Growth and physiology of mycorrhizal plants in soil containing enhanced non-segregating oil sands tailings

by

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Abstract

Open-pit mining of oil sands in Alberta, Canada, has resulted in a large-scale land disturbance of the boreal forest ecosystem and produced large amounts of tailings. To improve the effectiveness of the tailings solidification process and to make the tailings less harmful to vegetation, the novel oil sands tailings management technologies of the enhanced non-segregating tailings (eNST) and enhanced-spiked non-segregating tailings (esNST) have been developed. However, the full impact of these tailings on vegetation remains to be determined. Most plant species form symbiotic associations with fungi, which benefit plants, especially in poor-quality soils and under stressful conditions. The effects of eNST and esNST on the growth of mycorrhizal plants used for oil sands reclamation are little known. Therefore, in my thesis research, I carried out two studies to examine the growth and physiology of mycorrhizal plants growing in soil containing eNST and esNST.

In the first study, velvetleaf blueberry (*Vaccinium myrtilloides*) and Labrador tea (*Ledum groenlandicum*) were inoculated with *Oidiodendron maius* and *Pezoloma ericae* and grown in the peat mineral mix (PMM) that is commonly used as the reclamation soil, PMM/eNST (1:1, by volume), and PMM/esNST (1:1, by volume) for eight weeks. Then, the growth parameters, gas exchange, and tissue concentrations of chlorophyll and elements were measured in plants. The addition of eNST and esNST to PMM significantly increased the pH of the growth substrate and reduced its nutrient concentrations and water holding capacity. After eight weeks of treatments, both eNST and esNST significantly reduced velvetleaf blueberry and Labrador tea growth, reduced chlorophyll concentrations, net photosynthesis and transpiration rates, and increased leaf Na concentrations. Inoculation with the *P. ericae* and *O. maius* notably improved plant responses to tailings, with *P. ericae* showing more pronounced benefits. Compared with non-inoculated plants,

mycorrhizal plants had lower leaf Na and B concentrations, higher P concentrations, as well as net photosynthesis rates, and dry weights, especially when exposed to esNST.

In the second study, jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) were inoculated with *Laccaria bicolor* and subjected to PMM (control), PMM/eNST (1:1, by volume), PMM/esNST (1:1, by volume), eNST, and esNST treatments for eight weeks. The growth of jack pine and white spruce was severely impaired by eNST and esNST, with significant reductions in chlorophyll concentration, net photosynthesis, and total dry weights. This may be partly attributed to imbalanced nutrient absorption due to high Na concentrations in the leaves. Jack pine was more sensitive to tailings compared with white spruce and exhibited greater growth reductions compared with white spruce. However, the addition of PMM significantly increased the concentrations of K and Ca in jack pine and white spruce needles, and the improved nutrient balance likely contributed to the alleviation of tailings effects on the physiology and growth of plants. Inoculation with *L. bicolor* further enhanced plant tolerance to tailings, reduced needle B concentrations in jack pine seedlings treated with eNST, and needle B and Na concentrations in white spruce seedlings treated with eNST and esNST. The improvement of physiology and growth of plants exposed to eNST and esNST by *L. bicolor* demonstrates the importance of mycorrhizal associations for plants in reclamation areas affected by oil sands tailings and their potential for improving revegetation efforts.

Preface

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

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List of Abbreviations

ANOVA	analysis of variance
Chl	chlorophyll
CNRL	Canadian Natural Resources Ltd.
DCM	Dichloromethane
DMSO	dimethyl sulfoxide
DW	dry weight
E	transpiration rate
EC	electrical conductivity
ECM	ectomycorrhiza
eNST	enhanced non-segregating tailings
ERM	ericoid mycorrhiza
esNST	enhanced-spiked non-segregating tailings
FTIR	Fourier transform infrared spectroscopy
ICP-OES	inductively coupled plasma-optical emission spectroscopy
NST	non-segregating tailings
pH	power of hydrogen
PMM	peat mineral mix
Pn	net photosynthesis rate
PPFD	photosynthetic photon flux density
ROS	reactive oxygen species
RSHG	relative shoot height growth
RSDG	relative stem diameter growth
SE	standard error

Chapter 1 Introduction and Literature Review

1.1 Introduction

Oil sands are a naturally occurring petrochemical product, a mixture of sand, clay or other minerals, water, and bitumen that can be upgraded into a variety of hydrocarbon products, including gasoline and diesel fuels. Alberta's proven oil sands reserves are equal to about 158.9 billion barrels, and crude bitumen production (mined and in-situ) totaled approximately 3.3 million barrels per day (Government of Alberta 2024). While the oil sands mining industry has created huge economic value for the local economy, it has also disturbed large areas of boreal forest in Alberta and produced large amounts of tailings (Government of Alberta 2024). Oil sands mining companies are required to reclaim all disturbed lands to their natural equivalent land capacity (Fung and Macyk 2000), and reclamation certificates are only issued if monitoring through time demonstrates that these lands meet the criteria to return to self-sustaining ecosystems (Government of Alberta 2024).

In the process of recovering the bitumen, the oil sands slurry is moved into a large separation vessel and more hot water and different chemicals are added (Masliyah et al. 2004). These chemicals include Na_2CO_3, Na_2SiO_3 and NaOH, which make the slurry alkaline (Masliyah et al. 2004). The recovered bitumen is sent to the refineries for further processing, and the tailings, which contain clays, water, chemicals and trace amounts of bitumen as the major by-product of the extraction process, are pumped into tailings ponds (Fung and Macyk 2000). Since tailings are semi-liquid and their natural consolidation process may take more than a century, a variety of technical processes, possibly using chemical additives, can be used to promote their consolidation (Mikula et al. 1996, Fung and Macyk 2000). In order to improve the effectiveness of the tailings

solidification process, reduce emissions of greenhouse gases, use clean water, and reduce the potential harm of tailings to vegetation, CNRL has made efforts to further improve the tailings development process. These efforts have resulted in novel tailings technologies, which are referred to as enhanced non-segregating tailings (eNST) and enhanced-spiked non-segregating tailings (esNST) (Vedoy and Soares 2015, CNRL 2022).

Potentially harmful factors in revegetation associated with oil sands tailings include high pH and high salinity, due to the chemicals added during the bitumen extraction and consolidation process of tailings (Renault et al. 2000, Franklin et al. 2002). Furthermore, oil sands tailings may contain elevated levels of fluoride, boron and naphthenic acids, which may be phytotoxic to the revegetated plants at reclamation sites (Renault et al. 1998, Renault et al. 1999, Renault et al. 2000). Besides, the lack of nutrients in tailings impacted reclamation soils may also affect the growth of plants. Tailings also have low porosity and water permeability, which may result in poor soil aeration and root hypoxia (Redfield et al. 2003, Redfield et al. 2004, Fleurial et al. 2024). These factors are also known to alter soil microbial communities, including mycorrhizal fungi that are critical for plant survival.

It is known that the roots of most plants form symbiotic associations with fungi, and the presence of mycorrhizal fungi may enhance plant stress resistance (Hale and Orcutt 1987). Improved survival and growth of mycorrhizal plants in reclaimed areas have been observed in metal-contaminated (Grossnickle and Reid 1982, Walker 1999) and alkaline sites (Farghaly et al. 2022), as well as in non-toxic mine sites with low levels of mycorrhizal fungal inoculum (Malajczuk et al. 1994). Therefore, mycorrhizal fungi may have an important role in revegetation and improved reclamation (Quoreshi 2008).

In this thesis research, I examined the effects of enhanced non-separated tailings (eNST) and

enhanced-spiked non-separated tailings (esNST) on ericoid mycorrhizal (ERM) associations in velvetleaf blueberry (*Vaccinium myrtilloides* Michx.), Labrador tea (*Ledum groenlandicum*) and ectomycorrhizal (ECM) associations in jack pine (*Pinus banksiana* Lamb.) and white spruce [*Picea glauca* (Moench) Voss]. The ERM fungi used for plant inoculation were *Oidiodendron maius* and *Pezoloma ericae*, while the ECM fungus was *Laccaria bicolor*.

The specific objectives of the present study were to:

1) Characterize the chemical and physical properties of eNST and esNST tailings amended soils.

2) Characterize the effects of the eNST and esNST on four species of plants used for oil sands reclamation.

3) Identify the specific factors in eNST and esNST that may adversely affect plant growth and survival.

4) Examine the impact of ectomycorrhizal and ericoid mycorrhizal associations on the growth and physiological responses of plants impacted by eNST and esNST.

1.2 Literature review

1.2.1 Oil sands mining and reclamation

Alberta has the fourth-largest proven oil reserves in the world, after Venezuela, Saudi Arabia and Iran (Alberta Energy Regulator 2024). The Athabasca, Cold Lake, and Peace River areas of northern Alberta, contain huge oil sands deposits covering an area of more than 140,000 km^2 (Alberta Energy Regulator 2024). There are two mining methods based on the depth of oil sands deposits - surface mining is used to recover oil sands deposits less than 75 meters below the surface, and in-situ technologies are used to recover deeper deposits (Alberta Energy Regulator 2024). Approximately 5000 km^2 of oil sands deposits in northern Alberta are currently undergoing surface mining activities (Alberta Energy Regulator 2024).

Surface mining activities are preceded by the removal of all vegetation, soil (including peat, muskeg, and upland surface soil layers) and overburden (including rock, clay and non-bitumen sands). The removed soil and overburden material below the soil surface are stockpiled for later site reclamation. Then, the oil sands ore below the overburden material is transported from the mining site to the crushing facility where it is treated with hot water and pumped to the extraction plant to extract the bitumen. Thereafter, the oil slurry is transferred to a large separation vessel and more hot water and alkaline chemicals are added to recover the bitumen. After sending the recovered bitumen to the refineries, the tailings, which contain clays, water, chemicals and residual bitumen as the main by-product of the extraction process, are pumped into the tailings ponds and ready for land reclamation (CNRL 2022).

Since tailings are semi-liquid and would take a very long time to consolidate naturally, CNRL developed the non-segregating tailings (NST) technology. NST are produced by removing water from tailings streams to a density that promotes the capture of fines. This technology uses thickener and cyclone underflow and injects carbon dioxide into the process to increase the fine particle capture (Zhang et al. 2020). After that, CNRL developed the new enhanced non-segregating tailings (eNST), which captured more than 80% of the fines (CNRL 2022). The new technology combines tailings with a flocculant (anionic polyacrylamide) that clumps the solids together, which is then mechanically compacted between large metal plates lined with filter material (Vedoy and Soares 2015, CNRL 2022). The resultant clay fines cake is sufficiently dense and robust to be transported directly to a reclamation area (CNRL 2022). Based on the eNST, CNRL has developed and upgraded the enhanced-spiked non-segregating tailings (esNST) consolidation process, in which fluid tailings from the pond are recycled prior to flocculant addition. However, in the winter months, due to severe cold and freezing, it is impossible to produce esNST tailings, and thus its

use conditions are relatively limited.

After the tailings are solidified, they can be transported to the mining pit to be used for reclamation. Reclamation is the process of returning the disturbed land due to oil sands mining to the natural state of productivity that existed before industrial activity began. Overburden materials and oil sands tailings are returned to the reclamation sites on top of the mining pits to serve as substrate. Previously stripped soil stored at the start of the operation, such as peat-mineral soil mix (PMM), is placed over the overburden and oil sands tailings layers to support the growth of reclamation plants.

Since bitumen extraction requires the addition of various alkaline compounds, this results in high pH (8 to 10) and high salinity (4.6-5.5 mS cm^{-1}) in the by-product tailings (Fung and Macyk 2000). High pH and elevated salinity levels can affect the soil at the reclamation sites and impact the establishment and growth of vegetation and soil microorganisms (Renault et al. 2003, Zhang et al. 2015). Tailings have low air and water permeability, which can cause hypoxia, drought or waterlogging stress in plant roots (Howat 2000, Redfield et al. 2003). In addition, the low nutrient content and naphthenic acids of tailings are also potential harmful factors for revegetation (Renault et al. 1999, Renault et al. 2000, Franklin et al. 2002).

1.2.2 Biology of studied plant species

In the two research projects encompassed within this thesis, four species of native plants in the reclaimed areas were investigated, including velvetleaf blueberry (*Vaccinium myrtilloides* Michx.), Labrador tea (*Ledum groenlandicum*), jack pine (*Pinus banksiana* Lamb.), and white spruce [*Picea glauca* (Moench) Voss]. The biological characteristics of these four species are summarized below.

1.2.2.1 Velvetleaf Blueberry (*Vaccinium myrtilloides* Michx.)

Velvetleaf blueberry is a small perennial lowbush of the Ericaceae family, typically ranging from 10 to 50 cm in height, widely distributed in boreal forests and a native plant of Alberta (Moss et al. 1983). Velvetleaf blueberry is commonly found in acidic soils (pH from 4.0 to 5.5), sandy soils, peat bogs, muskegs, and mountain meadows (Carter and St-Pierre 1996). Grows well on dry, acidic soils and sandy loams in coniferous forests throughout Alberta (Inkpen and Eyk 2009). Velvetleaf blueberry is associated with ericoid mycorrhizal fungi that can improve plant stress resistance and increase the effectiveness of nutrient uptake (Fadaei et al. 2020, Mu et al. 2021).

1.2.2.2 Labrador tea (*Ledum groenlandicum*)

Labrador tea is a perennial aromatic shrub, typically 30 to 80 cm high (Flinn and Wein 1977). It is native to North America and widely inhabits in swamps, bogs and coniferous forests in northern Canada. Labrador tea has low nutrient requirements and high-water demand, so its usual habitat environment is wet, acidic, and nutrient poor (Jobidon 1995, Ringius et al. 1997). The experiments at Candle Lake, Saskatchewan, found that Labrador tea was most abundant at sites with pH between 2.9 and 4, and was also present at sites with pH values ranging from 4 to 6.9, but was not found at sites with a pH of 7 to 7.9 (Gucker 2006). Labrador tea is a member of the Ericaceae family and can form ericoid mycorrhiza with a diverse combination of endophytic fungi (Massicotte et al. 2005, Hébert and Thiffault 2011). Labrador tea also has important economic value and is often used by the indigenous community to make drinks and extract medicines (Hébert and Thiffault 2011, Dampc and Luczkiewicz 2015).

1.2.2.3 Jack pine (*Pinus banksiana* Lamb.)

Jack pine is an important economic timber species in northern North America, which ranks

among the top in terms of logging volume in Canada and is widely used for pulp and lumber (Reimenschneider 1982, Moore 1984). It is the most widespread pine tree in Canada (Farrar 1995), particularly in the boreal forest, and can grow well on dry, sandy, low-fertility sites where few other tree species grow. Jack pine grows rapidly and is always the first tree species to succeed after a forest fire or lumbering operations. As some of the first tree species to be re-established after disturbance or destruction, jack pine plays a crucial role in supporting biodiversity within forest ecosystems. Suitable soils for growing jack pine are acidic and neutral soils (pH 3.5-6.5), but in association with suitable mycorrhizal fungi, jack pine can also survive in alkaline soils (Limited 1989).

1.2.2.4 White spruce [*Picea glauca* (Moench) Voss]

White spruce is a slow-growing medium-to-large coniferous evergreen tree (Hassegawa et al. 2019). It's the cornerstone tree species of temperate-boreal forest and is widely distributed in northern Canada (Burns 1990). Due to its high wood density and stiffness, it is suitable for use in the production of pulp and lumber (Middleton and Zhang 2009). White spruce grows in a soil pH range of 4.7 to 7.0 (Sutton 1969, Brand et al. 1986, Xu et al. 2020), and can endure temperature extremes from -50 to 34 °C (Maini 1966). White spruce is an acidic conifer and produces organic acids that are released when plant litter accumulates on or becomes incorporated into the soil and acidifies soil pH over time (Brand et al. 1986, Nienstaedt and Zasada 1990).

1.2.3 Mycorrhizal associations

1.2.3.1 Introduction

Mycorrhizas are symbiotic associations between vascular plant roots and fungi that are present in 80% to 92% of surveyed land plant species and families (Wang and Qiu 2006). According to

the relationship of the fungus to the root cells, mycorrhizas are divided into two main types: ectomycorrhizas and endomycorrhizas (Janerette 1991). The hyphae of ectomycorrhizal fungi penetrate the spaces between cells without penetrating plant root cells, whereas the hyphae of endomycorrhizal fungi penetrate the cell wall of root cell and invaginate the cell membrane (Smith and Read 2010). Due to the diversity of endomycorrhizas, they are further classified as arbuscular mycorrhizas (AM), ericoid mycorrhizas (ERM), and orchid mycorrhizas (OM) (Smith and Read 2010). Mycorrhizal symbiosis plays an important role in plant nutrient and water absorption, and plant stress resistance (Al-Karaki 2013, Rasmussen and Rasmussen 2014, Van Der Heijden et al. 2015).

1.2.3.2 Ericoid mycorrhizas

Ericoid mycorrhiza (ERM) is a mutualistic relationship between members of the plant family Ericaceae and mycorrhizal fungi (Lewis 2016). Plants known to form ericoid mycorrhizas are widespread and are common components of heathland and boreal forest ecosystems (Specht 1979, Read 1991, Olson et al. 2001), often found in sandy or acidic soils with extremely poor nutrients (Luteyn 2002). This symbiotic relationship also represents an important adaptation of ericaceous plants to acidic and nutrient-poor soils (Cullings 1996). The main structural feature of ERM fungi is the formation of unique intracellular hyphal coils in the epidermal cells of the fine hair roots of the host plants (Read 1983). Hyphal coils are the site for transferring nutrients absorbed from the soil to the root cells by ERM fungi and carbohydrates fixed by plant photosynthesis to the fungi (Read 1983, Wei et al. 2022). Initially, the ERM fungi grow on the surface of hair roots, establishing loose networks of hyphae. Then, the hyphae penetrate the cortical cell walls, forming intracellular coils, which are called hyphal coils (Smith and Read 2010, Vohnik et al. 2012). Evidence suggests that ericoid mycorrhizal associations are short-lived, and coils are only

functional for a period of a few weeks (Mitchell and Gibson 2006, Smith and Read 2010). At the end of colonization, the structural integrity of plant organelles is lost, followed by degradation of the plant cytoplasm and loss of cell membrane integrity until the plant cell loses its integrity and the hyphal coils are completely degraded (Sumeet and Mukerji 2002). The establishment of roots and hyphal networks caused by mycorrhizal symbiosis greatly expands the surface area of plant roots, thereby increasing the efficiency of plant absorption of mineral nutrients (Wei et al. 2022).

ERM fungi have been shown to enhance the growth of ericaceous plants. The study by Wei et al. (2016) used the ERM fungus named *Oidiodendron maius* Om19 to inoculate *Rhododendron fortunei* seedlings, which significantly enhanced the growth of seedlings grown in peat-based substrate. The root growth and shoot growth of seedlings inoculated with Om19 were almost twice greater than those of uninoculated controls (Wei et al. 2016).

ERM fungi can promote N uptake by ericaceous plants. *R. fortunei* seedlings inoculated with *O. maius* Om19 absorbed more total nitrogen than those uninoculated controls (Wei et al. 2016). The qRT-PCR analysis showed that the expression of five genes related to N uptake and metabolism were highly upregulated in seedlings inoculated with Om19, including two nitrate transporters (NRT1-1 and NRT1-2), an ammonium transporter (AMT), glutamate synthase (GOGAT), and glutamine synthetase (GS), twofold to ninefold greater than the uninoculated control seedlings (Wei et al. 2016).

ERM also enable their host plants to access phosphorus sources that are not directly available to plants (Mitchell and Read 1981). The main forms of P in the poor soils where ericaceous plants grow are organic P that is not directly available to the plants, and the content of free inorganic phosphate is very low (Cosgrove 1967, Griffiths 1992). However, ERM fungi have been shown to produce and release extra-cellular and cell wall-bound phosphodiesterase enzymes to degrade the

phosphodiester DNA (Leake and Miles 1996).

Moreover, ERM fungi also have saprotrophic characteristics that allow them to degrade organic compounds and release organic nutrients that are inaccessible to plants (Read and Perez-Moreno 2003). ERM fungi genome is rich in extracellular enzyme-coding genes, such as carbohydrate-active enzymes (CAZymes), which can break down complex organic structures (Read et al. 2004, Perotto et al. 2012, Perotto et al. 2018). Some studies have suggested that in ERM fungi, *P. ericae* and *O. maius* were found to release a variety of enzymes to degrade tannic acid, cellulose, pectin, and chitin (Rice and Currah 2001, Thormann et al. 2002, Rice and Currah 2005). The ability of ERM fungi to mediate the degradation of soil organic matter (SOM) helps plants mobilize hard-to-use nutrients and improve the bioavailability of small compounds in soil (Wei et al. 2022).

In addition to growth enhancement, ERM fungi have also been shown to improve plant tolerance to environmental stresses such as salt, drought, and heavy metals (Birhane et al. 2012, Balestrini and Lumini 2018). Previous studies have shown that the inoculation of *M. variabilis* increased the root and shoot dry weight of velvetleaf blueberry (*Vaccinium myrtilloides*), Labrador tea (*Rhododendron groenlandicum*), and lingonberry (*Vaccinium vitis-idaea*) treated with NaCl, and significantly improved plant physiological parameters (Fadaei et al. 2020). ERM colonization can also alleviate drought stress in ericaceous plants. Inoculation with ERM fungi (*Pezicula ericae*, *Pezoloma ericae*, *Meliniomyces variabilis* and *Oidiodendron maius*) significantly enhanced the drought tolerance of upland and lowland blueberry seedlings (Mu et al. 2021). Among them, inoculation with *Pezicula ericae* had the most significant effect and improved the shoot water potential, net photosynthetic and transpiration rates of blueberry seedlings under drought stress (Mu et al. 2021). ERM fungi also enable plants to withstand heavy metal stress (Meharg and Cairney 1999). Studies have found that blueberry plants inoculated with ERM fungi can tolerate

high concentrations of Al and Mn, and can reduce the accumulation of Al and Mn in the host plants (Hashem 1995, Yang and Goulart 2000).

1.2.3.3 Ectomycorrhizas (ECM)

Ectomycorrhizal (ECM) associations exhibit a wide distribution worldwide, and they occur mainly on the roots of woody perennial plants, especially Pinaceae (northern conifer forests), Fagaceae (temperate deciduous woodlands), and Dipterocarpaceae (tropical rainforest) (Smith and Read 2010, Watkinson 2016). Among vascular plants, about 3% of plant species form ECM associations with thousands of species of fungi (Smith and Read 2010), which are usually from the Basidiomycota and Ascomycota phyla (Hibbett et al. 2000). The characteristic of ECM fungal association is that the fungus is always extracellular and does not form intracellular structures throughout the association (Watkinson 2016). ECM fungi do not penetrate the host cell walls and lack intracellular hyphae. Instead, they form a hyphal sheath, known as the mantle, on the surface of the host roots, and an intercellular hyphal network within the roots, known as the Hartig net (Hock 2012, Reddy and Saravanan 2013). ECM fungi obtain photosynthetic products from host plants and provide them with nutrients such as N and P, while some ECM fungal species can also obtain nutrients from coarse woody debris and have saprophytic growth capabilities (Courty et al. 2008).

ECM fungi can enhance the nutrition of host plants by expanding the surface area and range of nutrient absorption (Finlay and Read 1986). ECM associations are ubiquitous in boreal forest, where there are 350-650 meters of fungal hyphae per gram of mineral soil in forest podzolic soils (Söderström 1979). The hyphae of ECM fungi can also penetrate narrow soil pores, thereby improving soil structure and promoting root development in plants (Rillig and Mummey 2006, Lehto and Zwiazek 2011). Moreover, ECM mantle is connected to the underground hyphal

networks through extraradical hyphae, and is directly involved in the uptake and translocation of soil nutrients and water to the roots (Read 1984, Finlay and Read 1986). Due to the extensive contact area between the extraradical mycelium and the soil, ECM fungi have become crucial absorptive organs for plants, enhancing the host plant's uptake of water and nutrients (Smith and Read 2010). ECM fungi can also improve the uptake and utilization of nutrients by the host plants (Marschner and Dell 1994). The fungal symbionts of ECM plants secrete a variety of enzymes, such as chitinases, phosphatases, and proteases, which convert nutrients like N and P from organic matter into forms directly available to the host plants (Bending and Read 1995). Moreover, ECM can modify pH of their growth substrate by extruding organic acids and protons to increase the availability of ions such as K^+, Ca^{2+}, and Mg^{2+} (Arvieu et al. 2003).

It is known that ECM fungi provide a variety of advantages to the host and can improve the host plant's disease and stress resistance (Zak 1964, Lehto and Zwiazek 2011, Guerrero-Galán et al. 2019). After the formation of ECM on the host plant roots, the mantle enveloping the root tips acts as a physical barrier, making it difficult for pathogens to penetrate the roots, thereby protecting plant tissues from infection (Khullar et al. 2019). The hyphal network of ECM can harbor beneficial microorganisms, such as *Rhodococcus* and *Streptomycetes*, which are referred to as mycorrhiza helper bacteria (MHB) (Frey-Klett et al. 2007, Watkinson 2016). They colonize the surface of fungal hyphae, stimulate hyphal growth, and protect host plants from pathogens (Watkinson 2016). Studies have found that *Streptomyces* sp. AcH 505 could stimulate the growth of the mutualist *Amanita muscaria*, while inhibiting the growth of pathogens, such as *Heterobasidion annosum*, which causes wood decay in conifers, and the wide-host-range pathogen *Armillaria obscura* (Maier et al. 2004, Riedlinger et al. 2006). In addition, ECM associations can also improve the ability of host plants to tolerate drought and salt stress (Khullar and Reddy 2019).

Under drought conditions, ECM fungi improve their water availability by increasing stomatal conductance, shoot water potential, hydraulic conductance, and aquaporin function in host plants (Lehto and Zwiazek 2011). Moreover, the extensive extraradical hyphae of ECM fungi can also increase the surface area of the host plant roots for water uptake (Lehto and Zwiazek 2011). Additionally, ECM fungi can reduce the accumulation of Na^+ in plant tissues by stabilizing Na^+ efflux and limiting the transfer of Na^+ to photosynthetic organs, thereby protecting the plant's photosynthetic structures (Muhsin and Zwiazek 2002, Guerrero-Galán et al. 2019).

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Chapter 2 Ericoid mycorrhizae improve tolerance of velvetleaf blueberry (*Vaccinium myrtilloides*) and Labrador tea (*Ledum groenlandicum*) plants to oil sands tailings

2.1 Introduction

Open-pit mining of oil sands deposits leads to a large-scale land disturbance of the boreal forest ecosystem in the Athabasca region of Alberta, Canada (Government of Alberta 2024). Prior to mining, the forest soil and underneath overburden located above the oil sands deposits are removed and stockpiled for future land reclamation (Rowland et al. 2009). Oil sand ore is then mined and moved to the processing facility, where it is extracted with hot water containing alkaline chemicals and diluent to separate the bitumen from the sand (Fung and Macyk 2000, Giesy et al. 2010). This process generates large amounts of liquid tailings with suspended sand and clay particles, traces of bitumen, salts, and other compounds either extracted from the ore or added during the extraction process (Fung and Macyk 2000). These tailings are pumped into the Dedicated Disposal Areas (DDI), previously referred to as tailings ponds, to separate the solids from water so that the water can be recycled (Fung and Macyk 2000). Eventually, the tailings in the DDI areas are left to solidify before the site is reclaimed. For mine reclamation, the oil sands operators are legally required to return the disturbed land to a self-sustaining ecosystem with equivalent land capability through soil reconstruction and revegetation (Alberta Environment 2010, Government of Alberta 2024). Tailings sand or overburden are commonly used as capping substrates placed on top of the solidified tailings and then capped with a layer of topsoil or peat-mineral soil mixture (PMM),

which may be as thin as 15 cm (Fung and Macyk 2000, Audet et al. 2015, Howell 2015). PMM is a mixture of humic, mesic, and fibric forms of lowland organic peat deposits and sandy mineral forest soils (Ojekanmi and Chang 2014). It has high organic matter content and high water-holding capacity, which can benefit seedling establishment (Pinno and Errington 2015).

Since the natural consolidation and settlement process of fine tailings in DDI may take several decades (Mikula et al. 1996), Canadian Natural Resources Limited (CNRL) developed the non-seggregating tailings (NST) technology, which utilizes thickener underflow and cyclone underflow, and increases fine particle capture by injecting carbon dioxide into the tailings (Zhang et al. 2020). Further improvements of the NST technology by CNRL to accelerate tailings segregation and reduce greenhouse gas emissions have resulted in the development of tailings management processes referred to as the enhanced non-segregating tailings (eNST) and enhanced spiked non-segregating tailings (esNST) (Pathways Alliance 2022). The eNST technology combines the chemical treatment of NST with a polymer flocculant (anionic polyacrylamides), that causes the solids to clump together, by mechanically pressing the tailings between large metal plates lined with filter materials (CNRL 2022). Approximately 80% fine particles are captured in eNST (Vedoy and Soares 2015, CNRL 2022). The resulting clay fines cake is dense and strong enough to be shipped directly into a reclamation area. The esNST is a modification of the eNST. In the production of esNST, fluid tailings are pumped into the eNST before discharge. However, due to severe cold and freezing, the production of esNST tailings is not possible during the winter months, and its use may be more limited than that of eNST tailings.

During the final steps of oil sands mine reclamation, the site is revegetated with native plants.

However, plant establishment and growth are commonly impaired by the soil factors present in these areas, such as high pH, elevated salinity, presence of naphthenic acids, nutrient imbalance, low organic matter, and poor soil aeration (Franklin et al. 2002, Zhang et al. 2020). The pH of undisturbed soils in boreal forests is typically below 6.0, but the soil pH in reclaimed sites often exceeds 8.0 (Howat 2000). High pH can reduce the availability of mineral nutrients in the soil, disrupt plant water balance, and impair other plant physiological processes, leading to leaf chlorosis, reduced plant growth, and high plant mortality (Zhang et al. 2013, Zhang et al. 2015, Zhang and Zwiazek 2016). Elevated soil salinity is also of major concern for the revegetation of some of the reclamation sites. The recommended soil electrical conductivity (EC) for revegetation sites is below 2 mS cm^{-1} (Alberta Environment 2010), but in some oil sands reclamation sites, the soil EC values can be as high 5 mS cm^{-1} (Lazorko et al. 2012). High soil salinity can inhibit the growth and activity of soil microorganisms (Elmajdoub et al. 2014) and directly affect plant growth and survival by altering various physiological processes, including the uptake and transport of water and nutrients (Munns and Tester 2008, Duan et al. 2017, Vaziriyeganeh et al. 2018).

The restoration of boreal ecosystems requires re-establishment of the understory vegetation in addition to trees. The forest understory vegetation cover increases soil organic matter (Mummey et al. 2002), improves soil chemical and biological properties, and influences the development of plant communities (Lefrançois et al. 2010). Ericaceae plants, including velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) and Labrador tea (*Ledum groenlandicum*), play an important role in the boreal forest ecosystem, and their successful revegetation is among the high priorities for oil sands reclamation (Fadaei et al. 2021, Zhang et al. 2023). However, these plants are adapted to

acidic soils and are among the most susceptible woody plant species to oil sands tailings (Zhang et al. 2023).

ERM fungi have been proven to promote the growth of plants in the Ericaceae family (Wei et al. 2016). Studies have shown that the fresh and dry weights of the *Rhododendron fortunei* seedlings inoculated with *Oidiodendron maius* were 81% and 84% higher compared with the control group, respectively (Wei et al. 2016). Additionally, mycorrhizal fungi can improve plant stress tolerance through enhanced water and nutrient uptake (Xu et al. 2014, Frey 2019). Ericaceous plants commonly form ericoid mycorrhizal associations (ERM) with the ascomycete fungi. The ERM fungi used in the present study, *Oidiodendron maius* and *Pezoloma ericae,* have been previously demonstrated to improve drought and salt tolerance of velvetleaf blueberry (*Vaccinium myrtilloides*), Labrador tea (*Ledum groenladicum*), and lingonberry (*Vaccinium vitis-idea*) seedlings (Fadaei et al. 2021, Mu et al. 2021). ERM fungi can also enhance nutrient utilization in plants growing in poor soils by degrading organic compounds, helping them acquire otherwise inaccessible nutrients (Read and Perez‐Moreno 2003). The enhancement of plant growth by ERM fungi is primarily attributed to three following factors: (1) the symbiosis expands the surface area for nutrient acquisition; (2) ERM fungi can degrade organic matter, enhancing nutrient utilization; and (3) ERM symbiosis can upregulate the expression of genes related to the uptake and metabolism of N and P, thereby enhancing plant growth.

The objective of the present study was to investigate the effects of *O. maius* and *P. ericae* on the growth and physiological responses of velvetleaf blueberry and Labrador tea plants growing in the PMM soil containing the enhanced non-segregating tailings (eNST) and enhanced spiked

non-segregating tailings (esNST). The study hypotheses were: 1) the presence of eNST and esNST in the PMM soil would increase the soil pH and upset nutrient balance, 2) root inoculation of Labrador tea and velvetleaf blueberry with *O. maius* and *P. ericae* would alleviate the detrimental effects of both types of studied tailings on plants.

2.2 Materials and methods

2.2.1 Plant growth media

The growth substrates used in this study were the peat-mineral soil mix (PMM) and PMM supplemented with enhanced non-segregating tailings (eNST) or enhanced-spiked non-segregating tailings (esNST). PMM was collected at the reclamation site of Canadian Natural Resources Limited (CNRL) Horizon mine, north of Fort McMurray, Alberta, Canada (56° 43' 35.94" N, 111° 22' 44.1732" W). The eNST and esNST were generated by the CNRL's Horizon oil sands processing facility. PMM, eNST, and esNST were sealed in 20 L plastic pails and delivered to the University of Alberta. Large aggregates, stones, grass, and tree branches in PMM were removed from the PMM soil, and the soil was air-dried for 4-5 days before use. Dried PMM was ground and passed through a 2 mm sieve. For the treatments involving tailings, PMM and eNST or esNST were thoroughly mixed in the 1:1 ratio (by volume). Seedlings were planted in either 1) PMM (control), 2) PMM + eNST (1:1, by volume), or 3) PMM + esNST (1:1, by volume) and grown for eight weeks.

2.2.2 Plant material and growth conditions

Greenhouse-grown one-year-old velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) and Labrador tea (*Ledum groenlandicum*) seedlings were purchased from the plant nursery (Tree Time Services Inc., Edmonton, Alberta, Canada). The seedlings were grown in Styrofoam block containers (415D V77/170HS 164 cm^3 Styroblocks™, Beaver Plastics, Acheson, AB, Canada) from seeds, which were collected in the forest near Canadian Natural's Horizon oil sands mines in northeastern Alberta, Canada. Prior to the experiment, the seedlings were stored at 4 °C in the dark for two weeks.

The experiment was conducted in a controlled-environment growth room at a temperature of 22/18 °C (day/night), relative humidity of 50 ± 10%, and a 16-h photoperiod. Photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1} was provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

2.2.3 Fungal culture and root inoculation

Pezoloma ericae (D.J. Read) Baral and *Oidiodendron maius* (G.L. Barron) were isolated from the native ericaceous plants in the undisturbed boreal forest in the proximity to the oil sands reclamation areas (Fadaei 2019). Fungal cultures were first grown in the sterile potato dextrose agar (PDA) solid medium (Gams et al. 1975) in Petri dishes. The cultures were then transferred to the liquid modified Melin Norkrans (MMN) medium (Marx 1969, Pham et al. 2004) and placed on an orbital shaker at 120 RPM for 4 weeks in the dark at room temperature. The cultures were homogenized in a blender and diluted with distilled water to the final concentration of 0.5 mg dry

mycelia/mL for root inoculation (Calvo-Polanco et al. 2009).

The seedlings were inoculated twice by immersing the root plugs in the mycelial slurry in plastic tubs for one day. The first inoculation was carried out after the seedlings were removed from the cold storage. After the first inoculation, the plastic tubs with seedlings were placed in the growth room under the above growth conditions. Four weeks later, the seedlings were inoculated for the second time as above. For both inoculations, the autoclaved liquid MMN medium was used as an inoculation control. The seedlings were then transplanted to the different growth substrates in 500 cm^3 pots. There were 30 seedlings of each plant species inoculated with *O. maius*, 30 seedlings inoculated with *P. ericae*, and 30 inoculation control seedlings. The seedlings were spatially separated to minimize the possibility of cross-contamination.

2.2.4 Experimental set-up

The experiment was a 2 x 2 x 3 completely randomized factorial design with two ericoid mycorrhizal (ERM) fungi species (*P. ericae* and *O. maius*), two plant species (velvetleaf blueberry and Labrador tea) and three growth substrates (PMM, PMM/eNST, PMM/esNST). There were ten replicated seedlings per species per treatment. All plants were watered with distilled water every two days and fertilized with 14-14-14 Scotts Osmocote slow-release fertilizers (ICL Group, Dublin, OH, USA) once a week. The plants were grown in the media for eight weeks before the final measurements and harvest.

2.2.5 Examination of root fungal colonization

Four weeks after the second inoculation, three seedlings from each species and treatment were randomly selected to examine their roots for fungal colonization (Dodd et al. 2001). The roots were washed and cut into one-cm-long segments. The roots segments were cleared in 2.5% KOH at 60°C for 60 min, then washed twice with distilled water and followed by 5% acetic acid. The cleared root segments were stained with 5% ink in 5% acetic acid at 60°C for 20 minutes and rinsed with distilled water. Eight segments from each root were randomly selected, mounted on microscope slides, and examined for colonization rates. All segments were rated from number 0 to 5 as briefly explained below. The mycorrhizal colonization intensity (M%) in the root system was calculated using the following the following equation (Trouvelot et al. 1986).

M% = (95n5+70n4+30n3+5n2+n1)/ (n total)

n5 = number of root fragments rated 5 (more than 90 % intensity of mycorrhizal colonization)

n4 = number of root fragments rated 4 (between 50 to 90 % intensity of mycorrhizal colonization)

n3 = number of root fragments rated 3 (between 10 to 50 % intensity of mycorrhizal colonization)

n2 = number of root fragments rated 2 (between 1 to 10 % intensity of mycorrhizal colonization)

n1 = number of root fragments rated 1 (lower than 1 % intensity of mycorrhizal colonization)

n total = total number of root fragments

2.2.6 Analysis of growth substrates

Six replicates (n = 6) of 100 g were randomly collected from each of the three air-dried growth substrates (PMM, PMM/eNST, and PMM/esNST), and ground into fine powder with a mortar and

pestle. For each sample, 30g of powder was mixed with 60 mL distilled water and shaken for 1 h. The extracts were filtered using a clarifier syringe filter (0.45 μm) and the pH and electrical conductivity (EC) of the solutions were measured using an Orion STAR A111 pH meter (Thermo Fisher Scientific Inc., Waltham, MA) and a Traceable 15077-977 Expanded Range Conductivity Meter (Thermo Fisher Scientific Inc., Waltham, MA), respectively. The concentrations of Na, Mg, P, K, Ca, Fe, Mn, and Zn in the solution were analyzed using an ICP-OES (Thermo iCAP 6000 series) instrument (Zarcinas et al. 1987). To determine the water holding capacity (WHC) of each growth substrate, 25 g of dry powder was placed in a funnel with filter paper (Diameter 110 mm, CAT No. 17413182 Whatman® Prepleated Qualitative Filter Paper), 50 mL (V1) of distilled water was slowly added, and the gravimetrically drained extract was collected, and its volume (V2) measured with a graduate cylinder. The WHC (%) was calculated as: $WHC = \frac{\mathrm{V1-V2}}{25} X\ 100$ (Rowell 2014).

To determine naphthenic acid (NA) concentration, 20 mL of substrate extracted solution was passed through 0.45 μm Nylon filter. Then the pH of the filtered solution was adjusted to 2 by adding pure HCl (37.2%). Three grams of NaCl was added to the solution and dissolved to prevent the emulsion formation during shaking. Five mL dichloromethane (DCM) was added to the solution and shaken for two minutes. to extract the NAs. The extracted NAs were collected in a beaker, which was left under the fume hood to evaporate DCM. Commercial naphthenic acid mixture (MilliporeSigma Canada Ltd., Oakville, ON, Canada) was digested in DCM to prepare the standard solutions and a standard curve. The NAs concentrations were measured using a Fourier transform infrared (FTIR) spectroscopy at wavenumbers of 1743 and 1706 cm^{-1}, which

correspond to adsorption bands characteristic of monomeric and dimeric carboxylic groups, respectively (Meshref et al. 2020).

2.2.7 Gas exchange and biomass measurements

After 8 weeks of growth in the different substrates, gas exchange parameters (net photosynthetic and transpiration rates) of plants were measured in the fully developed uppermost leaves of six randomly selected seedlings per treatment (n = 6), conducted between 3 and 6 h following the onset of photoperiod using an infrared gas analyzer (LI-6400, LI-COR, Lincoln, Nebraska, USA). The reference CO_2 concentration was set to 400 μmol mol^{-1}, the flow rate was 200 μmol s^{-1}, the leaf chamber temperature was maintained at 20 ºC, and PPFD was 400 μmol $m^{-2}s^{-1}$. Following the measurements, the plants were harvested, and their dry shoot and root biomass of seedlings was determined after oven drying at 70°C for 96 h (n = 6).

2.2.8 Measurements of leaf chlorophyll and elemental concentrations

After eight weeks of treatment, chlorophyll and elemental concentrations were assessed in fully expanded uppermost leaves harvested from four randomly selected seedlings (n = 4) per treatment. The leaf samples were freeze-dried (Labconco Freeze Dry System 4.5 Liter) for four days and ground using a Thomas Wiley Mini-Mill (Thomas Scientific, Trenton, NJ, USA).

For leaf chlorophyll extractions, 10-mg samples of dry leaf powder were extracted with 8 mL dimethyl sulfoxide (DMSO) in a 15 mL Falcon tube, and the mixtures were placed in an oven at 65°C for 22 hours. Chlorophyll a and b concentrations were determined using a spectrophotometer

at wavelengths of 648 nm and 665 nm. The total chlorophyll concentrations were calculated using Arnon's equation (Sestak et al. 1971).

For leaf elemental (B, Ca, Fe, K, Mg, Mn, Na, P, Zn) concentration analysis, 0.2 g leaf powder was microwave digested with 10 mL 70% HNO_3 for 10 min at 185 ºC (Mars 5 Microwave Accelerated Reaction System, CEM) and diluted to 40 mL with Milli-Q water. The filtered extracts were analyzed with an ICP-OES instrument (Thermo iCAP 6000 series). The leaf total nitrogen (N) concentration was determined using the dry combustion method with a Thermo FLASH 2000 HT Plus Organic Elemental Analyzer (Thermo Fisher Scientific Inc) (Schumacher 2002).

2.2.9 Statistical analysis

The statistical analysis of the data was performed using one-way analysis of variance (ANOVA) in the R software (Version 4.3.2) to determine the significant differences ($p \leq 0.05$) between treatments. Comparisons between different treatment means were performed using the Holm test.

2.3 Results

2.3.1 Chemical and physical properties of the substrates

The pH of PMM (5.1) was significantly lower than compared with the PMM/eNST (5.4) and PMM/esNST (6.0) substrates (Figure 2.2), while the EC values, Mg, K, and Ca concentrations of PMM were significantly higher compared with PMM/eNST and PMM/esNST (Table 2.1). The EC values, Mg, and K concentrations of PMM/esNST were the lowest compared of the three examined growth substrates (Table 2.1). The concentrations of P, Fe, Mn, Zn, TN and WHC of

PMM were the highest, and no significant differences were found between PMM/eNST and PMM/esNST (Table 2.1). The WHC of the PMM growth substrate (100%) was significantly higher than that of PMM/eNST (48%) and PMM/esNST (44%) (Table 2.1).

2.3.2 Mycorrhizal root colonization

In both plant species, seedlings inoculated with *P. ericae* or *O. maius* had a significantly higher colonization rate (52% - 67.5%) compared with the non-inoculated groups (10% - 19%) (Table 2.2). In Labrador tea, seedlings grown in PMM and inoculated with *P. ericae* had a root fungal colonization rate of approximately 67.5%, which was the highest colonization rate among the treatments (Table 2.2). In velvetleaf blueberry, there were no significant differences in root colonization rates between the PMM/eNST and PMM/esNST treatments (Table 2.2).

2.3.3 Plant dry weights

In non-inoculation groups, both eNST and esNST treatments significantly reduced the shoot dry weights and total dry weights in Labrador tea and velvetleaf blueberry (Figure 2.3A, C; Figure 2.4A, C). In both Labrador tea and velvetleaf blueberry grown in PMM, inoculation with *O. maius* or *P. ericae* did not significantly affect the shoot, root and total dry weights (Figure 2.3, Figure 2.4A, C). In the PMM/eNST treatment, inoculation with *P. ericae* significantly increased the shoot dry weights in Labrador tea compared with the non-inoculated group (Figure 2.3A), while the inoculation with *O. maius* significantly increased the shoot, root, and total dry weights in velvetleaf blueberry (Figure 2.3C, D; Figure 2.4C). In the PMM/esNST treatment, inoculation of *P. ericae*

significantly increased the shoot, root (Figure 2.3A, B), and total dry weights (Figure 2.4A) in Labrador tea. It also significantly increased the root (Figure 2.3D) and total (Figure 2.4C) dry weights in velvetleaf blueberry but did not significantly affect the shoot dry weights (Figure 2.3B). Compared with non-inoculated plants, inoculation with *O. maius* significantly increased the shoot, root (Figure 2.3) and total dry weights (Figure 2.4A, C) in both Labrador tea and velvetleaf blueberry grown in the growth substrates amended with eNST and esNST. In the PMM/eNST treatment, inoculation with *P. ericae* and *O. maius* significantly reduced the shoot-to-root dry weight ratios in Labrador tea and velvetleaf blueberry compared with the non-inoculated plants (Figure 2.4B, D).

2.3.4 Leaf chlorophyll concentrations

In the non-inoculated Labrador tea and velvetleaf blueberry plants, the presence of eNST and esNST significantly reduced the total chlorophyll concentrations and chlorophyll a (chl a) to b (chl b) ratios compared with seedlings grown in PMM (Figure 2.5). In both Labrador tea and velvetleaf blueberry, inoculation of *P. ericae* in the PMM/eNST treatment significantly increased the chl a to chl b ratios compared with non-inoculated seedlings (Figure 2.5). In Labrador tea, in the PMM/esNST treatment, inoculation with *O. maius* or *P. ericae* significantly increased the total chlorophyll concentrations and Chl a to Chl b ratios compared with non-inoculated seedings (Figure 2.5A, B).

2.3.5 Gas exchange and chlorophyll fluorescence

In both PMM/eNST and PMM/esNST substrates, net photosynthesis and transpiration rates were reduced in both Labrador tea and velvetleaf blueberry compared with the seedlings grown in PMM (Figure 2.6A, B, D, E). In both Labrador tea and velvetleaf blueberry, inoculation with *P. ericae* or *O. maius* significantly increased the net photosynthesis rates in the PMM/eNST treatment compared with the non-inoculated plants (Figure 2.6A, D). In velvetleaf blueberry grown in PMM/esNST, inoculation of *P. ericae* or *O. maius* did not significantly affect the transpiration rates, while in Labrador tea, the inoculation of both fungi significantly increased the transpiration rates compared with non-inoculated seedlings (Figure 2.6B, E).

In both Labrador tea and velvetleaf blueberry fungal inoculation had no effect on the PSII maximum efficiency (Fv'/Fm') in seedlings grown in PMM (Figure 2.6C, F). In PMM/eNST treatment, inoculation with *P. ericae* or *O. maius* significantly increased Fv'/Fm' in Labrador tea, but only the inoculation with *O. maius* significantly increased the Fv'/Fm' in velvetleaf blueberry compared with the non-inoculated plants (Figure 2.6C, F). In both Labrador tea and velvetleaf blueberry, inoculation with *P. ericae* or *O. maius* significantly increased Fv'/Fm' in the PMM/esNST treatment compared with non-inoculated seedling (Figure 2.6C, F).

2.3.6 Leaf elemental concentrations

In the non-inoculated Labrador tea and velvetleaf blueberry, leaf Na concentrations were approximately two- to three-fold higher in seedlings treated with eNST and esNST amended substrates compared with seedlings grown in PMM (Table 2.3, 2.4).

Labrador tea seedlings grown in the PMM/eNST substrate had significantly higher leaf B, Ca, K, Mn, and Zn concentrations than the plants grown in PMM (Table 2.3). In addition, velvetleaf blueberry grown in the PMM/esNST substrate had significantly higher leaf Mg and Mn concentrations and lower Fe and P concentrations compared with the plants grown in PMM (Table 2.4).

Labrador tea seedlings inoculated with *P. ericae* seedlings and grown in PMM/eNST and PMM/esNST, leaf Ca, Mg and Zn concentrations were lower compared with the non-inoculated seedlings (Table 2.3). In both inoculated treatments, Labrador tea plants grown in the PMM/eNST and PMM/esNST substrates had significantly higher leaf B concentrations (Table 2.3). In velvetleaf blueberry inoculated with *P. ericae*, leaf Mg and Mn concentrations in seedlings grown in PMM/esNST substrate were significantly lower compared with the non-inoculated plants (Table 2.4).

Labrador tea seedlings inoculated with *O. maius* and grown in the PMM/esNST substrate had significantly lower leaf Ca and Zn concentrations compared with the non-inoculated plants (Table 2.3). In velvetleaf blueberry inoculated with *O. maius*, leaf Mg and Mn concentrations in plants grown in the PMM/esNST substrate were significantly higher compared with the non-inoculated plants (Table 2.4).

In Labrador tea, fungal inoculation did not significantly impact the K/Na ratios in plants grown in the PMM/eNST and PMM/esNST substrates, however in the PMM substrate, inoculation with *P. ericae* significantly increased the K/Na ratios in Labrador tea seedlings compared with the non-inoculated plants (Figure 2.7B). The K/Na ratios in non-inoculated velvetleaf blueberry were

significantly lower in plants grown in PMM/esNST compared with plants grown in PMM (Figure 2.7D).

2.4 Discussion

2.4.1 Effects of eNST and esNST on plant responses

In both plant species, eNST and esNST present in PMM significantly reduced plant total dry weights, which was associated with the reductions in chlorophyll concentrations, Fv'/Fm' ratios, net photosynthesis, and transpiration rates. The pH of PMM/eNST (5.4) and PMM/esNST (6.0) was significantly higher compared with PMM (5.1), and also higher than the preferred soil pH range by velvetleaf blueberry (pH 4.0 - 5.0) and Labrador tea (pH 2.9 - 4) (Gucker 2006, Mu 2021). A previous study also demonstrated that PMM amendment effectively lowered the pH of NST and enhanced the growth performance of paper birch (*Betula papyrifera*), white spruce (*Picea glauca*) and green alder (*Alnus viridis*) (Zhang et al. 2020). The concentrations of Mg, P, K, Ca, Fe, Zn and N in PMM/eNST and PMM/esNST substrates were all significantly lower compared with PMM, which was likely an important factor responsible for the reduced growth and physiological performance of velvetleaf blueberry and Labrador tea plants.

In this study, the NAs present in esNST solids (27 mg L^{-1}), eNST release water (65 mg L^{-1}) and esNST release water (57 mg L^{-1}) that I measured may also be a stress factor contributing to the impaired growth of seedlings. It has been reported that NAs impair photosynthesis, stomatal conductance, root hydraulic conductivity, and nutrient uptake by plants (Kamaluddin and Zwiazek 2002). In addition, the WHC of PMM/eNST and PMM/esNST were significantly lower compared

with PMM, which likely impaired plant growth through the effect on water relations.

2.4.2 Responses of Labrador tea inoculated with *P. ericae* or *O. maius* to eNST and esNST

In Labrador tea, both eNST and esNST treatments significantly increased leaf B and Na concentrations, while the inoculation with *P. ericae* and *O. maius* significantly decreased the leaf B and Na concentrations compared with the non-inoculated plants. Excessive B and Na accumulation in leaves can cause leaf chlorosis and necrosis (Apostol et al. 2002, Apostol and Zwiazek 2004, Han 2023). In the present study, leaf injury was widely observed in seedlings grown in PMM containing eNST and esNST. Reduced leaf chlorophyll synthesis and impaired photosynthesis have been commonly reported among the causes of high mortality and growth reductions in plants affected by the oil sands tailings and attributed to the presence of salts and NAs (Renault et al. 1999, Redfield et al. 2003, Zhang et al. 2023). Both *P. ericae* and *O. maius* were effective in alleviating salinity stress in ericaceous pants (Fadaei et al. 2020). In seedlings treated with PMM/esNST, *P. ericae* inoculation significantly increased leaf Fe concentrations, which was associated with higher chlorophyll concentrations. Numerous studies reported that Fe plays a pivotal role in chlorophyll synthesis and metabolism (Welch and Shuman 1995). The biomass growth was reduced in seedlings treated with eNST and esNST compared with PMM, and the resulting dilution effect (Jarrell and Beverly 1981) may likely explain the relatively lower foliar Ca, K, Mg and Zn concentrations in the PMM-grown seedlings.

Increased P uptake is one of the major benefits of mycorrhizae in plants growing in P deficient soils (Van't Padje et al. 2021). In my study, both eNST and esNST treatments significantly reduced

P concentrations in Labrador tea leaves. However, the inoculation with *P. ericae* and *O. maius* significantly increased leaf P concentration in seedlings grown in PMM/esNST substrate, which may have also contributed to the higher dry weights of these seedlings. However, the dry weights of seedlings grown in PMM/eNST were unaffected by the ERM inoculation, which suggests that the presence of some unidentified factors in eNST affected plant responses to fungal inoculation.

In Labrador tea seedlings grown in PMM/eNST, the shoot:root dry weight ratios were significantly reduced in plants inoculated with *P. ericae* and *O. maius* compared with the non-inoculated plants. The lack of a significant difference in the total dry weights of Labrador tea seedlings grown in PMM/eNST, regardless of inoculation, indicates that the effect of mycorrhizal fungi on root growth is greater than its effect on shoot growth. It is possible that the low WHC of eNST amendment affected water availability, and the plants allocated more photosynthates to roots compared with shoots, which is a common response of plants under environmental stress conditions (Xu et al. 2015). In the PMM/esNST treatment, the dry weights, net photosynthesis, Fv'/Fm' ratios, and chlorophyll concentrations of Labrador tea seedlings inoculated with *P. ericae* and *O. maius* were significantly higher compared with the non-inoculated plants, indicating that ERM fungi enhanced the tolerance of plants to esNST. Of the potentially phytotoxic factors present in tailings, research has primarily concentrated on the high salinity and elevated pH levels. Relative little is known about the effects of NAs on plants and whether these effects could be alleviated by mycorrhizae. Some of the microorganisms, including fungi can break down hydrocarbons, including NAs, however, this notion requires further investigation since it has not been investigated in ERM fungi.

2.4.3 Responses of velvetleaf blueberry inoculated *P. ericae* or *O. maius* to eNST and esNST

In velvetleaf blueberry, the addition of eNST and esNST significantly increased leaf Na concentrations. Elevated Na levels in tissues can disrupt photosynthesis and water uptake processes (Deinlein et al. 2014), which may account for the significant reductions in chlorophyll concentrations, net photosynthesis and total dry weights of seedlings treated with tailings. Interestingly, seedlings inoculated with *P. ericae* and grown in PMM/eNST had higher leaf Na concentrations compared with non-inoculated individuals, whereas in plants grown in esNST, leaf Na concentrations were significantly lower. The increase in leaf Na concentrations in plants inoculated with *P. ericae* suggests a possible root injury, which might have impaired the control of Na root uptake and (or) transfer to shoots. In non-inoculated velvetleaf blueberry seedlings grown in PMM/esNST, leaf P concentrations were significantly lower compared with plants grown in PMM, while leaf P concentrations were significantly higher compared with the plants inoculated with *P. ericae*. ERM increase plant P uptake by exploring greater soil volumes through fine hyphae (Tibbett 2000). ERM fungi can also help host plants acquire, otherwise inaccessible, P sources by producing and releasing extracellular and cell wall-bound phosphodiesterase enzymes to degrade phosphodiester DNA (Leake and Miles 1996). Moreover, ERM fungi can increase the uptake and metabolism of P by upregulating the expression of genes related to P uptake and metabolism (Wei et al. 2022).

Mn plays an essential role in the photosynthetic processes in plants, and also participates in redox reactions as a cofactor for different enzymes and is one of the important mechanisms for

plants to against oxidative stress (Bowler et al. 1994, Broadley et al. 2012). Mn concentration in lowbush blueberry leaves is normally lower than 289 mg/kg (Korcak 1988), and Mn toxicity in leaves has rarely been documented. In the present study, the leaf Mn concentration in non-inoculated velvetleaf blueberry in PMM was 453.8 mg/kg, in PMM/eNST 415.0 mg/kg, and in PMM/esNST 777.0 mg/kg, which all exceeded the typical concentrations. Therefore, it appears that the source of Mn was the PMM substrate used for oil sands revegetation, rather than the tailings. While the leaf concentration levels of Mn in the present study may not cause toxicity, it should be emphasized that the plants were grown in these growth substrates for only eight weeks in my study. Plants suffering from Mn toxicity have impaired chlorophyll synthesis, photosynthesis, and over-produce reactive oxygen species (ROS), which can induce oxidative stress in leaf cells (Boojar et al. 2008, Li et al. 2010, Sheng et al. 2016, Liu et al. 2019). In velvetleaf blueberry seedlings grown in PMM/esNST, inoculation with *P. ericae* significantly reduced the leaf Mn concentrations, indicating potential benefit of *P. ericae* in balancing plant mineral nutrition.

The total dry weights of velvetleaf blueberry seedlings grown in PMM/eNST were not affected by the inoculation with *P. ericae.* However, the shoot:root DW ratios were significantly lower, and net photosynthesis rates were significantly higher in the inoculated compared to non-inoculated plants. This suggests that *P. ericae* improved root growth more compared with shoot growth. Interestingly, in PMM/esNST, the shoot, root and total dry weights of velvetleaf blueberry inoculated with *O. maius* were significantly higher compared with the non-inoculated seedlings, while the net photosynthesis rates and chlorophyll concentrations were similar regardless of fungal

inoculation. A plausible explanation could be that the fungus had a greater effect on plant physiological responses shortly following inoculation compared with the later period of the experiment. However, more research would be needed to confirm this hypothesis.

In conclusion, the study demonstrated that the presence of both eNST and esNST in PMM significantly inhibited the growth of velvetleaf blueberry and Labrador tea, and negatively affected the physiological processes, including photosynthesis and chlorophyll synthesis. *P. ericae* and *O. maius* enhanced plant tolerance to tailings, improving growth and the measured physiological parameters in both plant species. The beneficial effects of *P. ericae* were more prominent compared with *O. maius*. The physical and chemical properties of the two tailings, eNST and esNST, are very similar, but the inoculation effect of Labrador tea is greater in esNST, and the inoculation effect of velvetleaf blueberry is greater in eNST. However, further long-term field studies need to be conducted to monitor the impact of ERM inoculation on plants in reclamation sites affected by eNST or esNST to take into consideration variable environmental factors present in planting sites.

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2.6 Tables

Table 2.1. The pH, electrical conductivity (EC, mS cm-1), elemental concentrations (mg kg-1, % for N and for C), and soil water holding capacity (WHC, %) of the treatment substrates at the beginning of the experiment. Means ± SE (n = 4) are shown. Different letters following the values in the same column indicate significant difference ($p \leq 0.05$) between treatments as determined by Holm test.

	pH	EC	Na	Mg	P	K	Ca	Fe	Mn	Zn	N	WHC
PMM	5.1 ±0.004 a	1.6 ±0.025 c	306.2 ±4.3 a	3220.5 ±33.1 c	372.5 ±11.3 b	3057.5 ±106.8 c	7750.8 ±347.6 c	12984.0 ±223.7 b	193.7 ±22.5 b	41.8 ±1.5 b	0.7 ±0.1 b	100
PMM/eNST	5.4 ±0.009 b	0.9 ±0.022 b	350.5 ±19.1 a	1596.0 ±91.1 b	136.3 ±7.0 a	2408.3 ±216.9 b	1802.8 ±99.6 a	7484.5 ±403.4 a	115.7 ±16.8 a	19.8 ±0.9 a	0.2 ±0.02 a	48
PMM/esNST	6.0 ±0.035 c	0.8 ±0.006 a	325.0 ±15.3 a	1320.8 ±75.2 a	150.2 ±8.1 a	1413.8 ±93.6 a	3016.5 ±144.6 b	6513.5 ±694.3 a	97.8 ±14.9 a	18.9 ±1.0 a	0.1 ±0.01 a	44

Table 2.2. Root mycorrhizal colonization rate in non-inoculated seedlings of velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) and

Labrador tea (*Ledum groenlandicum*), as well as those inoculated with *Oidiodendron maius* and *Pezoloma ericae* grown in PMM, PMM/eNST, and PMM/esNST substrates for eight weeks The values are means ± SE (n=3) and different letters indicate significant differences ($p \leq 0.05$) between treatments within each plant species according to Holm test.

	Non-inoculated	*Pezoloma ericae*	*Oidiodendron maius*
	Labrador tea		
PMM	18.8±1.5 a	67.5±2.4 b	61.5±3.4 a
PMM/eNST	11.9±2.4 a	55.8±1.5 a	59.8±3.4 a
PMM/esNST	13.4±1.2 a	57.0±1.3 a	59.3±2.3 a
	Velvetleaf blueberry		
PMM	19.0±2.4 b	58.8±0.6 a	60.8±1.5 a
PMM/eNST	10.3±1.8 a	52.7±1.5 a	55.4±3.3 a
PMM/esNST	13.2±1.0 ab	56.0±2.1 a	57.1±2.1 a

Table 2.3. Leaf elemental concentrations (mg kg-1, % for N) in Labrador tea (*Ledum groenlandicum*) seedlings grown for eight weeks

in treated with PMM/eNST, PMM/esNST and inoculated with *Pezoloma ericae*, *Oidiodendron maius* or non-inoculated. Means ± SE (n = 6) are shown. Different letters following the values in the same column indicate significant differences ($p \leq 0.05$) between treatments as determined by the Holm test.

	B	Ca	Fe	K	Mg	Mn	N	Na	P	Zn
Control	36.6±3.3 a	5696.7±393.6 abc	48.8±2.6 abc	8315.6±714.1 ab	2266.4±109.1 bcd	78.1±15.6 a	1.7±0.1 abc	2349.1±336.6 ab	1437.8±92.9 cd	10.8±0.7 a
P. ericae	71.2±6.5 b	6276.4±387.4 c	66.1±9.1 c	7422.8±203.9 a	2286.5±162.5 bcd	158.0±18.8 ab	1.9±0.1 c	1222.8±244.5 a	1292.2±95.2 abc	14.7±1.3 a
O. maius	45.3±2.1 a	5493.3±286.0 abc	57.2±3.2 abc	9387.6±331.2 abc	2009.2±257.2 abc	149.1±18.0 ab	1.7±0.1 bc	1776.8±205.5 a	1410.8±65.3 cd	10.9±0.6 a
PMM/eNST	133.2±2.0 d	10005.0±490.5 d	52.6±3.9 abc	11922.7±426.0 c	3049.7±274.1 d	201.4±28.3 b	1.6±0.1 abc	7785.7±246.9 d	1061.8±50.2 ab	31.7±2.1 c
PMM/eNST + *P. ericae*	77.8±5.9 b	5893.5±326.8 bc	52.6±3.8 abc	10842.8±799.7 bc	1971.6±132.8 abc	148.3±15.3 ab	1.7±0.02 bc	2678.3±283.0 ab	1268.0±61.9 abc	16.8±1.7 ab
PMM/eNST + *O. maius*	104.2±2.0 c	8456.7±526.9 d	48.1±1.8 abc	9401.8±1297.1 abc	2709.9±191.5 cd	181.8±15.8 b	1.5±0.1 ab	4281.1±311.3 bc	1017.1±32.4 ab	24.0±2.2 bc
PMM/esNST	101.1±6.7 c	6136.2±548.0 c	39.7±0.7 a	9416.7±1051.0 abc	2311.2±134.9 bcd	158.8±12.6 ab	1.6±0.1 abc	5405.8±907.6 cd	963.3±30.9 a	27.1±3.2 c
PMM/esNST + *P. ericae*	78.6±6.9 b	3906.5±234.5 a	59.9±0.5 bc	7642.8±316.4 ab	1412.8±30.7 a	155.2±26.9 ab	1.8±0.1 c	1762.0±798.9 a	1675.9±86.0 d	14.7±1.1 a
PMM/esNST + *O. maius*	74.5±2.8 b	4200.1±513.9 ab	42.7±3.4 ab	8793.5±347.3 abc	1839.0±164.2 ab	92.9±14.8 a	1.3±0.04 a	2788.2±167.7 ab	1334.9±44.2 bcd	17.2±1.6 ab

Table 2.4. Leaf elemental concentrations (mg kg-1, % for N) in velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) seedlings grown for eight weeks treated with PMM/eNST, PMM/esNST and inoculated with *Pezoloma ericae*, *Oidiodendron maius* or non-inoculated.

Means ± SE (n = 6) are shown. Different letters following the values in the same column indicate significant differences ($p \leq 0.05$) between treatments as determined by the Holm test.

	B	Ca	Fe	K	Mg	Mn	N	Na	P	Zn
Control	93.4±16.2	4445.4±398.3	100.2±5.3	15015.2±2035.1	2821.2±153.0	453.8±53.6	2.4±0.3	4110.0±485.6	1285.4±44.1	13.2±1.0
	a	ab	c	a	a	b	a	a	b	a
P. ericae	132.8±14.6	4882.4±121.9	43.2±1.6	16212.4±506.0	2864.6±127.1	235.3±26.5	1.8±0.3	4585.2±262.2	1029.0±31.0	10.3±0.6
	a	b	a	a	a	ab	a	ab	ab	a
O. maius	122.1±11.0	4605.0±346.9	44.8±7.4	14876.6±1142.2	2860.3±221.3	249.2±34.8	2.3±0.3	4701.2±346.7	998.8±104.0	10.2±0.8
	a	b	a	a	a	ab	a	ab	ab	a
PMM/eNST	192.2±18.1	3361.1±318.2	82.9±10.5	17296.8±824.5	2808.5±264.6	415.0±34.0	1.9±0.4	7156.2±1663.1	949.7±115.2	13.5±2.8
	a	ab	bc	a	ab	ab	a	bc	ab	a
PMM/eNST	148.9±14.5	3141.1±386.2	63.5±13.7	16339.3±571.7	2238.0±248.0	286.9±131.3	3.3±0.5	12209.7±701.8	1160.4±166.6	13.7±2.6
+ *P. ericae*	a	ab	ab	a	a	ab	a	de	ab	a
PMM/eNST	135.3±53.6	3936.2±163.2	38.4±4.8	18678.3±570.9	2422.8±178.2	315.1±7.7	2.1±0.3	9427.2±181.2	1079.2±179.8	8.3±0.8
+ *O. maius*	a	ab	a	a	a	ab	a	cd	ab	a
PMM/esNST	214.4±6.3	4454.4±724.2	56.7±7.3	19480.3±212.8	4045.5±381.9	777.0±73.1	2.1±0.4	13166.7±647.1	689.2±103.3	11.0±0.6
	a	ab	ab	a	b	c	a	e	a	a
PMM/esNST	142.0±33.8	2606.6±218.7	32.4±1.6	13764.8±609.7	2363.3±160.3	137.8±14.1	2.4±0.5	7383.3±831.6	1375.3±53.8	10.6±1.3
+ *P. ericae*	a	a	a	a	a	a	a	bc	b	a
PMM/esNST	239.7±49.8	3756.1±487.7	59.7±4.8	16594.0±1541.5	2099.0±305.2	385.7±84.3	1.6±0.2	10932.2±657.7	964.7±99.2	9.5±1.0
+ *O. maius*	a	ab	ab	a	a	ab	a	de	ab	a

2.7 Figures

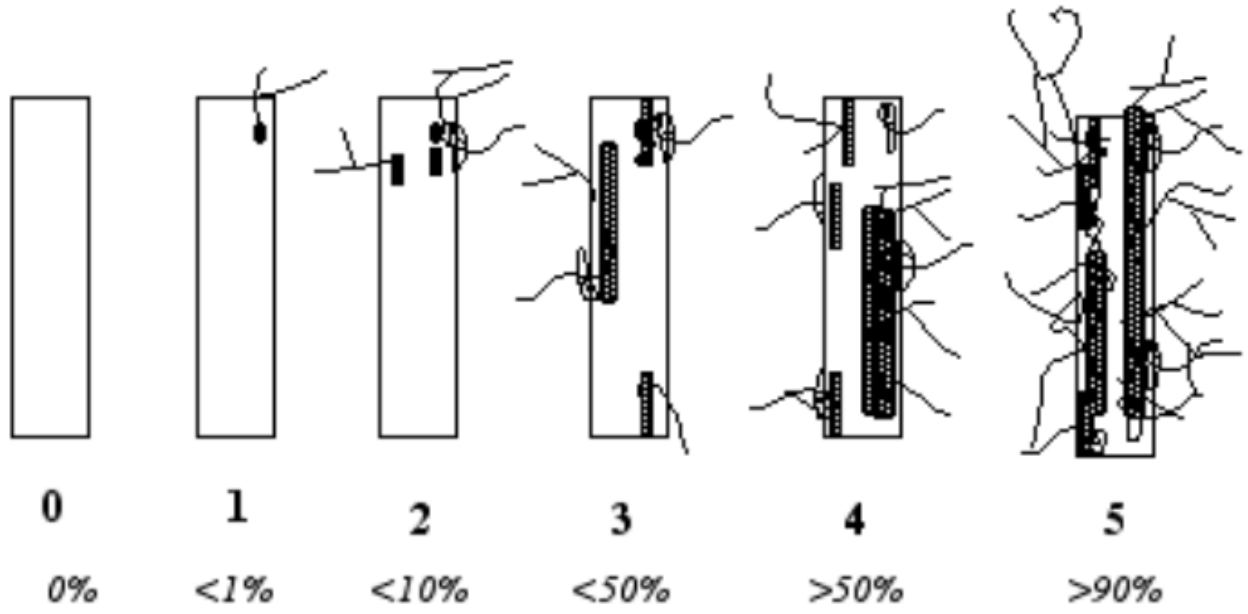

Figure 2.1. Examples of ERM fungi root colonization for calculating the intensity of mycorrhizal colonization (Trouvelot et al. 1986). Root sample rated number 5 for more than 90 % intensity of mycorrhizal colonization; root sample rated number 4 for between 50 to 90 % intensity of mycorrhizal colonization; root sample rated number 3 for between 10 to 50 % intensity of mycorrhizal colonization; root sample rated number 2 for between 1 to 10 % intensity of mycorrhizal colonization; root sample rated number 1 for lower than 1 % intensity of mycorrhizal colonization.

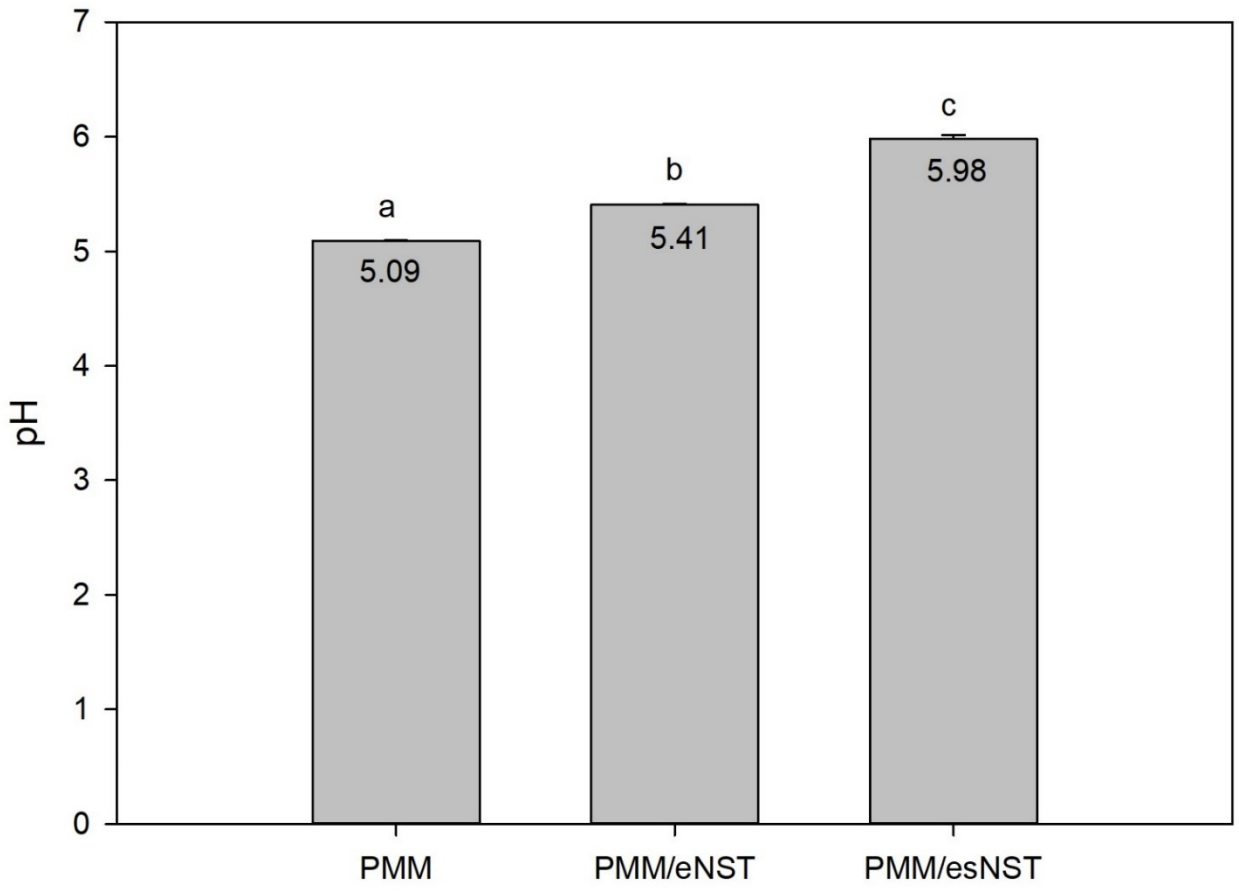

Figure 2.2. Initial soil pH of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 4). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

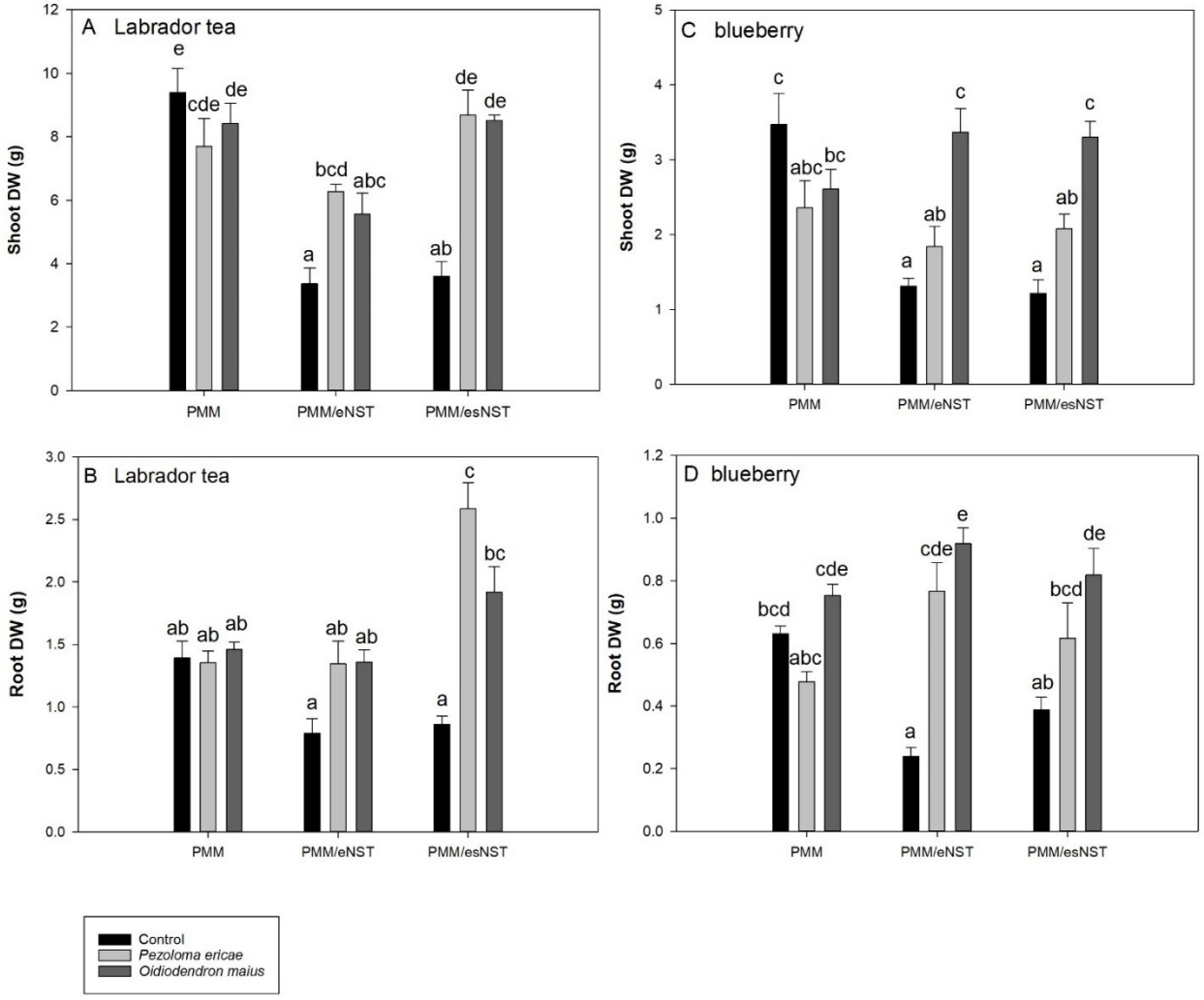

Figure 2.3. Shoot and root dry weights in Labrador tea (A, B) and velvetleaf blueberry (C, D). Plants were grown for eight weeks in the substrates of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

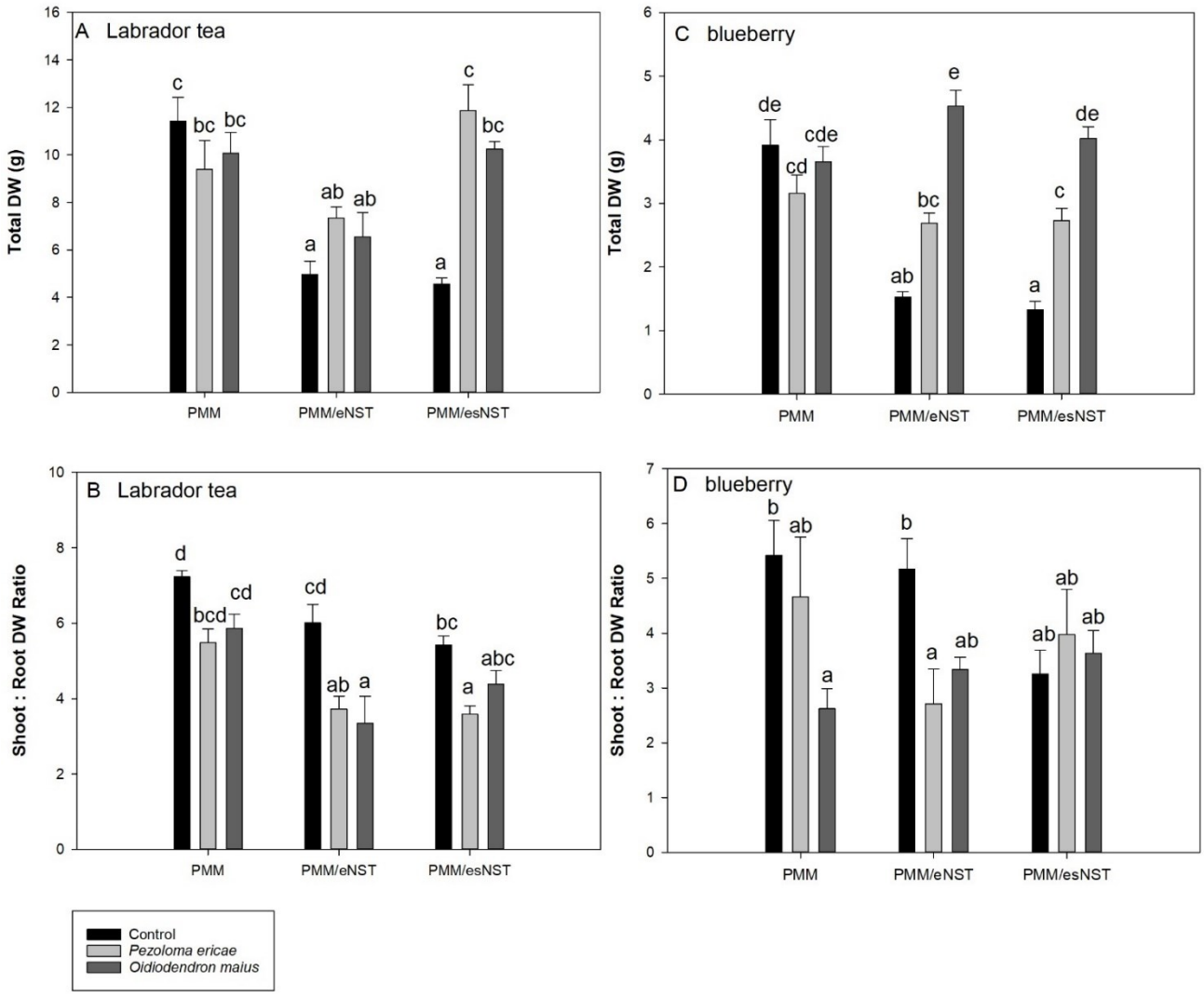

Figure 2.4. Total dry weight and shoot to root dry weight ratio in Labrador tea (A, B) and velvetleaf blueberry (C, D). Plants were grown for eight weeks in the substrates of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

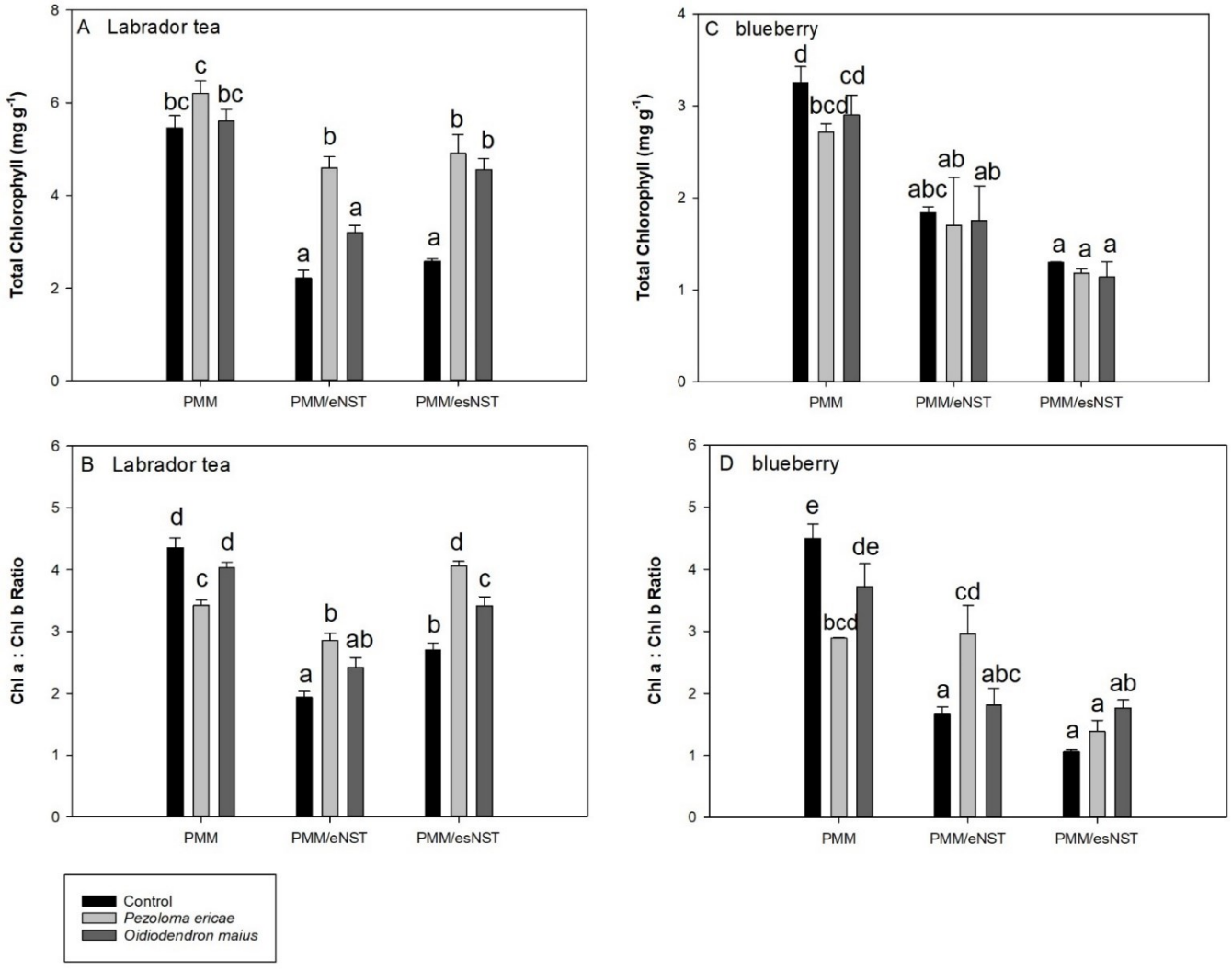

Figure 2.5. Total leaf chlorophyll concentrations and Chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios in Labrador tea (A, B) and velvetleaf blueberry (C, D). Plants were grown for eight weeks in the substrates of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 4). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

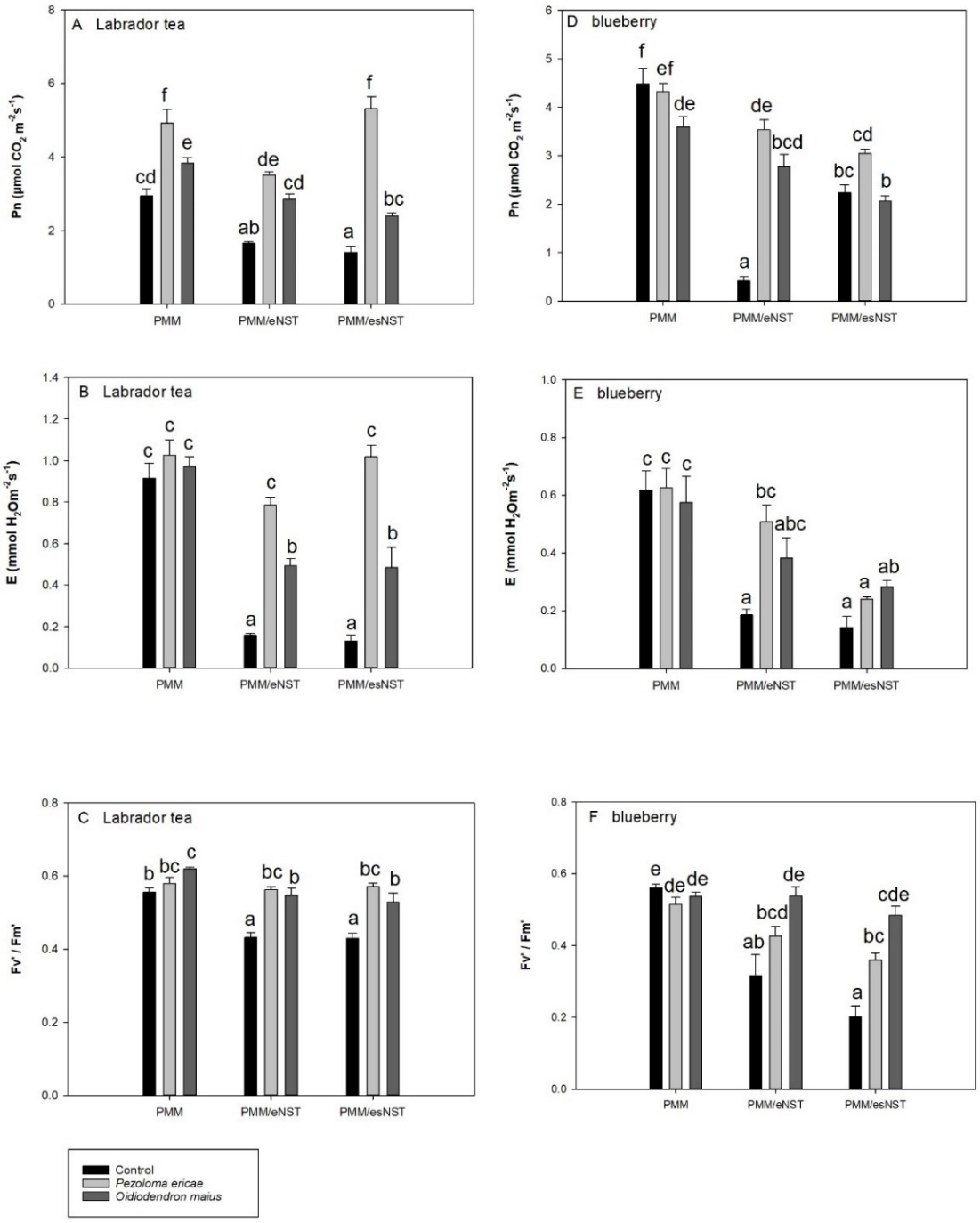

Figure 2.6. Net photosynthesis rate (Pn), transpiration rate (E) and photosystem II (PS II) maximum efficiency (Fv'/Fm') in Labrador tea (A, B, C) and velvetleaf blueberry (D, E, F). Plants were grown for eight weeks in the substrates of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

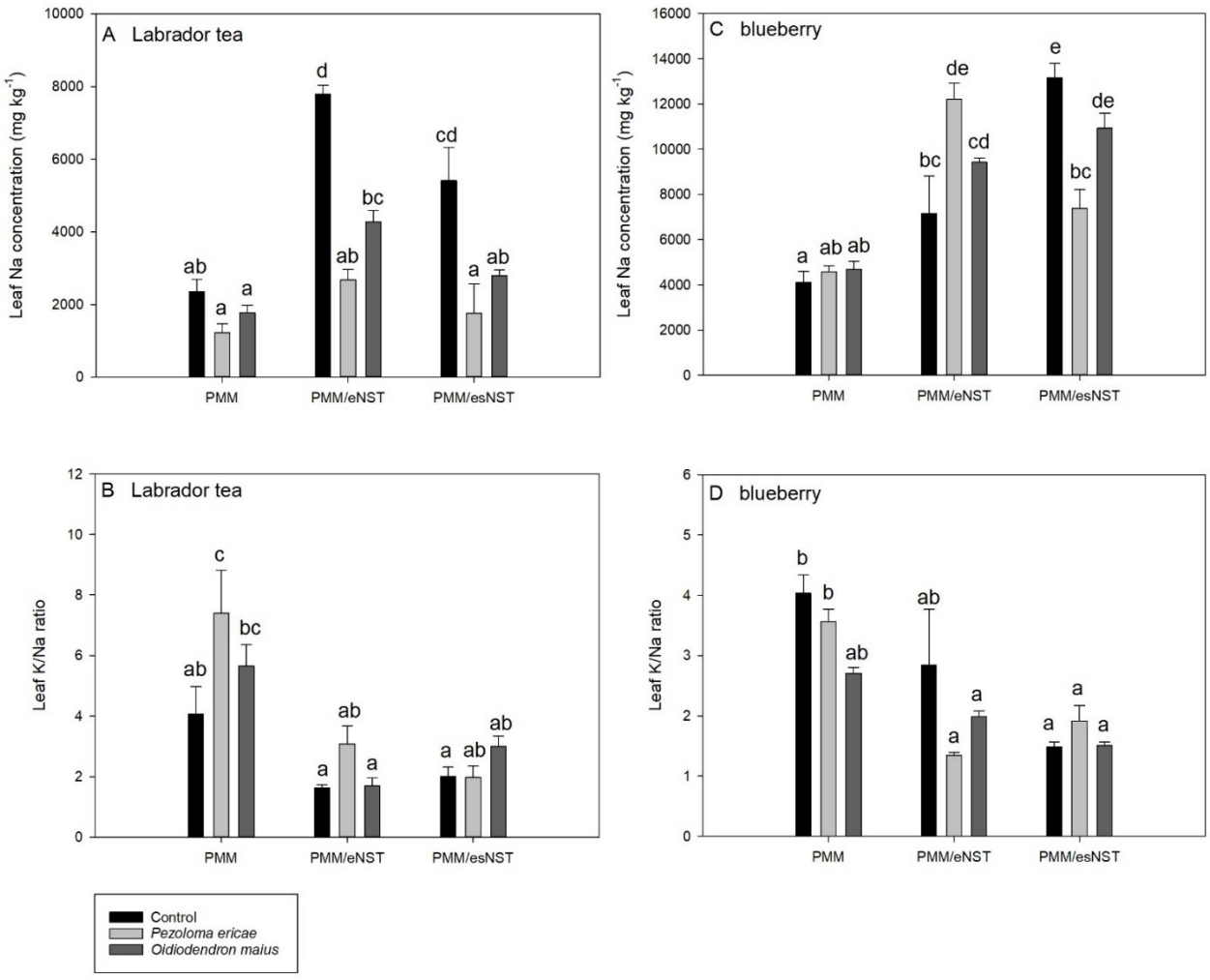

Figure 2.7. Leaf concentrations (mg kg-1) of Na, K/Na ratio in Labrador tea (A, C) and velvetleaf blueberry (B, D). Plants were grown for eight weeks in the substrates of PMM, PMM/eNST and PMM/esNST (Means ± SE, n = 4). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

Chapter 3 Impact of mycorrhization with *Laccaria bicolor* on responses of jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) seedlings to novel oil sands tailings

3.1 Introduction

The establishment and growth performance of tree species are among the most important criteria to evaluate the reclamation success of oil sands mining sites (Macdonald et al. 2015, Dhar et al. 2018). Mine operators have continued improving oil sands tailings with the aims of increasing water use efficiency, reducing carbon emissions, and ameliorating their phytotoxicity. Recently, Canadian Natural Resources Limited (CNRL) developed novel tailings technologies referred to as the enhanced non-segregating tailings (eNST) and enhanced spiked non-segregating tailings (esNST), which are further improvements of the earlier NST technology (Vedoy and Soares 2015, CNRL 2022). The responses of trees used for the revegetation of areas affected by these tailings to these novel tailings need to be comprehensively examined before their deposition in reclamation sites.

Since saline and alkaline chemicals are added in the oil sands processing and due to the properties of overburden placed in reclamation sites, reconstructed soils in these sites are often associated with high pH, elevated salinity, low organic matter, impaired microorganism community, and reduced water holding capacity (Fung and Macyk 2000, Dimitriu et al. 2010). As a result, the plants growing in oil sands reclamation sites may have high mortality, stunted growth (Zhang et al. 2015), and suffer from nutrient deficiencies (Zhang et al. 2013, Xu et al. 2019), root hypoxia (Fleurial et al. 2022), and impaired water uptake (Zhang et al. 2013, Zhang and Zwiazek

2016).

Jack pine (*Pinus banksiana*) and white spruce *(Picea glauca*) are among the dominant tree species in the boreal forests in Northeastern Alberta, Canada and are commonly used for the revegetation of oil sands areas. Jack pine prefers acidic sandy and loamy soils (Burns 1990), but with mycorrhizal association, it can grow on calcareous soils with pH as high as 8.2 (Rudolf 1965). Jack pine is regarded as one of the most sensitive boreal trees to oil sands tailings (Zhang et al. 2015, Zhang et al. 2023). White spruce is a mid- to late-successional species in the boreal forest (Abrahamson 2015) and is adapted to a wide range of soil and climatic conditions across the boreal region (Nienstaedt and Zasada 1990). White spruce is generally considered to be relatively tolerant to the presence of oil sands tailings (Zhang et al. 2013, Zhang et al. 2023).

Many species of boreal trees are associated with ectomycorrhizal (ECM) fungi. These fungi form hyphal sheaths referred to as the mantle on the surface of host roots and the intercellular Hartig net inside the root cortex (Lehto and Zwiazek 2011). ECM fungi are widely reported to enhance the growth of host trees under harsh environmental conditions by improving water and nutrient absorption as well as tolerance of abiotic and biotic stresses (Smith and Read 1997, Allen 2007). *Laccaria bicolor* is a common ECM fungus that frequently occurs in the early stages of fungal succession in young forests (Smith and Read 1997). This and other ECM fungi have been widely studied for their potential improvement of restoration and reclamation of disturbed ecosystems (Bois et al. 2005, Quoreshi 2008, Macdonald et al. 2012). *L. bicolor* was reported to improve the water uptake of hybrid poplar (*Populus trichocarpa* × *deltoides*) and white spruce

(*Picea glauca*) under drought conditions through its effect on the plant aquaporin expression and activity (Xu et al. 2015, Calvo-Polanco et al. 2019). A previous field study found that *L. bicolor* improved biomass growth of jack pine and white spruce in oil sands reclamation sites (Onwuchekwa et al. 2014). It was also demonstrated that *L. bicolor* in vitro culture showed superior tolerance and growth in the saline-alkaline media and in the media containing oil sands tailings water (Kernaghan et al. 2002).

The present study was conducted under controlled environmental conditions to examine the effects of mycorrhization of jack pine and white spruce seedlings with *Laccaria bicolor* on their responses to the presence of eNST or esNST in the soil. I hypothesized that *L. bicolor* inoculation would improve the growth and physiological responses of jack pine and white spruce to eNST and esNST and that this effect would involve enhanced water and mineral nutrient uptake by mycorrhizal associations.

3.2 Materials and methods

3.2.1 Plant material and growth conditions

One-year-old nursery grown jack pine (*Pinus banksiana* Lamb.) and white spruce [*Picea glauca* (Moench) Voss] seedlings were obtained from Tree Time Services Inc., Edmonton, Alberta, Canada. Seeds of the plants were collected in the undisturbed boreal forest near CNRL's Horizon oil sands mine and grown in Styrofoam block containers (415D V77/170HS 163 cm^3 Styroblocks™, Beaver Plastics, Acheson, AB, Canada). All seedlings were stored for two weeks at 4 °C prior to the commencement of the experiment. After removing from cold storage, the

seedlings were placed in PVC trays (59 cm x 39 cm x 18 cm, Curver 32QT LM Box Clear, Home Depot, Edmonton, AB, Canada) for fungal inoculation and then transplanted into 500 cm^3 pots. The plants were grown for eight weeks in a controlled-environment growth chamber with 22/18 °C (day/night) temperature, 50±10% relative humidity and 16-h photoperiod with 400 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPDF) provided by full-spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). The plants were watered every second day and fertilized by adding 500 mL 100% Hoagland's mineral solution (Epstein and Bloom 1853) to each pot once a week.

3.2.2 Fungal culture and inoculation

The fungal strain *Laccaria bicolor* UAMH8232 was provided by the University of Alberta Microfungus Collection and Herbarium. Mycelia of the *L. bicolor* strains grown on solid modified Melin-Norkans (MMN) medium (Marx and Davey 1969, Pham et al. 2004) were cut into pieces and transferred to flasks containing liquid modified Melin-Norkans (MMN) medium. The flasks were placed on a rotary shaker in the dark at 20 °C and were shaken at 120 rpm for one month (Xu et al. 2015). For root inoculation, the cultures were homogenized in a blender and diluted with distilled water to the final concentration of 0.5 mg dry mycelia/mL (Calvo-Polanco et al. 2009).

The seedlings were inoculated twice by immersing the root plugs in the mycelial slurry in plastic tubs for one day. The first inoculation was carried out in the controlled-environment growth room after the seedlings were moved from cold storage. After the first inoculation, the seedlings were bundled in groups of 10, and their root plugs were wrapped in Saran wrap and placed in tubs in

the controlled-environment growth room to resume growth. The root plugs were kept moist by watering the seedlings two to three times per week. After one month, the seedlings were inoculated for the second time, as above and kept in the growth room for another month. Then, the seedlings were microscopically examined for fungal colonization (Brundrett et al. 1996) and transplanted into 500 cm^3 pots filled with the different treatment substrates. For both inoculations, autoclaved fungal-free liquid MMN medium was used as mycorrhizal control. The seedlings were spatially separated to minimize the possibility of cross-contamination. There were ten replicates of seedlings per species in each treatment.

3.2.3 eNST and esNST treatments

The substrates used in this study were the peat-mineral soil mix (PMM), enhanced non-segregating tailings (eNST), enhanced-spiked non-segregating tailings (esNST), and PMM supplemented with eNST (1:1, by volume) or with esNST (1:1, by volume). PMM was collected from the boreal forest site near the oil sands mining areas of the Canadian Natural Resources Limited (CNRL) Horizon lease, north of Fort McMurray, Alberta, Canada (56° 43' 35.94" N, 111° 22' 44.1732" W). The eNST and esNST were by-products of the oil sands extraction process by Canadian Natural Resources Limited (CNRL). These materials were sealed in 20 L plastic pails and delivered to the University of Alberta. Prior to experimentation, large stones and chunks of wood debris were removed from PMM, and the soil was air-dried for 4-5 days.

Therefore, five types of growth substrates were used in total for this study, including 1) 100% PMM (control); 2) PMM + eNST (1:1, by volume); 3) PMM + esNST (1:1, by volume); 4) 100% eNST; 5) 100% esNST.

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

Immediately after transplanting to growth substrates, shoot heights in ten seedlings per treatment were measured from the root collar to the shoot tip (n = 10). Stem diameters were measured twice above the root collar at the perpendicular directions, and the mean values were taken for the analysis (n = 10). Prior to harvesting the plants (after eight-weeks of treatments), shoot heights and stem diameters were measured again. The RSHG and RSDG were calculated by dividing the differences between the initial and final measurements by the initial measurement values.

3.2.5 Leaf chlorophyll concentrations

After eight weeks of treatment, chlorophyll concentrations were determined in fully expanded leaves harvested from six randomly selected seedlings per treatment (n = 6). The leaf samples were freeze-dried (Labconco Freeze Dry System 4.5 Liter) for 96 h and ground into powder with a Thomas Wiley Mini-Mill (Thomas Scientific, Trenton, NJ, USA).

For chlorophyll analysis, 10 mg samples of dry leaf powder were extracted with 8 mL dimethyl sulfoxide (DMSO) in 15 mL Falcon tubes and placed in an oven at 65 ℃ for 22 h. The chlorophyll a and chlorophyll b concentrations were measured with a spectrophotometer (Ultrospec,

Pharmacia LKB, Uppsala, Sweden) at 648 nm and 665 nm. The total chlorophyll concentrations were calculated by the Arnon's equation (Sestak et al. 1971).

3.2.6 Analysis of treatment substrates

At the beginning of the experiment, four replicated samples (n = 4), each consisting of 100 g of treatment substrates (PMM, PMM/eNST, PMM/esNST, eNST, and esNST) were collected. To measure the water holding capacity (WHC), 25 g dry powder of each treatment substrate was placed in a funnel lined with filter paper (110 mm diameter, CAT No. 17413182 Whatman® Prepleated Qualitative Filter Paper). Gradually, 50 mL (V1) of distilled water was added. The gravity-drained extract was collected, and its volume (V2) was measured using a graduated cylinder.

The WHC (%) was calculated as: $WHC = \frac{V1-V2}{25} X\ 100$ (Rowell 2014).

For each sample, 30 g of powder was mixed with 60 mL of distilled water and shaken for 1 h. The slurry was then filtered using a 0.45 µm Teflon membrane filter to obtain the growth substrates extract solution. The pH of the solution was measured using an Orion STAR A111 pH meter (Thermo Fisher Scientific Inc., Waltham, MA), and the electrical conductivity (EC) was measured using a Traceable 15077-977 Expanded Range Conductivity Meter (Thermo Fisher Scientific Inc., Waltham, MA). The concentrations of Na, Mg, P, K, Ca, Fe, Mn, and Zn in the substrate solutions were analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Thermo iCAP 6000 series) (Zarcinas et al. 1987).

To determine the concentration of naphthenic acids (NAs), 20 mL of substrate filtered extract solution was adjusted to a pH of approximately 2 using pure HCl (37.2%). Each acidified water sample was then extracted twice with 10 mL of dichloromethane (DCM). The organic layers were evaporated to dryness under a fume hood to collect the final NAs extract. Commercial mixture of naphthenic acids (NAs) (MilliporeSigma Canada Ltd., Oakville, ON, Canada) was used to prepare standard solutions and calibration curves. NA's were measuredusing Fourier transform infrared (FTIR) spectroscopy with the peak heights recorded at 1743 and 1706 cm^{-1} wavenumbers. The concentrations of NAs in the samples were calculated from the total of recorded peak heights compared with the heights in calibration curves (Meshref et al. 2020).

3.2.7 Chlorophyll and elemental concentrations in leaves

After eight weeks of treatment, six seedlings (n = 6) were randomly selected from each treatment and the fully expanded uppermost leaves were harvested to measure chlorophyll and elemental concentrations. The harvested leaves were freeze-dried in a Labconco Freeze Drying System (4.5 L) for four days and then ground into powder using a Thomas Wiley Mini Mill (Thomas Scientific, Trenton, NJ, USA).

For leaf chlorophyll concentrations, 10 mg of the dried leaf powder samples were weighed and mixed with 8 mL dimethyl sulfoxide (DMSO) in a 15 mL Falcon tube, and the mixtures were placed in an oven at 65°C for 22 h. Chlorophyll a and b concentrations were determined using a spectrophotometer at wavelengths of 648 nm and 665 nm. The total chlorophyll concentrations were calculated using Arnon's equation (Sestak et al. 1971).

For the analysis of leaf elemental concentrations (B, Ca, Fe, K, Mg, Mn, N, Na, P, Zn), 0.2 g leaf powder of each treatment was digested in 10 mL of 70% HNO_3 at 185 ºC for 10 minutes using a microwave digestion system (Mars 5 Microwave Accelerated Reaction System, CEM). The digests were then diluted to 40 mL with Milli-Q water. The filtered extracts were analyzed by ICP-OES (Thermo iCAP 6000 series). Total nitrogen (N) concentration in the leaves was determined by a dry combustion method using a Thermo FLASH 2000 HT Plus Organic Element Analyzer (Thermo Fisher Scientific Inc) (Schumacher 2002).

3.2.8 Gas exchange, shoot water potential and dry mass

At the end of the experiment, net photosynthetic rates (P_n), transpiration rates (E), and PSII maximum efficiency (Fv'/Fm') of plants were measured in the needles of six randomly selected seedlings per treatment (n = 6) between 09:00 and 12:00 using the infrared gas analyzer (LI-6400, LI-COR, Lincoln, Nebraska, USA) at 400 μmol $m^{-2}s^{-1}$ photosynthetic photon flux density (PPDF), 400 μmol mol^{-1} reference CO_2 concentration, 200 μmol s^{-1} flow rate, and 20°C leaf chamber temperature. The needles of jack pine were spread out in the six cm^2 leaf chamber to cover the entire chamber. For white spruce, a 3-cm distal section of needles was inserted into the leaf chamber.

Terminal shoots with a length of 10 – 15 cm were excised at midday and immediately placed into a Scholander pressure chamber (PMS instruments, Corvallis, OR, USA) for shoot water potential (Ψ_{shoot}) measurements (Scholander et al. 1965, Wan et al. 1999). The reading of the

pressure provided at the first sight of xylem sap emerging from the cut section was recorded in MPa (n = 6).

All plants were harvested, and their shoots and roots were separated and placed in paper bags. Dry weights (DW) of shoots and roots were determined after oven drying at 70°C for 96 h (n = 6).

3.2.9 Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using the R software (Version 4.3.2) to determine the significant differences ($p \leq 0.05$) between treatments within each species. Comparisons between different treatment means were carried out using the Holm test.

3.3 Results

3.3.1 Substrate properties

The pH of PMM was about 5.1, significantly lower than the pH of PMM/eNST (5.4), PMM/esNST (6.0), eNST (7.1), and esNST (7.1) (Table 3.1). The EC of the substrates decreased with increasing tailings concentration (Table 3.1). PMM had the highest EC of 1.6 mS cm^{-1} among all substrates, while the EC of PMM/eNST and PMM/esNST were 0.9 and 0.8 mS cm^{-1}, respectively; and eNST and esNST had EC values of 0.4 and 0.5 mS cm^{-1}, respectively (Table 3.1). No significant differences were observed in Na concentrations between PMM and tailings amended substrates, and the lowest Na concentrations were observed in pure tailings (Table 3.1). The concentrations of Mg, P, K, Ca, Fe, Mn, and Zn were the highest in PMM and the lowest in tailings substrates (Table 3.1). The concentrations of NAs in the eNST released water (65 mg/L)

and esNST released water (57 mg/L) were significantly higher compared with the esNST sediment (27 mg/L) (Table 3.1). The WHC of the PMM (100%) was significantly higher than that of PMM/eNST (48%) and PMM/esNST (44%) (Table 3.1). Although the WHC of eNST and esNST was not measured, it can be inferred that the WHC of pure tailings would be lower compared with tailings amended substrates.

3.3.2 Plant relative stem diameter growth (RSDG) and relative shoot height growth (RSHG)

The RSDG and RSHG of non-inoculated jack pine seedlings grown in PMM/esNST and pure tailings were significantly lower compared with the plants grown in PMM, (Figure 3.2 A, B). Jack pine seedlings grown in PMM and PMM/esNST substrates inoculated with *L. bicolor* had significantly greater RSDG compared with non-inoculated plants (Figure 3.2A). For both inoculated and non-inoculated jack pine seedlings, the pure tailings had greater impact on RSHG compared with seedings grown in tailings supplemented with PMM and PMM (Figure 3B).

In non-inoculated white spruce, the RSDG of seedlings grown in PMM were significantly greater than those treated with tailings containing substrates (Figure 3.2C). For the *L. bicolor* inoculated white spruce, the seedlings grown in PMM and tailings amendments had greater RSDG than those grown in pure tailings (Figure 3.2C). In both PMM/eNST and PMM/esNST treatments, inoculated seedlings had greater RSDG compared with the non-inoculated white spruce (Figure 3.2C). White spruce seedlings inoculated with *L. bicolor* and grown in PMM/eNST and esNST had significantly higher RSHG compared with the non-inoculated seedlings (Figure 3.2D). For the non-inoculated seedlings, the plants that were grown in PMM had significantly higher RSHG

compared with the plants that were grown in the substrates containing tailings (Figure 3.2D). Compared with PMM, only pure tailings significantly reduced the RSHG in seedlings inoculated with *L. bicolor* (Figure 3.2D).

3.3.3 Shoot water potentials (*Ψshoot*)

In non-inoculated jack pine seedlings, tailings did not significantly impact *Ψshoot* (Figure 3.3A). However, in the inoculated jack pine, *Ψshoot* of seedlings grown in pure tailings were significantly lower than those grown in PMM (Figure 3.3A). In both inoculated and non-inoculated white spruce seedlings, all tailings treatments significantly decreased *Ψshoot* compared with PMM (Figure 3.3B). For both jack pine and white spruce, compared with the non-inoculated seedlings, *L. bicolor* inoculation did not significantly impact *Ψshoot* in seedlings grown in the same substrate (Figure 3.3B).

3.3.4 Dry weights

In both non-inoculated and inoculated jack pine and white spruce, the total dry weights of seedlings grown in tailings amendments were significantly lower compared with those grown in PMM, but significantly higher than the seedlings grown in pure tailings (Figure 3.4A, C). In white spruce inoculated with *L. bicolor*, the total dry weights of seedlings grown in esNST were significantly higher than the non-inoculated seedlings (Figure 3.4C).

In non-inoculated jack pine, the shoot-to-root dry weight ratios of seedlings growing in PMM were significantly lower compared with those grown in tailings containing substrates (Figure 3.4B).

For both jack pine and white spruce grown in the same substrate, *L. bicolor* inoculation did not significantly impact the shoot to root dry weight ratios (Figure 3.4B, D). For white spruce inoculated with *L. bicolor*, the shoot to root dry weight ratios of seedlings grown in PMM and PMM/eNST were significantly lower than those grown in eNST (Figure 3.4D).

3.3.5 Leaf chlorophyll concentrations

In both jack pine and white spruce with or without *L. bicolor* inoculation, all tailings treatments significantly reduced the total leaf chlorophyll concentrations in seedlings compared with the seedlings grown in PMM, with the lowest total chlorophyll concentrations of jack pine observed in plants treated with pure tailings (Figure 3.5A, C). However, for both species of plants grown in the same substrates, the effect of *L. bicolor* inoculation on total leaf chlorophyll concentrations were non-significant (Figure 3.5A, C).

The non-inoculated jack pine and white spruce, seedlings grown in PMM had significantly higher chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios compared with the seedlings grown in tailings containing substrates (Figure 3.5B, D). In jack pine seedlings treated with PMM/esNST, inoculation with *L. bicolor* significantly increased the Chl a to Chl b ratios compared with the non-inoculated plants (Figure 3.5B). For white spruce, *L. bicolor* inoculation had no significant effect on Chl a to Chl b ratios in seedlings grown in the same substrates (Figure 3.5D).

3.3.6 Net photosynthesis (Pn), transpiration (E) rates and chlorophyll fluorescence

The Pn of jack pine seedlings grown in PMM was significantly higher compared with the seedlings grown in tailings containing substrates (Figure 3.6A). In all treatments except eNST, the *L. bicolor* inoculated jack pine seedings had higher Pn compared with non-inoculated counterparts (Figure 3.6A). The E of non-inoculated jack pine seedlings grown in PMM was significantly higher than those grown in PMM/eNST and pure tailings (Figure 3.6B). However, *L. bicolor* inoculated jack pine seedlings grown in PMM had the highest E, and the seedlings grown in pure tailings had the lowest E (Figure 3.6B). Unlike Pn, inoculation with *L. bicolor* did not significantly impact E of jack pine seedlings in any of the studied treatments (Figure 3.6B).

The non-inoculated white spruce seedlings grown in PMM had the highest Pn followed by PMM tailings mixtures and the pure tailings (Figure 3.6D). In the inoculated white spruce, the Pn was the highest in PMM and PMM/esNST treatments, followed by PMM/eNST and pure tailings (Figure 3.6D). *L. bicolor* inoculation significantly increased the Pn only in seedlings grown in tailings mixtures compared with the non-inoculated ones (Figure 3.6D).

In non-inoculated white spruce, E was the highest in seedlings grown in PMM, followed by PMM/eNST, and was the lowest in PMM/esNST and pure tailings treatments (Figure 3.6E). For the inoculated group, the E was the highest in seedlings grown in PMM and PMM/esNST, intermediate in PMM/eNST and esNST, and the lowest in eNST (Figure 3.6E). In the PMM/esNST and esNST treatments, *L. bicolor* inoculation significantly increased the E of white spruce seedlings compared with the non-inoculated plants (Figure 3.6E).

In both jack pine and white spruce, the PSII maximum efficiency (Fv'/Fm') were significantly reduced by pure tailings compared with PMM and tailings amendments (Figure 3.6C, F). In white spruce grown in eNST, the seedlings inoculated with the *L. bicolor* had significantly higher Fv'/Fm' compared with the non-inoculated ones (Figure 3.6F).

3.3.7 Leaf elemental concentrations

In the inoculated and non-inoculated jack pine, the leaf N concentrations were significantly lower in substrates containing tailings compared with PMM. Non-inoculated jack pine seedlings grown in PMM/eNST, PMM/esNST, and eNST had significantly higher leaf Mn concentrations than those grown in PMM, whereas plants grown in PMM/eNST and pure tailings had significantly lower leaf K and Ca concentrations compared with those grown in PMM (Table 3.2). Leaf B, Fe and Na concentrations measured in seedlings grown in pure tailings were significantly higher than those grown in PMM (Table 3.2). For seedlings grown in PMM, PMM/esNST, eNST, and esNST, the *L. bicolor* inoculation significantly increased the leaf N concentration compared with the non-inoculated plants. Seedlings inoculated with *L. bicolor* grown in PMM/eNST had significantly higher leaf Ca and K concentrations than the non-inoculated seedlings. In PMM/esNST, inoculated jack pine seedlings had significantly lower Mg, Mn and Fe concentrations compared with non-inoculated plants. In eNST, inoculated jack pine seedlings had lower B and Mn concentrations compared with non-inoculated ones (Table 3.2).

Non-inoculated and inoculated white spruce seedlings grown in PMM had significantly higher N concentrations compared with the seedlings treated with tailings containing substrates, while the

L. bicolor inoculation did not significantly impact leaf N concentrations. (Table 3.3). For non-inoculated white spruce seedlings, the Ca concentrations were significantly lower when grown in tailings containing substrates than in PMM (Table 3.3). In addition, non-inoculated seedlings grown in pure tailings had significantly higher leaf B and Na concentrations than those grown in PMM (Table 3.3). *L. bicolor* inoculated white spruce seedlings had lower B and P concentrations than the non-inoculated plants (Table 3.3).

In non-inoculated jack pine and white spruce, both eNST and esNST treatments significantly reduced K/Na ratios, with seedlings grown in PMM having the highest K/Na ratios (Figure 3.7B, D). For white spruce grown in PMM/eNST, the inoculation of *L. bicolor* significantly increased K/Na ratios compared with the non-inoculated seedlings (Figure 3.7D).

3.4 Discussion

Results of the present study showed that both eNST and esNST significantly impaired the growth of jack pine and white spruce seedlings by altering nutrient composition and impairing water balance. Both tailings negatively impacted the net photosynthesis, transpiration, chlorophyll concentrations, and mineral nutrition in both plant species, while the presence of PMM in tailings significantly benefited the growth of plants. Compared with white spruce, jack pine was more sensitive to oil sands tailings and suffered more severe growth reduction compared with white spruce. Overall, eNST and esNST had similar effects on the growth and physiological responses in both tree species. However, the inoculation of *L. bicolor* effectively increased the tolerance of plants to oil sands tailings and improved plant growth and physiological performance in seedlings under the eNST and esNST treatments.

The successful establishment of tree species is one of the crucial criteria for the evaluation of reclamation success at Alberta's oil sands mines (Alberta-Environment 2010). However, the revegetation of conifers may be more challenging due to their relatively slow growth rate compared with deciduous tree species. Previous studies reported that jack pine is one of the most sensitive species to oil sands tailings (Zhang et al. 2015, Olivier et al. 2019, Zhang et al. 2023). However, white spruce exhibited a higher tolerance of oil sands tailings compared with the other studied boreal trees (Zhang et al. 2013, 2023). In the present study, the dry weight of jack pine grown in pure tailings was about 40% of the mean value measured in seedlings grown in PMM, while in white spruce, the mean dry weight was approximately 60% of that in the seedlings grown in PMM These results are consistent with the previous studies, which showed that jack pine was more sensitive to oil sands tailings compared with white spruce (Zhang et al. 2013, 2023). Since jack pine is a pioneer species in the boreal ecosystem succession (Rudolph and Laidly 1990), the successful establishment of jack pine in oil sands reclamation sites plays an important role in the functioning of the reclaimed ecosystem.

In this study, the responses of seedlings to tailings were measured after a relatively short treatment duration and may not be representative of longer-term responses to tailings. The water holding capacity of pure tailings was significantly lower than that of the PMM containing substrates and upon drying, the tailings formed a hard, cement-like, structure that was impermeable to water. The plants of both species growing in eNST and esNST suffered from the water deficit stress as evidenced by the decreased shoot water potential and transpiration rates. The sodium

concentrations in both eNST (65 mg kg^{-1}) and esNST (86 mg kg^{-1}) were much lower compared with those reported for NST (175 mg kg^{-1}) and other oil sands tailings (156 – 1385 mg kg^{-1}) (Renault et al. 2000, Degenhardt et al. 2023). This demonstrates that the addition of polymer flocculant in the chemical treatment of oil sands tailings effectively lowered the concentration of sodium. The pH values of both tailings were about 7, also significantly lower than the values reported for NST (pH 8.8) (Zhang et al. 2020), but still higher than the typical soil pH of 5 – 6 in the boreal forest near the oil sands mining areas (Howat 2000). The reported soil pH tolerance range of jack pine is 4.5 – 6.5 and it ranges from 4.7 to 7.0 for white spruce (Limited 1989, Abrahamson 2015). In the present study, the growth of jack pine was also impaired more than white spruce, which may be partly due to its higher sensitivity to high soil pH.

In this study, the EC and sodium concentration of the pure solid tailings were significantly lower than those of the tailings release water, which is consistent with the previous study (Zhang et al. 2020). This discrepancy might be due to the fact that the tailings samples used in the present study were obtained from already consolidated tailings in previously opened containers rather than fresh fluid tailings. Given the high solubility and mobility of Na in the aqueous phase, it tends to settle at the bottom of the tailings as water evaporates and the pore water level decreases.

Interestingly, the sodium concentration in PMM (306 mg kg^{-1}) was much higher than that of tailings and also higher than the PMM used in the previous study (47 mg kg^{-1}) (Zhang et al. 2020). PMM is produced by mechanically mixing with excavators the peat from lowlands and the dry mineral soil at the oil sands mining areas. Since the vicinity of oil sands mining sites contains

naturally occurring saline wetlands (Purdy et al. 2005), the source of peat might significantly affect the sodium concentration of PMM. However, since the EC of PMM was about 1.5 mS cm^{-1}, it is still within the range of acceptable salinity (0- 2 mS cm^{-1}) for the oil sands reclamation sites according to the guidelines (Howat 2000). Therefore, salinity of the growth media did not appear to be a major factor in this study. On the other hand, the leaf Na concentrations in both jack pine and white spruce grown in tailings-addition treatments were ten to hundreds of times higher than in plants grown in PMM, and elevated Na levels could lead to nutrient imbalance (Zhang et al. 2020). Na toxicity can also severely impair most of the major physiological processes of plants (Deinlein et al. 2014). In this study, the high Na concentrations in seedlings grown in eNST and esNST is likely to be a major cause of the reductions in net photosynthesis, transpiration and chlorophyll concentrations. Similarly to Na, leaf B concentration increased in plants growing in substrates containing tailings, although the magnitude of the increase was not as high as for Na. Elevated foliar B concentration is commonly reported for plants growing in saline soils, including those affected by oil sands tailings (Renault et al. 1998, Polanco et al. 2008). High concentrations of B negatively affect plants by impairing chlorophyll synthesis, reducing photosynthesis, retarding cell wall formation (Nable et al. 1997), and affecting root growth (Apostol et al. 2002).

In both jack pine and white spruce with or without fungal inoculation, in the eNST and esNST treatments, the leaf Na concentrations were markedly higher compared with seedlings grown in PMM, which was correlated with the reductions of leaf Ca and K concentrations. K and Na have the same chemical valence and compete for the uptake site in root cells (Zhu 2001). It has been

widely reported that treatments with Ca can significantly ameliorate salinity stress in plants (Hadi and Karimi 2012) by enhancing plasma membrane integrity and preventing an influx of Na into the cytosol (Cramer 2002). The excessively high K/Na ratios in plants affected by tailings indicate an ionic imbalance within the plant tissues, which may also contribute to the impairment of physiological processes. The poor aeration and low water holding capacity of the tailings may also exacerbate Na accumulation in the leaves. Saturation of the growing substrates with water can also lead to waterlogging, which reduces oxygen availability to plant roots (Fleurial et al. 2022). Previous studies have shown that under hypoxic conditions, salt stress in jack pine and black spruce is intensified in eNST treatments (Han 2023), and hypoxia enhanced Na uptake in trembling aspen subjected to NST treatments (Fleurial et al. 2022). Additionally, very low levels of beneficial soil microorganisms in the tailings may also contribute to the excessive accumulation of Na in seedlings (Fadaei 2019).

Leaf N concentration of 1.5% is regarded as the critical value for jack pine, while for white spruce this number is 1.25% (Ballard and Carter 1986). In the present study, non-inoculated jack pine treated with eNST and esNST suffered from N deficiency, while the leaf N concentrations of inoculated jack pine grown in pure tailings were at the critical value. This demonstrates that *L. bicolor* effectively improved N uptake by jack pine in these growth substrates. However, the effect of *L. bicolor* inoculation on white spruce seedlings to tailings treatment was not significant, since all the leaf N concentrations in white spruce seedlings in the eNST and esNST treatments were

lower than 1.25%. Further studies should explore the mechanisms that could explain the differences in N nutrition between the two tree species in response to mycorrhization.

The NA concentration in esNST extraction solution was approximately 27 mg/L, which was about half of the concentration present in the eNST release water. A previous study (Leishman et al. 2013) showed that a concentration of 10 mg/L NAs had an inhibitory effect on the germination of Arabidopsis seeds and also impaired the growth of seedling roots. NAs were also reported to affect plant water relations and gas exchange (Crowe et al. 2001, Kamaluddin and Zwiazek 2002, Apostol et al. 2004), probably due to the disruption of cell membranes (Quagraine et al. 2005, Frank 2008). At the oil sands reclamation sites, the thickness of capping soil may be lower than the root length of some tree species. More attention should focus on the potential phytotoxic impacts of the NAs on plants when the roots penetrate the tailings layer.

It appeared that *L. bicolor* had greater benefits for both plant species in the esNST treatment, since the net photosynthesis and transpiration rates of both species were higher in inoculated seedlings when grown in esNST, while in the eNST treatment, the differences were not significant. In the present study, *L. bicolor* inoculation lowered the B concentrations in jack pine seedlings treated with eNST, and both B and Na concentrations in white spruce treated with both eNST and esNST. However, the exact mechanisms of how *L. bicolor* symbiosis improved the water uptake and reduced the Na and B uptake will need to be investigated in future studies.

In conclusion, the results of this study showed that both eNST and esNST significantly affected the growth and physiological processes in jack pine and white spruce, largely due to excessive

accumulation of Na and the effects of tailings on mineral nutrition, while *L. bicolor* inoculation benefited the growth of seedlings exposed to tailings. As expected, the presence of PMM in the growth substrate with tailings significantly improved plant responses. Balanced nutrient supply and mycorrhizal associations should be viewed as important factors to consider for successful revegetation of sites impacted by eNST and esNST.

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3.6 Tables

Table 3.1. The pH, electrical conductivity (EC, mS cm-1), elemental and naphthenic acids concentrations (mg kg-1), and soil water holding capacity (WHC, %) of the treatment substrates and tailings release water at the beginning of the experiment. Means ± SE (n = 4) are shown. Different letters following the values in the same column indicate significant differences ($p \leq 0.05$) between treatments as determined by Holm test.

	pH	EC	Na	Mg	P	K	Ca	Fe	Mn	Zn	NAs	WHC
	5.1	1.6	306.2	3220.5	372.5	3057.5	7750.8	12984.0	193.7	41.8	NM	100
PMM	±0.004	±0.025	±4.3	±33.1	±11.3	±106.8	±347.6	±223.7	±22.5	±1.5		
	a	c	b	d	c	d	d	c	c	c		
	5.4	0.9	350.5	1596.0	136.3	2408.3	1802.8	7484.5	115.7	19.8	NM	48
PMM/eNST	±0.009	±0.022	±19.1	±91.1	±7.0	±216.9	±99.6	±403.4	±16.8	±0.9		
	b	b	b	c	b	c	b	b	b	b		
	6.0	0.8	325.0	1320.8	150.2	1413.8	3016.5	6513.5	97.8	18.9	NM	44
PMM/esNST	±0.035	±0.006	±15.3	±75.2	±8.1	±93.6	±144.6	±694.3	±14.9	±1.0		
	c	b	b	b	b	b	c	b	b	b		
	7.1	0.4	65.0	0.3	0.0048	2.5	0.8	0.2	0.0038	0.0045	NM	NM
eNST	±0.018	±0.027	±2.7	±0.02	±0.0018	±0.1	±0.03	±0.04	±0.001	±0.002		
	d	a	a	a	a	a	a	a	a	a		
	7.1	0.5	86.6	0.4	0.006	3.3	1.1	0.1	0.007	0.0085	27	NM
esNST	±0.05	±0.076	±13.7	±0.04	±0.002	±0.4	±0.14	±0.02	±0.002	±0.004		
	d	a	a	a	a	a	a	a	a	a		
NST water	9.0	5.5	1096	NM	NM	NM	NM	NM	NM	NM	133	NM
eNST water	9.1	4.5	1032	NM	NM	NM	NM	NM	NM	NM	65	NM
esNST water	9.0	4.0	NM	NM	NM	NM	NM	NM	NM	NM	57	NM

Table 3.2. Elemental concentrations (mg kg-1, % for N) in needles of jack pine (*Pinus banksiana* Lamb.) seedlings grown for eight weeks in treated with PMM, PMM/eNST, PMM/esNST, eNST, esNST and inoculated with *Laccaria bicolor* or non-inoculated. Means ± SE (n = 6) are shown. Different letters following the values in the same column indicate significant differences ($p \leq 0.05$) between treatments as determined by the Holm test.

	B	Ca	Fe	K	Mg	Mn	N	Na	P	Zn
PMM	42.4±0.7	6773.9±231.8	42.9±1.4	4169.6±395.3	2140.0±98.0	97.8±9.3	1.8±0.1	44.0±8.3	1316.2±51.4	49.2±1.9
	ab	d	ab	c	bc	ab	d	a	bc	cd
L. bicolor	39.5±3.0	6280.5±336.3	33.9±2.2	3396.4±124.2	1864.6±25.3	80.4±6.5	2.2±0.1	50.1±9.3	1088.5±33.3	31.7±2.2
	a	d	a	bc	ab	a	e	a	abc	a
PMM/eNST	56.5±2.3	3934.2±358.6	53.5±7.5	2298.5±205.3	2093.1±112.0	171.3±13.8	1.0±0.1	596.1±120.7	986.5±63.8	43.9±4.2
	ab	ab	abc	ab	bc	cde	a	a	a	bcd
PMM/eNST	66.5±5.1	6009.4±259.5	43.9±3.9	4025.0±594.0	2432.9±112.6	136.6±14.9	1.2±0.03	1319.8±368.5	1066.8±23.8	52.7±2.6
+ *L. bicolor*	b	cd	ab	c	c	abcd	ab	a	ab	d
PMM/esNST	65.0±3.6	5399.2±389.1	73.9±6.9	3792.8±265.6	2474.2±87.3	195.5±12.0	0.9±0.1	859.8±523.8	1172.2±71.4	41.9±2.1
	b	bcd	cd	bc	c	e	a	a	abc	abcd
PMM/esNST	51.9±2.5	4485.0±544.3	39.5±2.8	4527.8±186.9	1892.9±103.3	120.7±10.5	1.4±0.1	799.2±345.7	1090.0±32.0	38.4±2.7
+ *L. bicolor*	ab	abc	ab	c	ab	abc	bc	a	abc	abc
eNST	155.1±5.5	3090.1±258.8	84.6±6.2	834.6±97.8	1606.1±107.8	180.9±16.3	1.0±0.1	10846.4±881.4	1364.9±31.9	37.8±1.7
	d	a	d	a	a	de	a	b	c	abc
eNST	128.9±6.5	3475.8±225.6	60.1±5.3	957.1±82.1	1479.9±98.8	112.2±15.4	1.5±0.1	10663.7±935.7	1345.6±106.5	35.0±3.1
+ *L. bicolor*	c	a	bcd	a	a	abc	cd	b	bc	ab
esNST	121.5±7.6	3295.0±236.0	76.3±6.3	1639.7±412.5	1613.6±62.6	143.3±8.0	0.9±0.04	10598.3±779.7	1300.5±56.4	35.1±1.4
	c	a	cd	a	a	bcde	a	b	bc	ab
esNST	131.8±10.8	3759.5±473.3	56.9±8.1	1343.8±275.8	1798.1±112.4	121.2±13.3	1.5±0.1	8512.5±829.0	1219.2±63.8	38.5±2.3
+ *L. bicolor*	cd	a	abc	a	ab	abc	cd	b	abc	abc

Table 3.3. Elemental concentrations (mg kg-1, % for N) in needles of white spruce [*Picea glauca* (Moench) Voss] seedlings grown for eight weeks treated with PMM, PMM/eNST, PMM/esNST, eNST, esNST and inoculated with *Laccaria bicolor* or non-inoculated. Means ± SE (n = 6) are shown. Different letters following the values in the same column indicate significant differences ($p \leq 0.05$) between treatments as determined by the Holm test.

	B	Ca	Fe	K	Mg	Mn	N	Na	P	Zn
PMM	18.4±4.4	10296.0±1140.5	56.4±4.5	3571.0±188.6	1651.2±109.7	101.0±5.6	1.79±0.1	44.0±8.3	1441.8±71.8	36.3±3.3
	a	e	a	c	a	a	b	a	a	ab
L. bicolor	17.8±3.2	8734.3±532.9	69.4±7.2	3510.7±204.6	1497.0±62.1	104.6±9.3	1.8±0.1	50.1±9.3	1448.5±90.4	38.4±2.7
	a	de	a	c	a	a	b	a	a	ab
PMM/eNST	26.7±2.1	5043.0±400.5	64.1±8.7	2534.4±212.4	1567.5±169.1	127.8±9.1	0.8±0.1	596.1±120.7	1447.8±24.9	41.5±3.1
	a	abc	a	abc	a	a	a	a	a	ab
PMM/eNST	25.5±3.0	4236.6±71.3	52.7±6.7	3464.6±331.4	1624.7±126.9	94.7±8.4	0.9±0.04	60.6±8.6	1376.0±94.8	47.2±6.8
+ *L. bicolor*	a	ab	a	c	a	a	a	a	a	ab
PMM/esNST	26.3±2.2	6116.0±485.9	60.3±11.5	3127.2±280.4	1483.2±58.8	118.0±5.2	1.0±0.1	459.5±121.5	1543.0±149.0	45.0±2.1
	a	bc	a	bc	a	a	a	a	a	ab
PMM/esNST	28.7±3.7	7179.0±855.1	43.1±6.8	3344.0±407.9	1648.7±145.7	124.6±13.2	0.9±0.1	504.2±85.5	1261.8±110.7	57.4±7.2
+ *L. bicolor*	a	cd	a	bc	a	a	a	a	a	b
eNST	193.8±5.7	3579.8±243.2	83.9±15.4	1424.3±247.9	1405.7±98.5	93.5±6.2	1.0±0.1	12059.3±753.0	2485.7±270.8	43.2±1.8
	d	ab	a	a	a	a	a	d	b	ab
eNST	155.7±10.8	3458.8±379.7	82.5±13.4	1382.6±228.5	1223.5±66.5	87.1±14.3	0.7±0.04	9239.9±952.0	1761.2±179.1	46.0±6.2
+ *L. bicolor*	c	a	a	a	a	a	a	c	a	ab
esNST	161.9±12.7	3430.3±209.4	84.8±4.7	1991.3±155.8	1201.3±62.9	105.2±7.0	1.0±0.1	9154.1±602.2	1808.7±140.5	41.2±2.5
	c	a	a	ab	a	a	a	c	ab	ab
esNST	122.3±8.7	2567.3±233.7	48.6±2.6	2502.8±653.8	1228.8±115.6	94.3±16.3	1.0±0.1	5306.1±1309.2	1977.7±163.8	34.1±4.2
+ *L. bicolor*	b	a	a	abc	a	a	a	b	ab	a

3.7 Figures

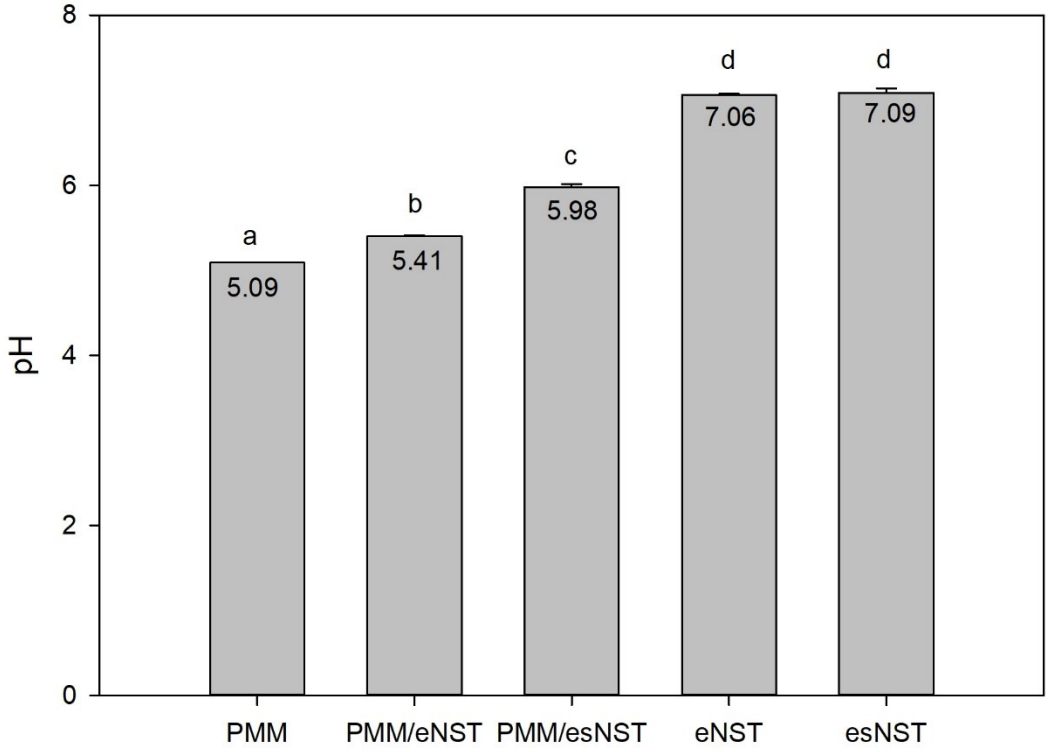

Figure 3.1. Initial soil pH of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 4). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

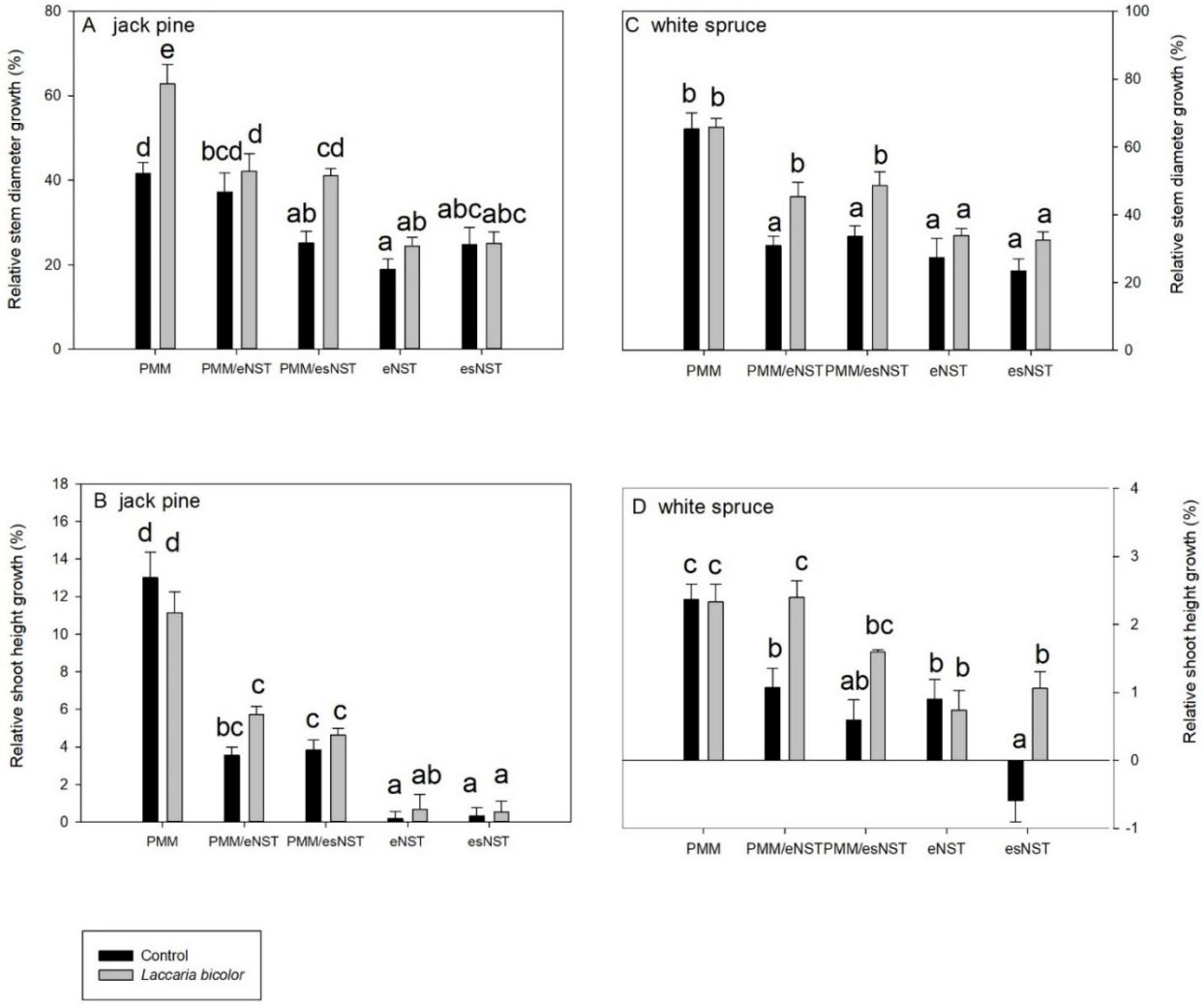

Figure 3.2. Relative stem diameter growth (RSDG) and relative shoot height growth (RSHG) in jack pine (A, B) and white spruce (C, D) seedlings with or without *L. bicolor* inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 10). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

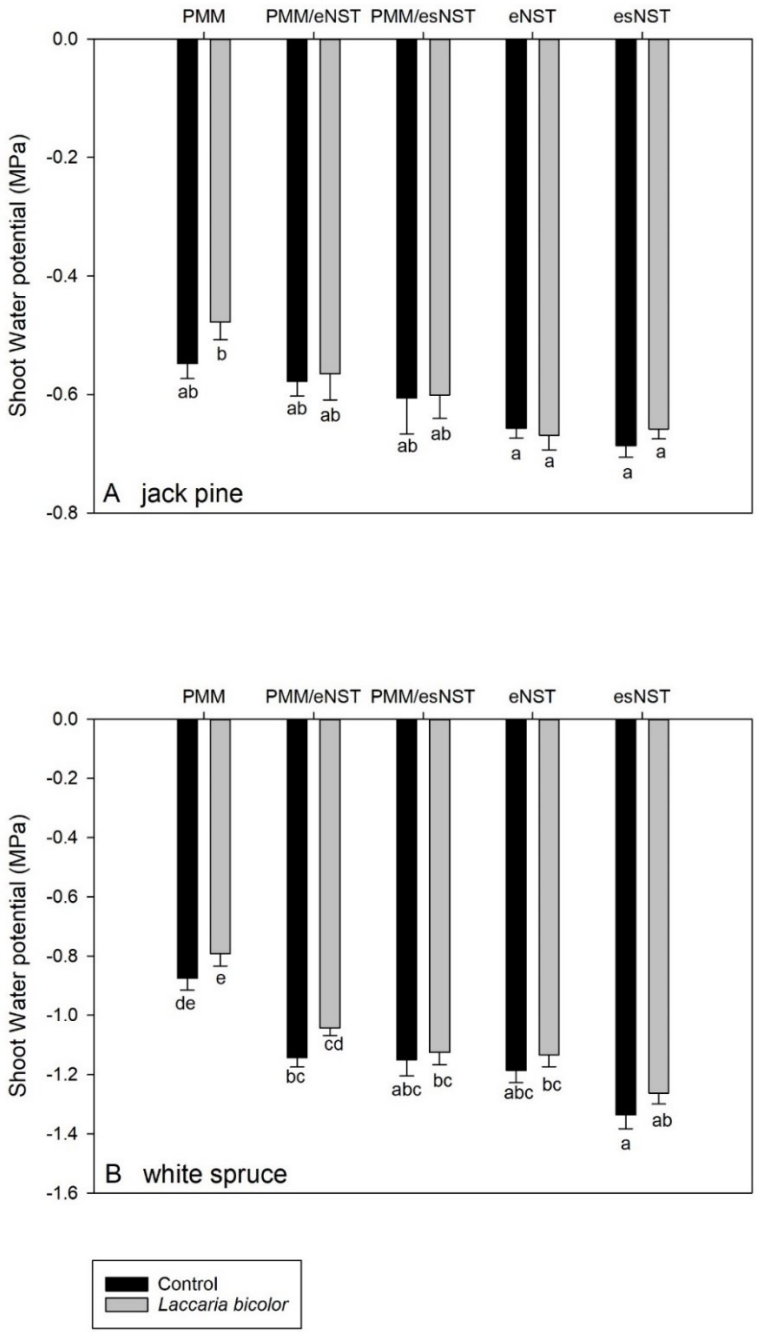

Figure 3.3. Shoot water potentials in jack pine (A) and white spruce (B) seedlings with or without *L. bicolor* inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 8). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

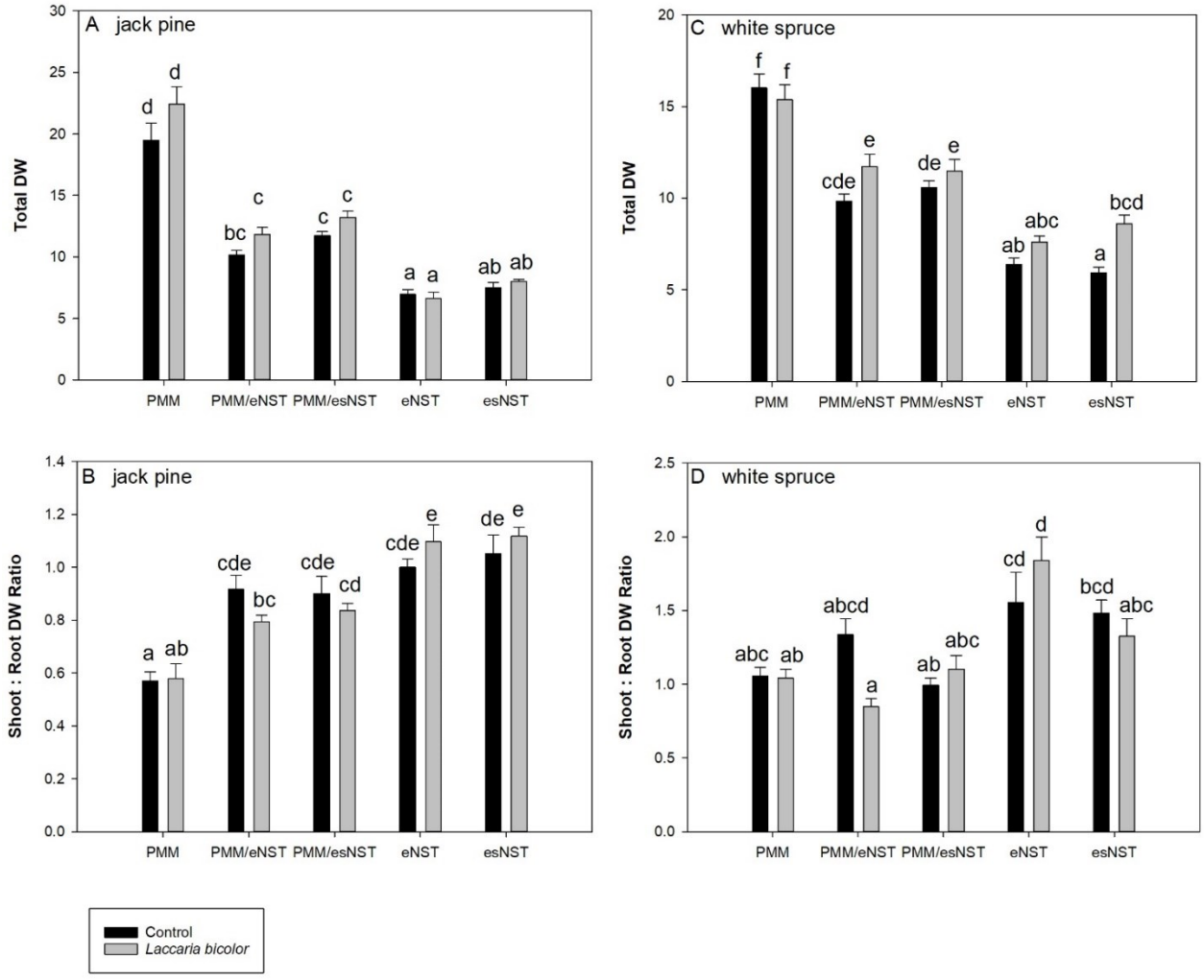

Figure 3.4. Total dry weight (DW) and shoot to root dry weight ratio in jack pine (A, B) and white spruce (C, D) seedlings with or without *L. bicolor* inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 10). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

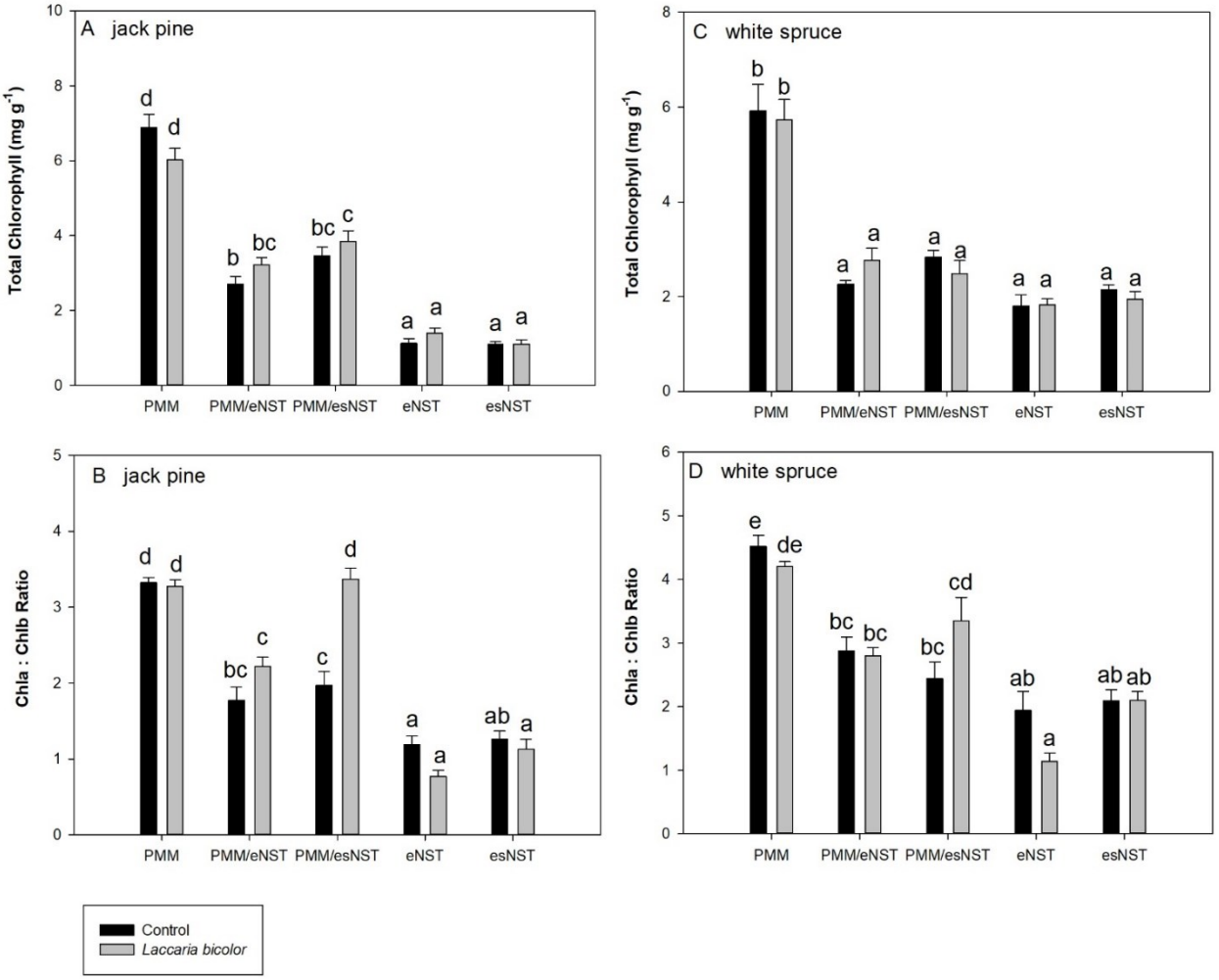

Figure 3.5. Total leaf chlorophyll concentrations and chlorophyll a (Chl a) to chlorophyll b (Chl b) ratios in jack pine (A, B) and white spruce (C, D) seedlings with or without *L. bicolor* inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

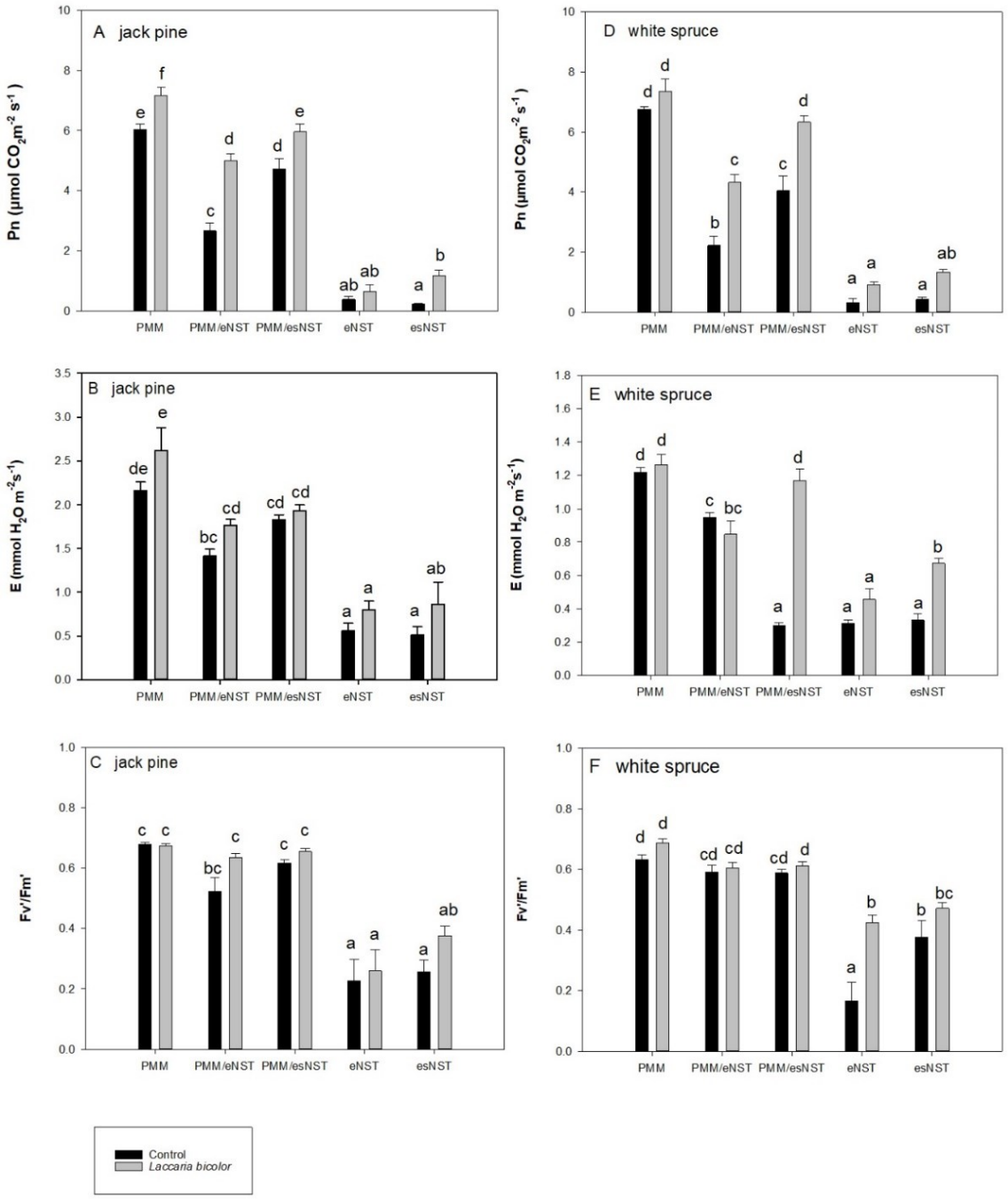

Figure 3.6. Net photosynthesis (Pn), transpiration rate (E) and photosystem II (PS II) maximum efficiency (Fv'/Fm') in jack pine (A, B, C) and white spruce (D, E, F) seedlings with or without *L. bicolor* inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences (α = 0.05) between treatments determined by the Holm test.

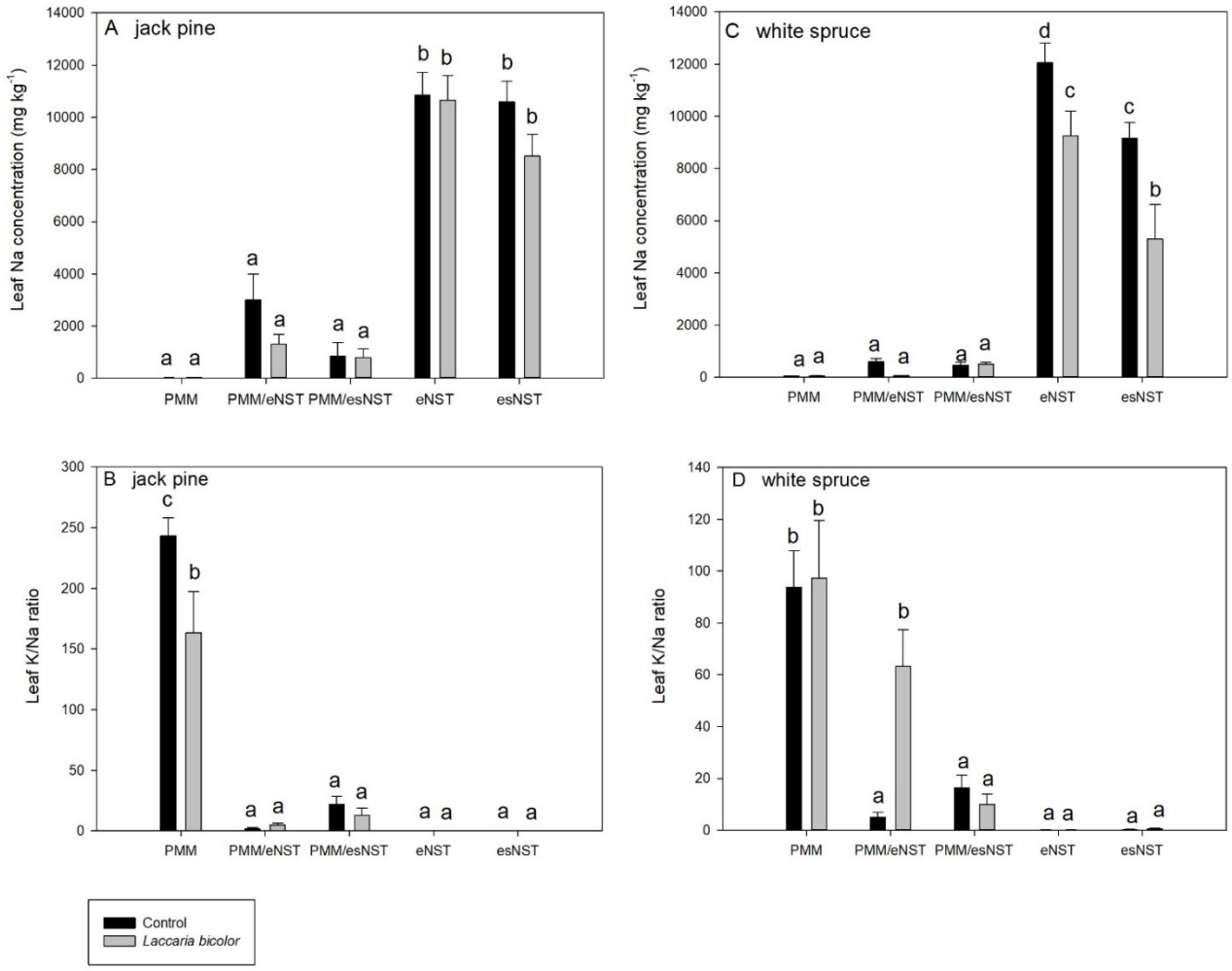

Figure 3.7. Leaf Na concentration (mg kg-1) and K/Na ratio in jack pine (A, B) and white spruce (C, D) seedlings with or without L. bicolor inoculation. Plants were grown for eight weeks in the substrate of PMM, PMM/eNST, PMM/esNST, eNST and esNST (Means ± SE, n = 6). Different letters above the bars indicate significant differences ($\alpha = 0.05$) between treatments determined by the Holm test.

Chapter 4 General Discussion and Conclusions

4.1 General discussion

In my first study, I compared the effects of eNST and esNST tailings in PMM on the growth and physiology performance of mycorrhizal and non-mycorrhizal velvetleaf blueberry and Labrador tea seedlings. The study showed that after adding eNST and esNST to PMM, the pH of PMM/eNST (5.4) and PMM/esNST (6.0) were significantly higher compared with PMM (5.1), and exceeded the optimal soil pH range for velvetleaf blueberry (pH 4.0 - 5.0) and Labrador tea (pH 2.9 - 4) (Gucker 2006, Mu et al. 2021). Moreover, the presence of eNST and esNST reduced the nutrient concentrations of the growth substrate and decreased the water holding capacity to 48% (PMM/eNST) and 44% (PMM/esNST) of the measured in PMM. These adverse factors and the presence of phytotoxic compounds, such as NAs, in eNST and esNST led to leaf chlorophyll reductions and reduced growth of velvetleaf blueberry and Labrador tea. These findings are consistent with previous studies showing that oil sands tailings impaired photosynthesis and plant nutrient balance in plants and, in consequence, negatively impacted their growth (Renault et al. 1999, Redfield et al. 2003, Zhang et al. 2023). ERM fungi were previously reported to help host plants enhance stomatal conductance, promote tissue osmotic regulation and balance nutrient uptake, thereby increasing plant tolerance to adverse environmental conditions and promoting the growth of ericaceous plants (Wei et al. 2022). In my study, the inoculation with mycorrhizal fungi *Oidiodendron maius* and *Pezoloma ericae* enhanced growth performance and tolerance to tailings in velvetleaf blueberry and Labrador tea by reducing the excessive accumulation of Na and B and promoting balanced uptake of mineral nutrients. *O. maius* was more effective compared with *P.*

ericae in protecting velvetleaf blueberry plants against the effects of eNST and esNST. In contrast, *P. ericae* inoculation was more effective in alleviating the effects of esNST in Labrador tea. Mu et al. (2021) investigated the effects of four ericoid mycorrhizal fungi and showed that *Pezicula ericae* was the most effective of the studied fungi in promoting growth and improving physiological parameters in velvetleaf blueberry under drought and non-drought conditions. Another study examining the response of ERM velvetleaf blueberry plants to NaCl also found that the degree of protection fungi against plant stress varied between the ERM fungi (Fadaei 2019, Fadaei et al. 2020). This suggests that it is critical to focus on developing optimized ecological restoration programs based on specific interactions between mycorrhizal fungal and plant species and the type of tailings.

In my second study, I examined the impact of mycorrhization with ectomycorrhizal fungus *Laccaria bicolor* on the responses of jack pine and white spruce seedlings to novel oil sands tailings eNST and esNST. The study showed that both eNST and esNST significantly inhibited the growth parameters and physiological processes in jack pine and white spruce. The harmful effects of eNST and esNST on plants can be partly attributed to elevated Na levels, which lead to nutrient imbalance. This is consistent with the impact of traditional non-segregating tailings (NST), where NST treatment resulted in excessive Na accumulation in the leaves of white spruce, with concentrations exceeding those found in PMM treatments by over a hundredfold (Zhang et al. 2020). Previous studies demonstrated that NST severely reduced the growth of jack pine, while the effects on white spruce were relatively weak (Zhang et al. 2023). In my study, I also found that

jack pine was more sensitive to oil sands tailings compared with white spruce. The higher sensitivity of jack pine to tailings could be partly attributed to the imbalanced uptake of mineral elements and a higher sensitivity of jack pine seedlings to higher soil pH. Previous studies pointed out that PMM improved nutrient cycling and microbial activity in reclaimed soil (Rowland et al. 2009), while PMM mixed with NST improved the growth and physiological responses of paper birch, white spruce, and green alder seedlings (Zhang et al. 2020). My study also showed that the presence of PMM significantly improved the physical characteristics of the growth substrates, enhanced the water holding capacity, and increased organic matter content, which benefited seedling responses to tailings. The addition of PMM significantly increased the concentrations of leaf K and Ca in jack pine and white spruce, while the Na concentration was three- to ten-fold lower than in pure eNST and esNST tailings. Furthermore, inoculation with *L. bicolor* alleviated the negative impact of tailings on seedlings, significantly reduced leaf B concentration in jack pine seedlings treated with eNST, and leaf B and Na concentrations in white spruce seedlings treated with eNST and esNST. It also significantly increased RSDG and Pn in jack pine and white spruce seedlings treated with PMM/esNST. In this study, the primary factors leading to the reduction in growth and physiological parameters of jack pine and white spruce were attributed to Na toxicity and the effects of tailings on mineral nutrition, while inoculation with *L. bicolor* and PMM showed promise, helping the plants with balanced uptake of mineral nutrients.

4.2 General conclusion

My first study demonstrated that both eNST and esNST presented significant challenges to plant growth and physiological processes by affecting soil physical structure, chemical properties, and nutrient availability. These factors resulted in reduced biomass and impaired physiological functions in velvetleaf blueberry and Labrador tea seedlings. However, the application of mycorrhizal fungi, *P. ericae* and *O. maius*, may offer a helpful strategy for ameliorating these negative effects by facilitating nutrient uptake and mitigating the uptake and accumulation of potentially toxic compounds from the tailings. The study also showed that different species of ericoid mycorrhizal fungi vary in their effectiveness of alleviating the effects of tailings depending on the ericaceous plant species and the growth substrate. Therefore, it is essential to match ERM fungi with specific ericaceous plant species and reclamation site conditions.

My second study demonstrated the detrimental effects of eNST and esNST on jack pine and white spruce seedlings and emphasized the importance of addressing nutritional factors and Na toxicity in reclamation strategies. As expected, the addition of PMM significantly mitigated these effects, highlighting its potential as an effective amendment in reclamation sites. Additionally, the symbiotic relationship with *L. bicolor* emerged as an effective approach to enhance seedling tolerance of eNST and esNST tailings.

4.3 Suggestions for oil sands reclamation and future research

The findings of my studies demonstrate that the addition of PMM and the inoculation with mycorrhizal fungi alleviate the adverse effects of eNST and esNST on plant growth and

physiological processes. Balanced nutrient supply, optimal soil pH, and effective mycorrhizal symbiosis are crucial factors to be considered for the revegetation of oil sands reclamation sites. Mycorrhizal fungi have also shown the ability to enhance plant resilience and promote growth under tailings-induced stress, highlighting their potential role in ecological restoration efforts. In my first study, the growth and physiological processes of velvetleaf blueberry inoculated with *O. maius* in PMM/eNST treatment and Labrador tea inoculated with *P. ericae* in PMM/esNST treatment were significantly improved. In my second study, white spruce showed greater tolerance than jack pine under tailings stress conditions, while *L. bicolor* showed greater benefits to both jack pine and white spruce in esNST treatment. Future research should focus on developing optimized ecological restoration protocols based on specific interactions between mycorrhizal species, tailings types, and host plant species. The short duration of both studies limits the understanding of long-term cumulative effects and environmental stress responses. Future research should explore the long-term impacts of tailings on the establishment of reclamation plants and the mechanisms by which mycorrhizal fungi enhance plant resistance. My studies were conducted under controlled environment conditions, which may not fully reflect reclamation site conditions. Further research should be carried out to evaluate plant performance under field conditions. Given the differences in chemical properties of tailings sediments and tailings release water, further studies should also explore the specific nutrient dynamics and physical properties of tailings to develop strategies that can help mitigate these adverse effects and improve plant growth in these substrates. Additionally, understanding the impact of naphthenic acids on the physiology of

mycorrhizal plants would contribute to developing more effective plant reclamation strategies, thereby improving tailings management and ecological restoration effectiveness.

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