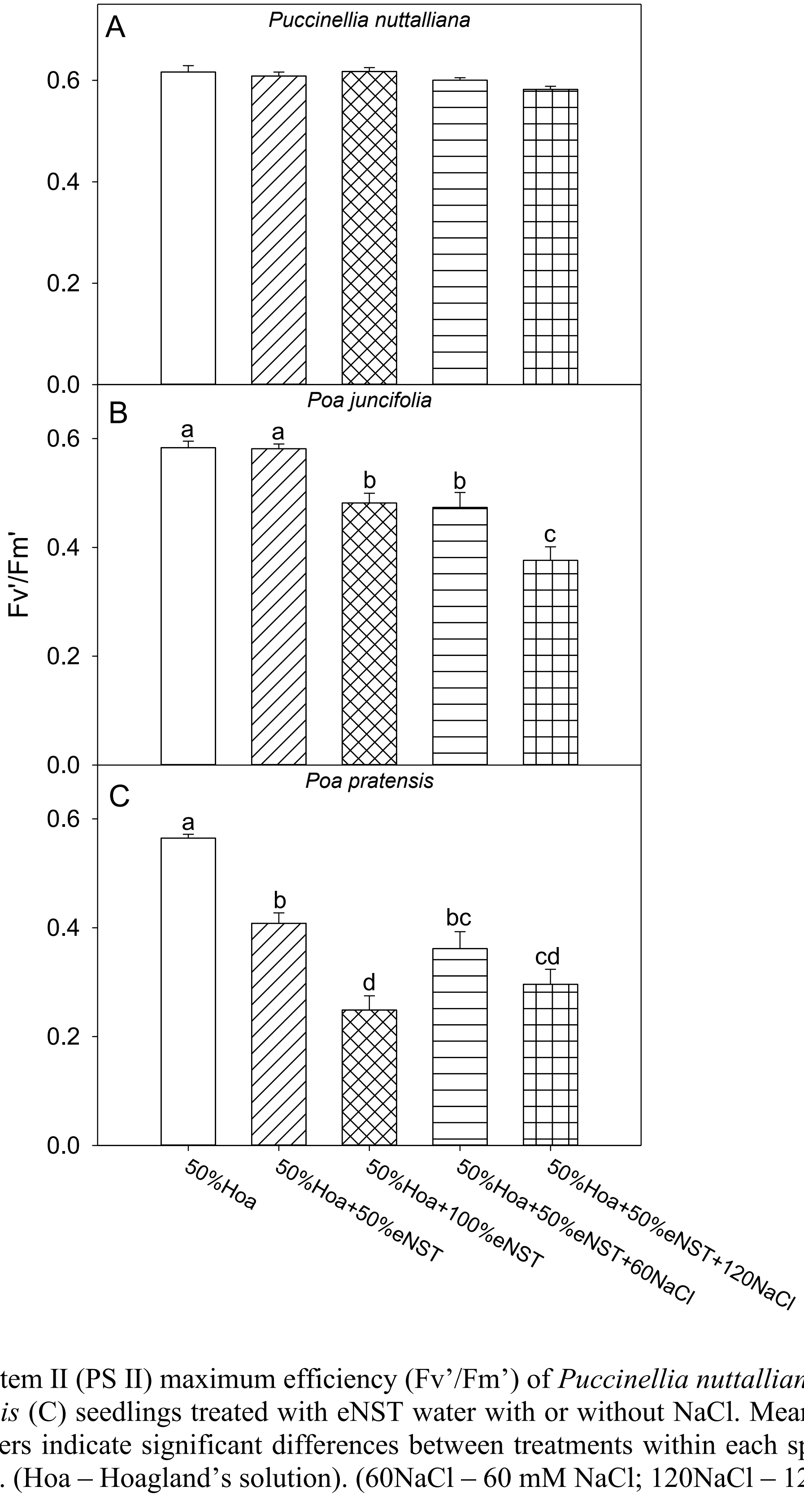

Figure 3. 6: Photosystem II (PS II) maximum efficiency (Fv'/Fm') of *Puccinellia nuttalliana* (A), *Poa juncifolia* (B), and *Poa pratensis* (C) seedlings treated with eNST water with or without NaCl. Means (n=8-10) ± SE are shown. Different letters indicate significant differences between treatments within each species determined by Holm's test ($P<0.05$). (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

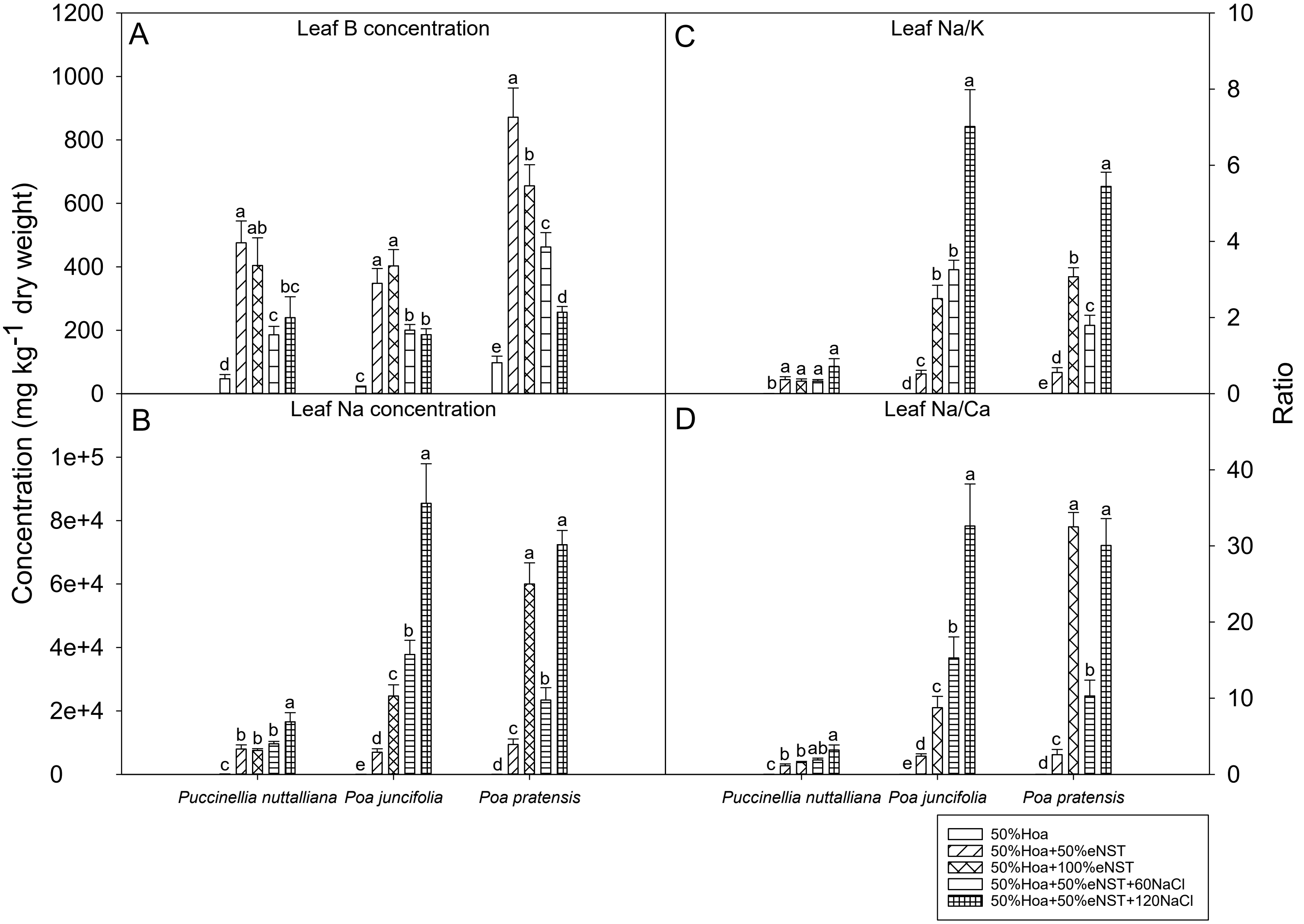

Figure 3. 7: Leaf concentrations (mg kg^{-1}) of B (A), Na (B), Na/K ratio (C), and Na/Ca ratio (D) in *Puccinellia nuttalliana*, *Poa juncifolia*, and *Poa pratensis* seedlings treated with eNST water with or without NaCl. Means (n=6) ± SE are shown. Different letters indicate significant differences between treatments within each species determined by Holm's test (P<0.05). (Hoa – Hoagland's solution). (60NaCl – 60 mM NaCl; 120NaCl – 120 mM NaCl).

Chapter 4 General Discussion and Conclusions

4.1 General discussion

The first study showed that eNST water significantly decreased the growth parameters in paper birch. The leaf chlorophyll concentrations and gas exchange rates in paper birch and trembling aspen were also significantly reduced by the eNST water. These detrimental effects are largely due to the high pH (> 9) and high salinity (4 mS cm^{-1}) of eNST water, which can reduce nutrient availability, disturb plant ion balance, and induce osmotic stress (Marschner, 2011, Jouyban, 2012, Arif et al., 2020). When the root hypoxia was combined with the eNST water, it aggravated the negative effects of eNST water on the growth and physiological processes in paper birch, which can be attributed to the interference of salt exclusion ability by the root hypoxia (Drew, 1997, Li et al., 2022). Moreover, compared with the aerated eNST water, the combination of root hypoxia and eNST water also reduced leaf chlorophyll concentrations in jack pine and black spruce, which were partially due to the reduction of leaf nutrient concentrations and deactivation of enzymes induced by oxygen deficiency (Steffens et al., 2005, Arif et al., 2020).

Results of the second study showed that *Puccinellia nuttalliana* was more salt tolerant than *Poa juncifolia* and *Poa pratensis*, which is consistent with the previous studies (Vaziriyeganeh et al., 2018, 2021, 2022, 2023). The high salt tolerance of *Puccinellia nuttalliana* is likely partially due to its salt exclusion ability, allowing the plants to maintain low leaf Na concentrations, low leaf Na/K ratios, and low leaf Na/Ca ratios. All applied treatments significantly inhibited the growth and physiological processes in *Poa pratensis* confirming that this glycophyte is sensitive to high salinity conditions (Mishra and Tanna, 2017, Vaziriyeganeh et al., 2018). On the other hand, 50% eNST water did not affect the relatively salt tolerant *Poa juncifolia* (Flowers and Colmer, 2008, Vaziriyeganeh et al., 2018). Besides, when the 50% eNST water concentration was increased to

100% or NaCl was added to 50% eNST water, the salinity exceed the salt tolerance threshold of *Poa juncifolia*, resulting in significant decreases in chlorophyll concentrations, net photosynthesis, and total dry weights. In this study, the reductions of growth and physiological parameters of *Poa juncifolia* and *Poa pratensis* can be attributed to the osmotic stress, ion toxicity, and reduced nutrient uptake induced by high salinity (Munns and Tester, 2008, Marschner, 2011).

4.2 General conclusion

The first study demonstrated that eNST water impaired the growth and physiological processes in four tree species that are commonly used for oil sands reclamation. The high sodium and boron concentrations in the eNST water significantly increased leaf sodium and boron concentrations in all four tree species. The high pH and high salinity of eNST water reduced the leaf nutrient elemental concentrations, chlorophyll concentrations, and net photosynthesis rates of paper birch and trembling aspen. Besides, the net photosynthesis rates of jack pine and black spruce were more sensitive to root hypoxia than to eNST water. Root hypoxia can aggravate the negative effects of eNST water on the growth and physiological processes of paper birch and trembling aspen. In addition, the needle chlorophyll contents in jack pine and black spruce were also reduced by the combination of eNST water and root hypoxia.

The second study showed that *Puccinellia nuttalliana* is more tolerant to eNST water than *Poa juncifolia* and *Poa pratensis*. The growth and physiological processes of *Puccinellia nuttalliana* were unaffected in all treatment groups, which was partially due to its salt exclusion ability. Therefore, *Puccinellia nuttalliana* may be a useful species for improving the success of oil sands land reclamation. Besides, *Poa pratensis* was the most sensitive species to all applied treatments among the three grass species. The performances of three grass species corresponded to the ranking of their salt tolerance demonstrating that salinity plays a main role in the detrimental effects of eNST water on reclamation plants. The 50% eNST water did not affect *Poa juncifolia*. The 100% eNST water or 50% eNST water plus NaCl exceeded the salt tolerance threshold of *Poa juncifolia*, leading to decreases in the total dry weight, leaf chlorophyll concentration, and net photosynthesis of *Poa juncifolia*. Besides, compared with the 50% eNST water treatment, the addition of NaCl to 50% eNST water aggravated the negative effects of the eNST water on *Poa pratensis*.

4.3 Suggestions for oil sands revegetation and future research

The results of this thesis studies show that root hypoxia can aggravate the negative effects of eNST water on the growth and physiological processes of plants. Therefore, for oil sands land reclamation, attention should be paid to the drainage of reclamation sites to avoid waterlogging and root hypoxia. Besides, the plant species inherently growing in saline soil should be considered reliable candidates to increase the success of oil sands reclamation.

Future research should examine the performance of other halophytes in oil sands reclamation. Results from these studies can provide more plant species options to increase the success of oil sands reclamation. Besides, some researchers suggested that removing the halophytes growing in saline soil can desalinate the soil environment (Sarath et al., 2021). Therefore, more studies should be conducted to investigate the land desalination capabilities of *Puccinellia nuttalliana* in the fieldwork. Moreover, future studies should compare the desalination capabilities of different halophytes to screen the optimal species for oil sands tailings reclamation.

4.4 Application for oil sands reclamation

This thesis research was conducted in the hydroponic system under controlled environment conditions, which may not reflect land reclamation conditions. Additional studies should be carried out to examine plant performance under the field conditions.

The first study showed that root hypoxia can aggravate the negative effects of eNST water on the growth and physiological processes of reclamation plants. Therefore, the landform design of reclamation sites should emphasize adequate drainage to avoid waterlogging and root hypoxia.

The second study showed that *Puccinellia nuttalliana* is highly tolerant of the tailings release water. Therefore, land reclamation managers should examine the performance of *Puccinellia nuttalliana* in the oil sands reclamation sites. In addition, other halophytes that share similar traits with *Puccinellia nuttalliana* shouldbe examined in future research to investigate their potential for oil sands reclamation.

4.5 References

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. & Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156**,** 64-77.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Biology,* 48**,** 223-250.

Flowers, T. J. & Colmer, T. D. 2008. Salinity tolerance in halophytes. *New phytologist***,** 179, 945-963.

Jouyban, Z. 2012. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

Li, Y., Zeng, H., Xu, F., Yan, F. & Xu, W. 2022. H^+-ATPases in plant growth and stress responses. *Annual Review of Plant Biology,* 73**,** 495-521.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. *Academic Press*. London, UK. 447 p.

Mishra, A. & Tanna, B. 2017. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8**,** 829-839. doi: 10.3389/fpls.2017.00829.

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

Sarath, N. G., Sruthi, P., Shackira, A. & Puthur, J. T. 2021. Halophytes as effective tool for phytodesalination and land reclamation. *Frontiers in Plant-Soil Interaction.* In *Frontiers in Plant-Soil Interaction* (pp. 459-494). Academic Press. Elsevier. doi:10.1016/B978-0-323-90943-3.00020-1.

Steffens, D., Hutsch, B., Eschholz, T., Losak, T. & Schubert, S. 2005. Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment,* 51**,** 545-552.

Vaziriyeganeh, M., Carvajal, M., Du, N. & Zwiazek, J. J. 2022. Salinity tolerance of halophytic grass *Puccinellia nuttalliana* is associated with enhancement of aquaporin-mediated water transport by sodium. *International Journal of Molecular Sciences,* 23**,** 5732-5750.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2021. Transcriptome and metabolome analyses reveal potential salt tolerance mechanisms contributing to maintenance of water balance by the halophytic grass Puccinellia nuttalliana. *Frontiers in Plant Science,* 12**,** 760863-760887.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2023. Analysis of aquaporins in northern grasses reveal functional importance of Puccinellia nuttalliana PIP2; 2 in salt tolerance. *Plant, Cell & Environment*, 46, 1033-1418.

Vaziriyeganeh, M., Lee, S. H. & Zwiazek, J. J. 2018. Water transport properties of root cells contribute to salt tolerance in halophytic grasses *Poa juncifolia* and *Puccinellia nuttalliana*. *Plant Science,* 276**,** 54-62.

Bibliography

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J. & Hernandez, J. A. 2017. Plant responses to salt stress: adaptive mechanisms. *Agronomy,* 7**,** 18. doi: 10.3390/agronomy7010018.

Aer. 2023. Environmental protection and enhancement act [Online]. *Alberta Energy Regulator*. Available: https://open.alberta.ca/publications/e12#summary [Accessed 2022 February]. 134 p.

Ahmad, P., Azooz, M. M. & Prasad, M. N. V. 2013. Ecophysiology and responses of plants under salt stress. *Springer*, 32-33. doi: 10.1007/978-1-4614-4747-4.

Alberta, G. O. 2010. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. Second edition [Online]. Available: https://open.alberta.ca/publications/9780778588252 [Accessed 2023 September]. 43 p.

Alberta, G. O. 2013. Oil Sands Mine Reclamation and Disturbance Tracking by Year [Online]. Available: http://osip.alberta.ca/library/Dataset/Details/27 [Accessed 2023 February].

Alberta, G. O. 2023. Oil Sands Overview [Online]. Available: https://www.alberta.ca/oil-sands-overview.aspx [Accessed 2021 July].

Alberta, P. O. 2022. Environmental protection and enhancement act, conservation and reclamation regulation. 134 p.

Alejandro, S., Höller, S., Meier, B. & Peiter, E. 2020. Manganese in plants: from acquisition to subcellular allocation. *Frontiers in Plant Science,* 11**,** 300. doi: 10.3389/fpls.2020.00300.

Aleksandrov, V. 2022. Identification of nutrient deficiency in plants by artificial intelligence. *Acta Physiologiae Plantarum,* 44**,** 29. doi: 10.1007/s11738-022-03363-0.

Ali, A. & Yun, D.-J. 2017. Salt stress tolerance; what do we learn from halophytes. *Journal of Plant Biology,* 60**,** 431-439.

Alloway, B. J. 2008. Micronutrient deficiencies in global crop production. *Springer Science & Business Media*. 230 p. doi: https://doi.org/10.1007/978-1-4020-6860-7.

Ana Flor, E.-M., Daniela, E. & Katrin, E. 2016. Chloroplast iron transport proteins - function and impact on plant physiology. *Frontiers in Plant Science,* 7, 1-12. doi: 10.3389/fpls.2016.00178

Andresen, E., Peiter, E. & Küpper, H. 2018. Trace metal metabolism in plants. *Journal of Experimental Botany,* 69**,** 909-954.

Andrews, J. A., Johnson, J. E., Torbert, J. L., Burger, J. A. & Kelting, D. L. 1998. Minesoil and site properties associated with early height growth of eastern white pine. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Vol. 27, No. 1, pp. 192-199.

Andrews, J. A., Johnson, J. E., Torbert, J. L., Burger, J. A. & Kelting, D. L. 1998. Minesoil and site properties associated with early height growth of eastern white pine. *Journal of Environmental Quality,* 27**,** 192-199.

Apostol, K. & Zwiazek, J. J. 2003. Hypoxia affects root sodium and chloride concentrations and alters water conductance in salt-treated jack pine (*Pinus banksiana*) seedlings. *Trees,* 17**,** 251-257.

Apostol, K. G., Zwiazek, J. J. & Mackinnon, M. D. 2004. Naphthenic acids affect plant water conductance but do not alter shoot Na^+ and Cl^- concentrations in jack pine (*Pinus banksiana*) seedlings. *Plant and Soil,* 263**,** 183-190.

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A. & Hayat, S. 2020. Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry,* 156**,** 64-77.

Arno, S. F. & Hammerly, R. P. 2020. Northwest trees : identifying and understanding the region's native trees. *Mountaineers Books.* 601 p.

Ashraf, M. 2012. Waterlogging stress in plants: a review. *African Journal of Agricultural Research,* 7 (13), 1976-1981.

Aer. 2021. Reclamation process and criteria for oil and gas sites [Online]. *Alberta Energy Regulator*. Available: https://www.aer.ca/regulating-development/projectclosure/reclamation/oil-and-gas-site-reclamation-requirements/reclamation-process-and-criteria-for-oil-and-gas-sites [Accessed 2021 July].

Bai, T., Li, C., Ma, F., Shu, H. & Han, M. 2009. Exogenous salicylic acid alleviates growth inhibition and oxidative stress induced by hypoxia stress in *Malus robusta Rehd*. *Journal of Plant Growth Regulation,* 28**,** 358-366.

Bansal, R. & Srivastava, J. P. 2015. Effect of waterlogging on photosynthetic and biochemical parameters in pigeonpea. *Russian Journal of Plant Physiology,* 62**,** 322-327.

Barrett-Lennard, E. G. & Shabala, S. N. 2013. The waterlogging/salinity interaction in higher plants revisited–focusing on the hypoxia-induced disturbance to K^+ homeostasis. *Functional Plant Biology,* 40**,** 872-882.

Barros, N. L. F., Marques, D. N., Tadaiesky, L. B. A. & De Souza, C. R. B. 2021. Halophytes and other molecular strategies for the generation of salt-tolerant crops. *Plant Physiology and Biochemistry,* 162**,** 581-591.

Blumwald, E., Aharon, G. S. & Apse, M. P. 2000. Sodium transport in plant cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes,* 1465**,** 140-151.

Boukhalfa, H. & Crumbliss, A. L. 2002. Chemical aspects of siderophore mediated iron transport. *Biometals,* 15**,** 325-339.

Bradford, K. J. & Hsiao, T. C. 1982. Stomatal behavior and water relations of waterlogged tomato plants. *Plant Physiology,* 70**,** 1508-1513.

Brady, N. 1984. The nature and properties of soils (ninth edition). *New York: Macmillan*.

Brdar-Jokanović, M. 2020. Boron toxicity and deficiency in agricultural plants. *International Journal of Molecular Sciences,* 21**,** 1424-1444.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. & Lux, A. 2007. Zinc in plants. *New pPhytologist,* 173**,** 677-702.

Búfalo, J., Rodrigues, T. M., De Almeida, L. F. R., Dos Santos Tozin, L. R., Marques, M. O. M. & Boaro, C. S. F. 2016. PEG-induced osmotic stress in *Mentha x piperita L.*: structural features and metabolic responses. *Plant Physiology and Biochemistry,* 105**,** 174-184.

Burns, R. M. & Honkala, B. H. 1990. Silvics of North America. Volume 2: Hardwoods. USDA Forest Service. *Agriculture Handbook,* 654. 158 p.

Bushman, B. S., Amundsen, K. L., Warnke, S. E., Robins, J. G. & Johnson, P. G. 2016. Transcriptome profiling of kentucky bluegrass (*Poa pratensis L.*) accessions in response to salt stress. *BMC Genomics,* 17**,** 1-12.

Çalişkan, B. & Çalişkan, A. C. 2019. Potassium nutrition in plants and its interactions with other nutrients in hydroponic culture. *Improvement of Quality in Fruits and Vegetables Through Hydroponic Nutrient Management,* 9 p. doi: 10.5772/intechopen.71951.

Capp. 2022. Land Reclamation [Online]. *Canadian Association of Petroleum Producers*. Available: https://www.capp.ca/explore/land-reclamation/ [Accessed 2022 September].

Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M. & Husted, S. 2018. The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant physiology,* 177**,** 271-284.

Centofanti, T. & Bañuelos, G. 2015. Evaluation of the halophyte *Salsola soda* as an alternative crop for saline soils high in selenium and boron. *Journal of Environmental Management,* 157**,** 96-102.

Cheeseman, J. M. 2015. The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytologist,* 206**,** 557-570.

Chen, G., Amoanimaa-Dede, H., Zeng, F., Deng, F., Xu, S. & Chen, Z.-H. 2022. Chapter One - Stomatal regulation and adaptation to salinity in glycophytes and halophytes. *In:* SHABALA, S. (ed.) *Advances in Botanical Research.* Academic Press, 103, 1-42.

Chen, G., Amoanimaa-Dede, H., Zeng, F., Deng, F., Xu, S. & Chen, Z.-H. 2022a. Chapter One - Stomatal regulation and adaptation to salinity in glycophytes and halophytes. *In:* Shabala, S. (ed.) *Advances in Botanical Research.* Academic Press, 103, 1-42.

Chen, L., Zhao, R., Yu, J., Gu, J., Li, Y., Chen, W. & Guo, W. 2022b. Functional analysis of plasma membrane H^+-ATPases in response to alkaline stress in blueberry. *Scientia Horticulturae,* 306**,** 111453.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S. A. & Tzortzakis, N. 2019. The combined and single effect of salinity and copper stress on growth and quality of *Mentha spicata* plants. *Journal of Hazardous Materials,* 368**,** 584-593.

CNRL. 2017. CNRL 2017 Stewardship report to stakeholders [Online]. *Canadian Natural Resources Limited*. Available: https://www.cnrl.com/upload/report/112/05/2017-stewardship-report-to-stakeholders.pdf [Accessed 2021 July]. 19 p.

Context Energy Examined. 2023. Myth: The oil sands are destroying Canada's boreal forests [Online]. Available: https://context.capp.ca/energy-matters/2019/mythbuster-oil-sands-extraction-and-canadas-boreal-forest/ [Accessed 2023 May].

Cosgrove, D. J. 2022. Plant cell growth and cell wall enlargement. *Encyclopedia of Life Sciences.* 854 p. doi: https://doi.org/10.1002/9780470015902.a0029421.

Cosgrove, D. J. 1996. Plant cell enlargement and the action of expansins. *BioEssays,* 18**,** 533-540.

Cosgrove, D. J. 2005. Growth of the plant cell wall. *Nature Reviews. Molecular Cell Biology,* 6**,** 850-61.

Cramer, G. R., LäUchli, A. & Polito, V. S. 1985. Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells: a primary response to salt stress. *Plant Physiology,* 79**,** 207-211.

Dai, Q. & Chung, K. H. 1996. Hot water extraction process mechanism using model oil sands. *Fuel,* 75**,** 220-226.

Dassanayake, M. & Larkin, J. C. 2017. Making plants break a sweat: the structure, function, and evolution of plant salt glands. *Frontiers in Plant Science,* 8, 406-426.

De Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., De Abreu, C. E. B. & Gomes-Filho, E. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany,* 56**,** 87-94.

Dhar, A., Comeau, P. G., Karst, J., Pinno, B. D., Chang, S. X., Naeth, A. M., Vassov, R. & Bampfylde, C. 2018. Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. *Environmental Reviews,* 26**,** 286-298.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Biology,* 48**,** 223-250.

Drew, M. C. 1997. Oxygen deficiency and root metabolism: Injury and acclimation under hypoxia and anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology,* 48**,** 223-250.

Duarte, B., Santos, D., Marques, J. C. & Caçador, I. 2013. Ecophysiological adaptations of two halophytes to salt stress: photosynthesis, PS II photochemistry and anti-oxidant feedback – Implications for resilience in climate change. *Plant Physiology and Biochemistry,* 67**,** 178-188.

Düzyol, S. An evaluation of toxicity effect on flocculants usage in environmental aspects. 792 p.

E-Flora, E. a. O. T. F. O. B. C. 2023. *Puccinellia nuttalliana (Schult.) Hitchc. Nuttall's alkaligrass Poaceae (Grass family)* [Online]. Available: https://linnet.geog.ubc.ca/Atlas/Atlas.aspx?sciname=Puccinellia%20nuttalliana [Accessed 2023 March].

Epstein, E. & Bloom, A. J. 1853. Mineral nutrition of plants: principles and perspectives. *Sinauer*.

Evans, G. & Hughes, A. 1961. Plant growth and the aerial environment: I. Effect of artificial shading on *Impatiens parviflora*. *New Phytologist,* 60**,** 150-180.

Farhat, N., Elkhouni, A., Zorrig, W., Smaoui, A., Abdelly, C. & Rabhi, M. 2016. Effects of magnesium deficiency on photosynthesis and carbohydrate partitioning. *Acta Physiologiae Plantarum,* 38**,** 145-155. doi: 10.1007/s11738-016-2165-z.

Feis, F. E. I. S. 2023a. *Index of Species Information species: Betula papyrifera* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/betpap/all.html [Accessed 2021 July].

Feis, F. E. I. S. 2023b. Index of Species Information species: *Pinus banksiana*.

Feis, F. E. I. S. 2023c. Index of Species Information species: *Pinus banksiana* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/pinban/all.html [Accessed 2021 August].

Feis, F. E. I. S. 2023d. Index of Species Information species: *Poa pratensis* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/graminoid/poapra/all.html [Accessed 2022 August].

Feis, F. E. I. S. 2023e. Index of Species Information species: *Poa secunda* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/graminoid/poasec/all.html [Accessed 2022 August].

Feis, F. E. I. S. 2023f. Index of Species Information species: *Populus tremuloides* [Online]. Available: https://www.fs.usda.gov/database/feis/plants/tree/poptre/all.html [Accessed 2022 August].

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.

Fleurial, K. G., Zhang, W.-Q., Vassov, R. & Zwiazek, J. J. 2022. Root hypoxia aggravates the effects of saline tailings water on the growth and physiology of woody plants. *Plant and Soil***,** 481, 1-18.

Flowers, T. J. & Colmer, T. D. 2008. Salinity tolerance in halophytes. *New Phytologist,* 179**,** 945-963.

Flowers, T. J. & Colmer, T. D. 2008a. Salinity tolerance in halophytes. *New Phytologist***,** 179, 945-963.

Flowers, T. J. & Colmer, T. D. 2008b. Salinity tolerance in halophytes. *New Phytologist,* 179**,** 945-963.

Flowers, T. J. & Colmer, T. D. 2015. Plant salt tolerance: adaptations in halophytes. *Annals of Botany,* 115**,** 327-331.

Fung, M. Y. & Macyk, T. M. 2000. Reclamation of oil sands mining areas. *Reclamation of Drastically Disturbed Lands,* 41**,** 755-774.

Geology. 2023. What are oil sands [Online]. Available: https://geology.com/articles/oil-sands/ [Accessed 2021 June].

George, E., Horst, W. J. & Neumann, E. 2012. Adaptation of plants to adverse chemical soil conditions. Marschner's mineral nutrition of higher plants. In *Marschner's mineral nutrition of higher plants* (pp. 409-472). Academic Press.

Giesy, J. P., Anderson, J. C. & Wiseman, S. B. 2010. Alberta oil sands development. *Proceedings of the National Academy of Sciences,* 107**,** 951-952.

Goldberg, S. & Forster, H. 1991. Boron sorption on calcareous soils and reference calcites. *Soil Science,* 152**,** 304-310.

Government of Alberta. 2010. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region [Online]. Available: https://open.alberta.ca/publications/9780778588252 [Accessed 2021 August]. 43 p.

Government of Alberta. 2023. Oil sands – Overview [Online]. Available: https://www.alberta.ca/oil-sands-overview.aspx [Accessed 2021 July].

Grieve, C. M. & Poss, J. A. 2000. Wheat response to interactive effects of boron and salinity. *Journal of Plant Nutrition,* 23**,** 1217-1226.

Grondin, A., Rodrigues, O., Verdoucq, L., Merlot, S., Leonhardt, N. & Maurel, C. 2015. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *Plant Cell,* 27**,** 1945-1954.

Guo, R., Shi, L., Yan, C., Zhong, X., Gu, F., Liu, Q., Xia, X. & Li, H. 2017. Ionomic and metabolic responses to neutral salt or alkaline salt stresses in maize (*Zea mays L.*) seedlings. *BMC Plant Biology,* 17**,** 1-13.

Gupta, R. K. & Abrol, I. 1990. Salt-affected soils: their reclamation and management for crop production. *Advances in Soil Science: Soil Degradation Volume 11***,** 223-288.

Hasegawa, P. M., Bressan, R. A., Zhu, J.-K. & Bohnert, H. J. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Biology,* 51**,** 463-499.

Hniličková, H., Hnilička, F., Hejnák, V. & Orsák, M. 2019. Effect of salt stress on growth, electrolyte leakage, Na^+ and k^+ content in selected plant species. *Plant, Soil and Environment,* 65**,** 90-96-96.

Howat, D. 2000. Acceptable salinity, sodicity and pH values for boreal forest reclamation. *Environmental Sciences Division Edmonton*, AB, Canada. 8 p.

Howat, D. 2000a. Acceptable salinity, sodicity and pH values for boreal forest reclamation. *Environmental Sciences Division Edmonton, AB, Canada*, p. 191. 8 p.

Howat, D. 2000b. Acceptable salinity, sodicity and pH values for boreal forest reclamation. *Alberta Environment, Environmental Service, Environmental Sciences Division*, p. 191. 8 p.

Howell, D. M. 2015. Influence of amendments and soil depth on available nutrients and microbial dynamics in contrasting topsoil materials used for oil sands reclamation. 8 p.

Hu, H. & Sparks, D. 1991. Zinc deficiency inhibits chlorophyll synthesis and gas exchange in *Stuart Pecan*. *HortScience,* 26**,** 267-268.

Hu, X., Wang, D., Ren, S., Feng, S., Zhang, H., Zhang, J., Qiao, K. & Zhou, A. 2022. Inhibition of root growth by alkaline salts due to disturbed ion transport and accumulation in *Leymus chinensis*. *Environmental and Experimental Botany,* 200**,** 104907-104918.

Islam, A., Edwards, D. & Asher, C. 1980. pH optima for crop growth: results of a flowing solution culture experiment with six species. *Plant and Soil,* 54**,** 339-357.

Jauregui, M. A. & Reisenauer, H. 1982. Dissolution of oxides of manganese and iron by root exudate components. *Soil Science Society of America Journal,* 46**,** 314-317.

Jones Jr, J. B. 2012. Plant nutrition and soil fertility manual. *CRC press*. 91 p.

Jouyban, Z. 2012. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

Jouyban, Z. 2012a. The effects of salt stress on plant growth. *Tech J Engin & App Sci,* 2**,** 7-10.

Jouyban, Z. 2012b. The effects of salt stress on plant growth. *Technical Journal of Engineering and Applied Sciences,* 2**,** 7-10.

Kamaluddin, M. & Zwiazek, J. J. 2002a. Ethylene enhances water transport in hypoxic aspen. *Plant Physiology,* 128**,** 962-969.

Kamaluddin, M. & Zwiazek, J. J. 2002. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. *Tree Physiology,* 22**,** 1265-1270.

Kamaluddin, M. & Zwiazek, J. J. 2002b. Naphthenic acids inhibit root water transport, gas exchange and leaf growth in aspen (*Populus tremuloides*) seedlings. *Tree Physiology,* 22**,** 1265-1270.

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.

Khalid, R., Patrick Jr, W. & Gambrell, R. 1978. Effect of dissolved oxygen on chemical transformations of heavy metals, phosphorus, and nitrogen in an estuarine sediment. *Estuarine and Coastal Marine Science,* 6**,** 21-35.

Khan, M. M., Al-Mas'oudi, R. S., Al-Said, F. & Khan, I. Salinity effects on growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (*Cucumis sativus L.*). *International Conference on Food and Agricultural Sciences Malaysia: IACSIT Press*, 2013. 28-32.

Khayyat, M., Tehranifar, A., Akbarian, A., Shayesteh Nia, S. & Khabari, S. 2009. Effects of calcium forms on electrolyte leakage, total nitrogen, yield and biomass production by strawberry plants under NaCl salinity. *Journal of Central European Agriculture,* 10**,** 297-302.

Kiani-Pouya, A., Rasouli, F., Bazihizina, N., Zhang, H., Hedrich, R. & Shabala, S. 2019. A large-scale screening of quinoa accessions reveals an important role of epidermal bladder cells and stomatal patterning in salinity tolerance. *Environmental and Experimental Botany,* 168**,** 103885.

Kieko, S. & Masahiko, I. 2012. The function of roots of tea plant (*Camellia sinensis*) cultured by a novel form of hydroponics and soil acidification. *American Journal of Plant Sciences,* 4, 1-4.

Kopittke, P. M. & Menzies, N. W. 2005. Control of nutrient solutions for studies at high pH. *Plant and Soil,* 266**,** 343-354.

Kuboi, T. & Fujii, K. 1985a. Toxicity of cationic polymer flocculants to higher plants. *Soil Science and Plant Nutrition,* 31**,** 163-173.

Kuboi, T. & Fujii, K. 1985b. Toxicity of cationic polymer flocculants to higher plants: II. Hydroponic cultures. *Soil Science and Plant Nutrition,* 31**,** 163-173.

Lambers, H., Chapin, F. S. & Pons, T. L. 2008. Plant physiological ecology (Vol. 2, pp. 11-99). *New York: Springer*. 122 p.

Lamdan, N. L., Attia, Z., Moran, N. & Moshelion, M. 2012. The *Arabidopsis*-related halophyte *Thellungiella halophila*: boron tolerance via boron complexation with metabolites. *Plant, Cell & Environment,* 35**,** 735-746.

Läuchli, A. & Grattan, S. R. 2012. Soil pH extremes. *Plant Stress Physiology.* CABI Wallingford UK. 195 p.

Lazorko, H. & Van Rees, K. C. 2012. Root distributions of planted boreal mixedwood species on reclaimed saline–sodic overburden. *Water, Air, & Soil Pollution,* 223**,** 215-231.

Lee, S. H. & Zwiazek, J. J. 2015. Regulation of Aquaporin-Mediated Water Transport in *Arabidopsis* Roots Exposed to NaCl. *Plant and Cell Physiology,* 56**,** 750-758.

Lewis, O. A. & Lewis, O. A. 1991. Plants and nitrogen. *Cambridge University Press*. 52 p.

Li, Y., Zeng, H., Xu, F., Yan, F. & Xu, W. 2022. H^+-ATPases in plant growth and stress responses. *Annual Review of Plant Biology,* 73**,** 495-521.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996a. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183-183.

Liang, Y., Shen, Q., Shen, Z. & Ma, T. 1996b. Effects of silicon on salinity tolerance of two barley cultivars. *Journal of Plant Nutrition,* 19**,** 173-183.

Limited, H. B. 1989. Manual of plant species suitability for reclamation in Alberta: 2nd Edition [Online]. Available: https://www.cclmportal.ca/resource/manual-plant-species-suitability-reclamation-alberta-2nd-edition [Accessed 2021 July]. 178 p.

Lopes, L. D., Hao, J. & Schachtman, D. P. 2021. Alkaline soil pH affects bulk soil, rhizosphere and root endosphere microbiomes of plants growing in a Sandhills ecosystem. *FEMS Microbiology Ecology,* 97**,** fiab028.

Lopes, L. D. S., Carvalho, H. H. D., Miranda, R. D. S., Gallão, M. I. & Gomes-Filho, E. 2020. The influence of dissolved oxygen around rice roots on salt tolerance during pre-tillering and tillering phases. *Environmental and Experimental Botany,* 178**,** 104169.

López-Berenguer, C., García-Viguera, C. & Carvajal, M. 2006. Are root hydraulic conductivity responses to salinity controlled by aquaporins in broccoli plants. *Plant and Soil,* 279**,** 13-23.

López-Bucio, J., Guevara-García, A., Ramírez-Rodríguez, V., Nieto, M. & Herrera-Estrella, L. Agriculture for marginal lands: transgenic plants towards the third millennium. In *Plant genetic engineering: towards the third millennium: Proceedings of the International Symposium on Plant Genetic Engineering, Havana, Cuba, 6-10 December, 1999.* (pp. 159-165). *Elsevier Science Publishers*.

Luna, D. F., Aguirre, A., Pittaro, G., Bustos, D., Ciacci, B. & Taleisnik, E. 2017. Nutrient deficiency and hypoxia as constraints to *Panicum coloratum* growth in alkaline soils. *Grass and Forage Science,* 72**,** 640-653.

Luna Wolter, G. L., Dhar, A. & Naeth, M. A. 2021. Response of three native grass species on dry tailings reclamation substrate amended with petroleum coke. *Wiley Online Library*, (Vol. 50, No. 2, pp. 384-395*).*

Luna Wolter, G. L. & Naeth, M. A. 2014. Dry mature fine tailings as oil sands reclamation substrates for three native grasses. *Journal of Environmental Quality,* 43**,** 1510-1516.

Lyubimova, I., Pankova, Y. & Pestov, L. 2009. Amelioration of alkali (sodic/solonetz) soils. *Agricultural Land Improvement'Amelioration and Reclamation,* 2**,** 241-266.

Macdonald, S. E., Landhäusser, S. M., Skousen, J., Franklin, J., Frouz, J., Hall, S., Jacobs, D. F. & Quideau, S. 2015. Forest restoration following surface mining disturbance: challenges and solutions. *New Forests,* 46**,** 703-732.

Macke, A. J. & Ungar, I. A. 1971. The effects of salinity on germination and early growth of *Puccinellia nuttalliana*. *Canadian Journal of Botany,* 49**,** 515-520.

Mackinnon, M., Matthews, J., Shaw, W. & Cuddy, R. 2001. Water quality issues associated with composite tailings (CT) technology for managing oil sands tailings. *International Journal of Surface Mining, Reclamation and Environment,* 15**,** 235-256.

Mackenzie, D. 2012. Best management practices for conservation of reclamation materials in the mineable oil sands region of Alberta. *DesLibris*. 49 p.

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.

Malakar, P. & Chattopadhyay, D. 2021. Adaptation of plants to salt stress: the role of the ion transporters. *Journal of Plant Biochemistry and Biotechnology,* 30**,** 668-683.

Marschner, H. 2011. Marschner's mineral nutrition of higher plants. *Academic press*. London, UK .447 p.

Matthews, J., Shaw, W., Mackinnon, M. & Cuddy, R. 2002. Development of composite tailings technology at Syncrude. *International Journal of Surface Mining, Reclamation and Environment,* 16**,** 24-39.

Mcmillan, R., Quideau, S., Mackenzie, M. & Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. *Journal of Environmental Quality,* 36**,** 1470-1478.

Mehta, P., Jajoo, A., Mathur, S. & Bharti, S. 2010. Chlorophyll a fluorescence study revealing effects of high salt stress on Photosystem II in wheat leaves. *Plant Physiology and Biochemistry,* 48**,** 16-20.

Melton, J. R., Mahtab, S. K. & Swoboda, A. R. 1973. Diffusion of zinc in soils as a function of applied zinc, phosphorus, and soil pH. *Soil Science Society of America Journal,* 37**,** 379-381.

Mengel, K. & Kirkby, E. A. 2012. Principles of plant nutrition. *Springer Science & Business Media*, 93, 479-480.

Mikula, R. J., Kasperski, K. L., Burns, R. D. & Mackinnon, M. D. 1996. Nature and fate of oil sands fine tailings. *ACS Publications*.

Mishra, A. & Tanna, B. 2017. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8, 829-839. doi: 10.3389/fpls.2017.00829.

Mishra, A. & Tanna, B. 2017a. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8**,** 829-839. doi: 10.3389/fpls.2017.00829.

Mishra, A. & Tanna, B. 2017b. Halophytes: potential resources for salt stress tolerance genes and promoters. *Frontiers in Plant Science,* 8, 829-839. doi: 10.3389/fpls.2017.00829.

Moraghan, J. & Mascagni Jr, H. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxicities. *Micronutrients in Agriculture,* 4**,** 371-425.

Mu, X. & Chen, Y. 2021. The physiological response of photosynthesis to nitrogen deficiency. *Plant Physiology and Biochemistry*, *158*, 76-82.

Munns, R. & Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology,* 59**,** 651-681.

Munns, R. & Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review Plant Biology,* 59**,** 651-681.

Murat, A., Turan, M., Awadelkarim, A., Khalifa, A. E., Taban, N. & Taban, S. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research,* 4**,** 893-897.

Nable, R. O., Bañuelos, G. S. & Paull, J. G. 1997. Boron toxicity. *Plant and soil,* 193**,** 181-198.

Naeth, M. A., Wilkinson, S., Mackenzie, D., Archibald, H. & Powter, C. B. 2013. Potential of LFH mineral soil mixes for reclamation of forested lands in Alberta. *Oil Sands Research and Information Network*. 3 p.

Nero, V., Farwell, A., Lee, L., Van Meer, T., Mackinnon, M. & Dixon, D. 2006. The effects of salinity on naphthenic acid toxicity to yellow perch: Gill and liver histopathology. *Ecotoxicology and Environmental Safety,* 65**,** 252-264.

Neina, D. 2019. The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science,* 2019**,** 1-9.

Ondrasek, G., Rathod, S., Manohara, K. K., Gireesh, C., Anantha, M. S., Sakhare, A. S., Parmar, B., Yadav, B. K., Bandumula, N. & Raihan, F. 2022. Salt stress in plants and mitigation approaches. *Plants,* 11**,** 717.

O'neill, M. A., Ishii, T., Albersheim, P. & Darvill, A. G. 2004. Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review Plant Biology,* 55**,** 109-139.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M. & Dat, J. F. 2008. An overview of plant responses to soil waterlogging. *Plant Stress,* 2**,** 20-27.

Parent, C., Capelli, N., Berger, A., Crevècoeur, M. & Dat, J. 2008a. An overview of Pplant responses to soil waterlogging. *Plant Stress,* 2, 20-27.

Parent, C., Capelli, N., Berger, A., Crèvecoeur, M. & Dat, J. F. 2008b. An overview of plant responses to soil waterlogging. *Plant Stress,* 2**,** 20-27.

Passioura, J. B. 2010. Plant–water relations. *Encyclopedia of Life Sciences.* 60 p.

Penn, C. J. & Camberato, J. J. 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture,* 9**,** 120.

Perala, D., Burns, R. & Honkala, B. 1990. *Populus tremuloicies Michx.*-Quaking Aspen. *Silvics of North America: Hardwoods; Burns, RM, Honkala, BH, Eds***,** 555-569.

Phillips, T., Petrone, R., Wells, C. & Price, J. 2016. Characterizing dominant controls governing evapotranspiration within a natural saline fen in the Athabasca oil sands of Alberta, Canada. *Ecohydrology,* 9**,** 817-829.

Princi, M. P., Lupini, A., Araniti, F., Longo, C., Mauceri, A., Sunseri, F. & Abenavoli, M. R. 2016. Boron toxicity and tolerance in plants: Recent advances and future perspectives. *Plant Metal Interaction***,** 115-147. doi: 10.1007/978-3-030-20732-8.

Producers, C. a. O. P. 2023. Canada's oil industry [Online]. Available: https://www.capp.ca/oil/ [Accessed 2023 April].

Purdy, B. G., Ellen Macdonald, S. & Lieffers, V. J. 2005. Naturally saline boreal communities as models for reclamation of saline oil sand tailings. *Restoration Ecology,* 13**,** 667-677.

Raghothama, K. G. 2005. Phosphorus and Plant Nutrition: An Overview. *Phosphorus: Agriculture and the Environment.* 355 p.

Rámila, C. D. P., Contreras, S. A., Di Domenico, C., Molina-Montenegro, M. A., Vega, A., Handford, M., Bonilla, C. A. & Pizarro, G. E. 2016. Boron stress response and accumulation potential of the extremely tolerant species *Puccinellia frigida*. *Journal of Hazardous Materials,* 317**,** 476-484.

Raven, J. 1980. Short-and long-distance transport of boric acid in plants. *New Phytologist,* 84**,** 231-249.

Rawat, J., Pandey, N. & Saxena, J. 2022. Role of potassium in plant photosynthesis, transport, growth and yield. *Role of Potassium in Abiotic Stress,* 1, 1-14. doi: 10.1007/978-981-16-4461-0_1.

Redfield, E., Croser, C., Zwiazek, J., Mackinnon, M. & Qualizza, C. 2003. Responses of red ‐ osier dogwood to oil sands tailings treated with gypsum or alum. *Journal of Environmental Quality,* 32**,** 1008-1014.

Redfield, E. B., Durnie, S. M. & Zwiazek, J. J. 2004. Effects of hypoxia on ion accumulation in wild raspberry (*Rubus idaeus*) exposed to water released from saline oil sands mine tailings. *Environmental and Experimental Botany,* 52**,** 1-9.

Regulator, A. E. 2023. Oil sands [Online]. Available: https://www.aer.ca/providing-information/by-topic/oil-sands [Accessed 2023 February].

Renault, S., Croser, C., Franklin, J., Zwiazek, J. & Mackinnon, M. 2001. Effects of consolidated tailings water on red-osier dogwood (*Cornus stolonifera Michx*) seedlings. *Environmental Pollution,* 113**,** 27-33.

Renault, S., Lait, C., Zwiazek, 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., Mackinnon, M. & Qualizza, C. 2003. Barley, a potential species for initial reclamation of saline composite tailings of oil sands. *Journal of eEnvironmental Quality,* 32**,** 2245-2253.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J. & Mackinnon, M. 1999. Responses of boreal plants to high salinity oil sands tailings water. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, Vol. 28, No. 6, pp. 1957-1962.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J. & Mackinnon, M. 1999. Responses of boreal plants to high salinity oil sands tailings water. (Vol. 28, No. 6, pp. 1957-1962). *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*.

Renault, S., Qualizza, C. & Mackinnon, M. 2004. Suitability of altai wildrye (*Elymus angustus*) and slender wheatgrass (*Agropyron trachycaulum*) for initial reclamation of saline composite tailings of oil sands. *Environmental Pollution,* 128, 339-349.

Renault, S., Zwiazek, J., Fung, M. & Tuttle, S. 2000. Germination, growth and gas exchange of selected boreal forest seedlings in soil containing oil sands tailings. *Environmental Pollution,* 107, 357-365.

Rengel, Z. 2002. Handbook of plant growth pH as the master variable. *CRC Press*, 88, 1-23.

Rengel, Z. 2002. Handbook of plant growth pH as the master variable. *CRC Press*, Vol. 88, 1-23.

Robin, A., Vansuyt, G., Hinsinger, P., Meyer, J. M., Briat, J.-F. & Lemanceau, P. 2008. Iron dynamics in the rhizosphere: consequences for plant health and nutrition. *Advances in Agronomy,* 99, 183-225.

Saeed, R., Mirza, S. & Ahmad, R. 2014. Electrolyte leakage and relative water content as affected by organic mulch in okra plant (*Abelmoschus esculentus (L.) Moench*) grown under salinity. *FUUAST Journal of Biology,* 4, 221-227.

Sajal, R. & Nasrin, C. 2020. Salt stress in plants and amelioration strategies: A critical review. *In:* Shah, F., Shah, S., Yajun, C., Chao, W. & Depeng, W. (eds.) *Abiotic Stress in Plants.* Rijeka: IntechOpen. 459 p. doi: 10.5772/intechopen.93552.

Sarath, N. G., Sruthi, P., Shackira, A. & Puthur, J. T. 2021. Halophytes as effective tool for phytodesalination and land reclamation. *Frontiers in Plant-Soil Interaction,* pp. 459-494. doi:10.1016/B978-0-323-90943-3.00020-1.

Sarath, N. G., Sruthi, P., Shackira, A. & Puthur, J. T. 2021a. Halophytes as effective tool for phytodesalination and land reclamation. *Frontiers in Plant-Soil Interaction.* Elsevier. 459-494. doi:10.1016/B978-0-323-90943-3.00020-1.

Sarath, N. G., Sruthi, P., Shackira, A. M. & Puthur, J. T. 2021b. Chapter 16 - Halophytes as effective tool for phytodesalination and land reclamation. *In:* Aftab, T. & Hakeem, K. R. (eds.) *Frontiers in Plant-Soil Interaction.* Academic Press. 461 p.

Schneider, R., Fortin, M., Berninger, F., Ung, C.-H., Swift, D. E. & Zhang, S. 2011. Modeling jack pine (*Pinus banksiana*) foliage density distribution. *Forest Science,* 57**,** 180-188.

Schumacher, B. A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. *Ecological Risk Assessment Support Center Office of Research and Development*.

Scott, A. C., Young, R. F. & Fedorak, P. M. 2008. Comparison of GC–MS and FTIR methods for quantifying naphthenic acids in water samples. *Chemosphere,* 73**,** 1258-1264.

Sestak, Z., Catský, J. & Jarvis, P. G. 1971. Plant photosynthetic production. Manual of methods. The Hague, Netherlands.

Shahid, M. A., Sarkhosh, A., Khan, N., Balal, R. M., Ali, S., Rossi, L., Gómez, C., Mattson, N., Nasim, W. & Garcia-Sanchez, F. 2020. Insights into the physiological and biochemical impacts of salt stress on plant growth and development. *Agronomy,* 10**,** 938-972.

Sheldon, A. R., Dalal, R. C., Kirchhof, G., Kopittke, P. M. & Menzies, N. W. 2017. The effect of salinity on plant-available water. *Plant and Soil,* 418**,** 477-491.

Sims, J. T. & Sharpley, A. N. 2005. Phosphorus: agriculture and the environment. *American Society of Agronomy*. 1-22 p. doi: 10.2134/agronmonogr46.

Steffens, D., Hutsch, B., Eschholz, T., Losak, T. & Schubert, S. 2005. Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant Soil and Environment,* 51**,** 545-552.

Stiles, A. R., Bautista, D., Terry, N., Atalay, E. & Babaoğlu, M. 2010. Mechanisms of boron tolerance and accumulation in plants: A physiological comparison of the extremely boron-tolerant plant species, *Puccinellia distans*, with the moderately boron-tolerant *Gypsophila arrostil*. *Environmental Science and Technology,* 44**,** 7089-7095.

Subgroup, T. & Association, C. E. M. 2009. Guidelines for reclamation to forest vegetation in the Athabasca Oil Sands Region. *Alberta Environment*. 43 p.

Tan, X., Liu, M., Du, N. & Zwiazek, J. J. 2021. Ethylene enhances root water transport and aquaporin expression in trembling aspen (*Populus tremuloides*) exposed to root hypoxia. *BMC Plant Biology,* 21**,** 1-8.

Tan, X., Xu, H., Khan, S., Equiza, M. A., Lee, S. H., Vaziriyeganeh, M. & Zwiazek, J. J. 2018. Plant water transport and aquaporins in oxygen-deprived environments. *Journal of Plant Physiology,* 227**,** 20-30.

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. In:* Barrow, N. J. (ed.) *Plant Nutrition — from Genetic Engineering to Field Practice: Proceedings of the Twelfth International Plant Nutrition Colloquium,* 21–26 September 1993, Perth, Western Australia (pp. 763-765). Springer Netherlands.

Tarasoff, C., Mallory-Smith, C. & Ball, D. 2007. Comparative plant responses of *Puccinellia distans* and *Puccinellia nuttalliana* to sodic versus normal soil types. *Journal of Arid Environments,* 70**,** 403-417.

Therby-Vale, R., Lacombe, B., Rhee, S. Y., Nussaume, L. & Rouached, H. 2022. Mineral nutrient signaling controls photosynthesis: focus on iron deficiency-induced chlorosis. *Trends in Plant Science,* 27**,** 502-509.

Tomar, R. S., Kataria, S. & Jajoo, A. 2021. Behind the scene: Critical role of reactive oxygen species and reactive nitrogen species in salt stress tolerance. *Journal of Agronomy and Crop Science,* 207**,** 577-588.

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.

Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E. & Et Al. 2003a. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature,* 425**,** 393-397.

Tournaire-Roux, C., Sutka, M., Javot, H., Gout, E., Gerbeau, P., Luu, D.-T., Bligny, R. & Maurel, C. 2003b. Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. *Nature,* 425**,** 393-397.

Tozlu, I., Moore, G. A. & Guy, C. L. 2000. Effects of increasing NaCl concentration on stem elongation, dry mass production, and macro-and micro-nutrient accumulation in *Poncirus trifoliata*. *Functional Plant Biology,* 27**,** 35-42.

Turan, M. A., Elkarim, A. H. A., Taban, N. & Taban, S. 2009. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *African Journal of Agricultural Research,* 4**,** 893-897.

Turner, N. & Begg, J. 1981. Plant-water relations and adaption to stress. *Plant and Soil,* 58**,** 97-131.

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants***,** 179-192. doi: 10.1007/978-81-322-0807-5_7

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013a. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants*, 179-192. doi: 10.1007/978-81-322-0807-5_7

Upadhyaya, H., Sahoo, L. & Panda, S. K. 2013b. Molecular physiology of osmotic stress in plants. *Molecular Stress Physiology of Plants*, 179-192. doi: 10.1007/978-81-322-0807-5_7

Usda, U. S. D. O. a. N. R. C. S. 2023. *Poa secunda J. Presl* Sandberg bluegrass [Online]. Available: https://plants.sc.egov.usda.gov/home/plantProfile?symbol=POSE [Accessed 2023 February].

Vaziriyeganeh, M., Carvajal, M., Du, N. & Zwiazek, J. J. 2022. Salinity tolerance of halophytic grass *Puccinellia nuttalliana* is associated with enhancement of aquaporin-mediated water transport by sodium. *International Journal of Molecular Sciences,* 23**,** 5732-5750.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2021. Transcriptome and metabolome analyses reveal potential salt tolerance mechanisms contributing to maintenance of water balance by the halophytic grass Puccinellia nuttalliana. *Frontiers in Plant Science,* 12**,** 760863-760887.

Vaziriyeganeh, M., Khan, S. & Zwiazek, J. J. 2023. Analysis of aquaporins in northern grasses reveal functional importance of Puccinellia nuttalliana PIP2; 2 in salt tolerance. *Plant, Cell & Environment*, 46, 1033-1418.

Vaziriyeganeh, M., Lee, S. H. & Zwiazek, J. J. 2018. Water transport properties of root cells contribute to salt tolerance in halophytic grasses *Poa juncifolia* and *Puccinellia nuttalliana*. *Plant Science,* 276**,** 54-62.

Vedoy, D. R. & Soares, J. B. 2015. Water‐soluble polymers for oil sands tailing treatment: A Review. *Canadian Journal of Chemical Engineering,* 93**,** 888-904.

Viereck, L. A. & Johnston, W. F. 1990. *Picea mariana (Mill.)* BSP black spruce. *Silvics of North America,* 1**,** 227-237.

Wallace, A., Wallace, G. A. & Abouzamzam, A. 1986. Effects of excess levels of a polymer as a soil conditioner on yields and mineral nutrition of plants. *Soil Science,* 141**,** 377-380.

Wang, H., Ahan, J., Wu, Z., Shi, D., Liu, B. & Yang, C. 2012. Alteration of nitrogen metabolism in rice variety 'Nipponbare' induced by alkali stress. *Plant and Soil,* 355**,** 131.

Wang, H. & Jin, J. Y. 2005. Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. *Photosynthetica: International Journal for Photosynthesis Research,* 43**,** 591-596.

Watson, L. 1989. Manual of plant species suitability for reclamation in Alberta. *Alberta Land Conservation and Reclamation Council, Reclamation Research Technical Advisory Committee.* 178 p. doi: 10.5962/bhl.title.104055.

Wendel, G. W. & Smith, H. C. 1990. Silvics of North America: 1. Conifers. *Pinus strobus L. - Eastern White Pine***,** 476-488.

Wennerberg, S. & Skinner, M. 2007. Plant Guide—Kentucky Bluegrass (*Poa pratensis*). *USDA-NRCS: Washington, DC, USA***,** 1-4.

Wilkinson, S. 1999. PH as a stress signal. *Plant Growth Regulation,* 29**,** 87-99.

Wu, L., Ok, Y. S., Xu, X. L. & Kuzyakov, Y. 2012. Effects of anionic polyacrylamide on maize growth: a short term 14 C labeling study. *Plant and Soil,* 350**,** 311-322.

Xiong, B., Loss, R. D., Shields, D., Pawlik, T., Hochreiter, R., Zydney, A. L. & Kumar, M. 2018. Polyacrylamide degradation and its implications in environmental systems. *NPJ Clean Water,* 1**,** 1-17.

Xu, F. 2017. Effects of Mineral Nutrition and Iron Supply on Growth and Physiological Responses of Selected Boreal Plant Species to Root Zone pH. *Thesis, University of Alberta. Department of Renewable Resources.* 66 p. doi: 10.7939/R3CR5NT49

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.

Yang, C., Jianaer, A., Li, C., Shi, D. & Wang, D. 2008. Comparison of the effects of salt-stress and alkali-stress on photosynthesis and energy storage of an alkali-resistant halophyte *Chloris virgata*. *Photosynthetica,* 46**,** 273-278.

Yang, Y., Wassie, M., Liu, N.-F., Deng, H., Zeng, Y.-B., Xu, Q. & Hu, L.-X. 2022. Genotypic-specific hormonal reprogramming and crosstalk are crucial for root growth and salt tolerance in bermudagrass (*Cynodon dactylon*). *Frontiers in Plant Science,* 13, 956410-956426.

Yi, H., Polanco, M. C., Mackinnon, M. D. & Zwiazek, J. J. 2008. Responses of ectomycorrhizal *Populus tremuloides* and *Betula papyrifera* seedlings to salinity. *Environmental and Experimental Botany,* 62**,** 357-363.

Yokoi, S., Bressan, R. A. & Hasegawa, P. M. 2002. Salt stress tolerance of plants. *JIRCAS Working Report,* 23**,** 25-33.

Yordanova, R. Y. & Popova, L. P. 2007. Flooding-induced changes in photosynthesis and oxidative status in maize plants. *Acta Physiologiae Plantarum,* 29**,** 535-541.

Yruela, I. 2013. Transition metals in plant photosynthesis. *Metallomics,* 5**,** 1090-1109.

Yuan, F., Leng, B. & Wang, B. 2016. Progress in studying salt secretion from the salt glands in recretohalophytes: how do plants secrete salt. *Frontiers in Plant Science,* 7, 977-989.

Zhang, B., Zhang, C., Liu, C., Jing, Y., Wang, Y., Jin, L., Yang, L., Zhao, F., Lan, W., Fu, A., Shi, J. & Luan, S. 2018. Inner envelope CHLOROPLAST MANGANESE TRANSPORTER 1 supports manganese homeostasis and phototrophic growth in Arabidopsis. *Molecular Plant,* 11**,** 943-954-954.

Zhang, W., Calvo-Polanco, M., Chen, Z. & 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. Q., Fleurial, K., Moawad, M., Vassov, R., Macdonald, S. E. & Zwiazek, J. J. 2023. Growth responses of 20 boreal forest species to oil sands non-segregating tailings: significance for reclamation. *Restoration Ecology***,** e13874. doi: 10.1111/rec.13874

Zhang, W.-Q., 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**,** 113945-113953.

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, D., Oosterhuis, D. & Bednarz, C. 2001. Influence of potassium deficiency on photosynthesis, chlorophyll content, and chloroplast ultrastructure of cotton plants. *Photosynthetica,* 39**,** 103-109.

Zhao, Q., Suo, J., Chen, S., Jin, Y., Ma, X., Yin, Z., Zhang, Y., Wang, T., Luo, J., Jin, W., Zhang, X., Zhou, Z. & Dai, S. 2016. Na_2CO_3-responsive mechanisms in halophyte *Puccinellia tenuiflora* roots revealed by physiological and proteomic analyses. *Scientific Reports,* 6**,** 32717-32740.

Zhu, J.-K. 2001. Plant salt tolerance. *Trends in Plant Science,* 6**,** 66-71.

Zhu, J.-K. 2007. Plant Salt Stress. *Encyclopedia of Life Sciences.* 2 p.