Selected Aspects of Plant Responses to Elevated pH, Salinity and Drought: Implications for Oil Sands Revegetation

by

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

Doctor of Philosophy

in

Forest Biology and Management

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University of Alberta

Abstract

Elevated salinity, high pH, and drought are among the most challenging environmental factors affecting the growth and survival of plants in oil sands reclamation areas. In my thesis research, I focused on the selected aspects of these environmental stresses to better understand their potential impacts on plants. Three research studies were carried out as part of this thesis project.

In Study 1, I examined the processes of salt and water redistribution as well as physiological responses of trembling aspen (*Populus tremuloides*) plants subjected to heterogeneous distribution of NaCl in soil and soil water gradients. A vertical split-root growth setup was used to study the effects of soil salinity and water content gradients on plants under controlled-environment conditions. Trembling aspen seedlings were first subjected to drought treatment and then, 30 and 60 mM NaCl was applied to the lower part of the root system. I found that plant roots could transport water from deeper, moister, parts of the soil into the upper, drier, areas through the process known as the hydraulic lift. The water released to the dry soil through hydraulic lift increased the soil volumelic water content by about 13%. The salinity and drought caused decreases in net photosynthetic rates, transpiration rates, chlorophyll concentrations, and stem water potentials. Moreover, Na^+ accumulated in the lower part of the root system and no detectable Na^+ was released with hydraulically lifted water to the soil. It was concluded

that the trembling aspen could supply water from the deeper soil layers with elevated salinity without contributing to salt redistribition in the soil. This process could also benefit neighbouring shallow-rooted plants in reclamation areas during the periods of drought.

In Study 2, I focused on the responses of sweet yellow clover (*Agropyrum trachycaulum*) and slender wheatgrass (*Melilotus officinalis*) to NaCl when their roots were exposed to high pH in the lower soil layer. A vertical split-root setup was used to induce the salinity and pH stress in the lower soil layer in a controlled-environment study. The growth of slender wheatgrass was sharply inhibited when only 10% of the root system was exposed to NaCl and (or) high pH, but it was relatively less affected in yellow sweet clover. The NaCl and high pH treatments triggered a series of different physiological responses and lowered leaf photosynthetic rates, decreased transpiration rates, and reduced stem water potentials in the two studied plants, which contributed to growth reductions when only a relatively small part of the root system was exposed to NaCl and (or) high pH. Compared with high pH, NaCl was the main factor responsible for the decreased root distribution in the lower soil in slender wheatgrass. However, root growth in sweet yellow clover was stimulated by high (7.7-8.3) soil pH. The combined NaCl and pH were more harmful to wheatgrass and clover plants than the NaCl and pH stresses alone. More importantly, in wheatgrass, salt stress inhibited root distribution in

the deeper soil profile with elevated NaCl level, while, in sweet yellow clover, high pH stimulated root distribution in the deeper high pH soil layer.

In Study 3, I investigated the diversity of ericoid mycorrhizal (ERM) fungi in the roots of velvetleaf blueberry (*Vaccinium myrtilloides*) seedlings from the northeastern Alberta, and their role in drought resistance of upland and lowland populations. The ERM fungi enhanced the growth and survival of plants subjected to drought stress and increased net photosynthetic rates, transpiration rates and shoot water potentials. Of the examined ericoid mycorrhizal fungi, *Pezicula ericae* was the most effective in enhancing growth and physiological parameters of plants. I concluded that inoculation of velvetleaf blueberries plants with *Pezicula ericae* prior to planting could be an effective method to improve the establishment and growth of velvetleaf blueberry in oil sands reclamation areas and other sites exposed to harsh environmental conditions.

Preface

This thesis presents three research studies (Chapters 2, 3 and 4) that are intended for publication in refereed journals. All thesis work was carried out by myself, including the experiment implementation, data collection and analyses, and writing. The isolation and identification of ERM fungi in ericaceous plants root in Chapter 4 was part of the larger collective team effort involving the University of Alberta researchers that also included Dr. Alejandra Equiza, Dr. Beatriz Sanchez Romera, Dr. Maryamsadat Vaziriyeganeh, and Sepideh Fadaei. My supervisor, Dr. Janusz Zwiazek, was involved in concept formation, research supervision throughout my PhD program, and editing the thesis.

Acknowledgments

I would like to sincerely thank my supervisor Dr. Janusz J. Zwiazek, first of all, for giving me the opportunity of my PhD study, and for his patient guidance and consistent assistance in my research and paper writing. I also thank Dr. Scott X. Chang and Dr. Stephen Strelkov for acting as members of my supervisory committee.

My gratitude to the people from the Tree Physiology lab for their support throughout my research, including but not limited to Dr. Alejandra Equiza, Dr. Wenqing Zhang, Dr. Frank (Xiangfeng) Tan, Dr. Feng Xu, Dr. Hao Xu, Dr. Shanjida Khan, Dr. Seong Hee Lee, Dr. Nathan Lauer, Dr. Beatriz Sanchez Romera, Samantha Olivier, Sepideh Fadaei, Mengmeng Liu and Maryam Vaziri. My thanks also to all my friends who helped me with my study, research and daily life in Canada.

I especially thank my parents for their understanding and support of my research all the time. This accomplishment would not have been possible without them.

I would like to thank Canadian Natural Resources Ltd., Imperial Oil Ltd. And the Natural Sciences and Engineering Research Council of Canada (NSERC) for providing research funding.

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List of Abbreviations

SSOB	saline-sodic overburden materials
EC	electric conductivity
ERM	ericoid mycorrhizal fungi
TDR	time domain reflectometry
HL	hydraulic lift
ROS	reactive oxygen species
AM	arbuscular mycorrhiza
ECM	ectomycorrhizal
ORM	orchid mycorrhizas
DMSO	dimethyl sulfoxide
E	transpiration rate
Pn	net photosynthesis rate
ANOVA	analysis of variance
Ψ_L	leaf water potentials
ψ_w	shoot water potential
MN	modified Melin-Norkans medium
PDA	potato dextrose agar medium
ITS	internal transcribed sequence
NCBI	national centre for biotechnology information
V/V	volume/volume
S/R	shoot to root ratios

Chapter 1

Introduction and literature review

1.1 Introduction

According to the Alberta Energy Regulator (2018), the production of oil sands in Alberta, Canada, is expected to increase from 2.8 million barrels per day in 2017 to 3.9 million barrels per day in 2027. The expanding oil sands industry is widely regarded as one of the driving forces for the Canadian economy (Sauchyn et al. 2015). However, the existing and new developments create significant environmental concerns due to their impacts on boreal ecosystems, since most of the bitumen is recovered from sand through open-pit mining and affects large areas (Carrera-Hernandez et al. 2012). Before oil sands mining, all vegetation has to be removed from the future mining sites and the soil and subsoil layers stripped. Site reclamation following mining involves complex site reconstruction. In many reclaimed oil sands sites, elevated salinity persists in the lower parts of the soil due to the presence of saline-sodic overburden materials (SSOB) that are capped with peat and mineral soil layers (Kessler et al. 2010, Lazorko and Van Rees 2012). In addition, if present in the reclaimed sites, oil sands mine tailings may alter soil chemical properties and raise Na^+ levels (Zhang 2015).

The elevated salt levels in reclamation soils are often accompanied by high pH (Allen 2008, Lilles et al. 2012). Both of these soil factors are harmful to the majority of plants and can be further aggravated by other environmental factors, especially drought. Drought stress has been reported to increase in occurrence and intensity and trigger tree dieback and mortality in Canada's boreal forests (Allen et al. 2010, Peng et al. 2011, Michaelian et al. 2011). Therefore, combined salinity stress, high soil pH, and drought stress may pose serious challenges to plants in the oil sands reclamation areas.

Although some of the responses of plants to salinity and drought are similar, many morphological, physiological, and molecular mechanisms may significantly differ between salt- versus drought-stressed plants (Munns 2002, Chaves et al. 2009, Miller et al. 2010). More importantly, the physiological and biochemical responses of plants to the interactions between drought and salinity as well as high pH and salinity are unique and, therefore, cannot be directly extrapolated from the corresponding responses to each stress when applied separately (Shi and Sheng 2005, Mittler 2006).

Plants of many species can transport water from the lower, usually wetter, parts of the soil and release it to the drier soil through their root system. This process is referred to as hydraulic redistribution or hydraulic lift (Richards and Caldwell 1987). Hydraulic lift is believed to be a common and important phenomenon in plants. During water deficit periods, water redistributed through the hydraulic lift to drier soil, usually near the soil surface, is thought to help plants maintain the viability of their fine roots (Bauerle et al. 2008) and buffer shallow-rooted plants against drought stress (Xu et al. 2006). Although, many species of plants have been reported to redistribute water through their root system (Caldwell and Richards 1989, Querejeta et al. 2003), little is known about the hydraulic lift and its ecological significance in plants of the boreal forest. Additionally, the consequences of the observed structural heterogeneity in soil salinity and root distribution patterns to long-term plant productivity as well as potential redistribution of salt by the roots of plants need to be further investigated.

In general, large parts of the root system (~ 80%) are present in the upper 30 cm of the soil profile in both natural areas and oil sands reclamation sites (Purdy et al. 2005, Lilles et al. 2012). It is believed that successful survival of plants in high salinity sites may be related to their ability to distribute roots in the low salinity areas in the soil (Lazorko and Van Rees 2012). To date, little is known about the root responses of different plant species to salinity and high pH and of the rooting patterns that they

develop where increased salt concentrations are present, especially in the deeper soil profile.

Since most oil sands reclamation research has focused on several dominant tree species of the boreal forest, little is known about ecologically and economically important forest understory plants and their responses to challenging environmental conditions of the reclamation sites. Velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) is regarded as a cultural keystone species in northern Alberta (Garibaldi and Straker 2009). Plants of this species play an important role in the daily lives of native people. In natural areas, blueberry plants form symbiotic associations with ericoid mycorrhizal fungi (ERM). Many studies have focused on the diversity of the ERM fungal taxa (Sharples et al. 2000, Bougoure et al 2009) and genetic and functional differences of the ERM (Grelet et al 2008). However, the physiology of velvetleaf blueberry plants has been poorly studied and little is known about the regulation of their water relations and drought resistance processes. Therefore, understanding the role that the ERM associations play in conferring stress resistance to velvetleaf blueberry may be crucial for improving the revegetation success.

My thesis research has been designed to generate knowledge that could improve revegetation efforts of challenging reclamation sites following oil sands mining. Although numerous past studies examined the effects of drought, salinity, and pH, there are many aspects of these environmental stresses that have not received sufficient attention. These aspects include potential redistribution of water and salt by plant roots and the role of mycorrhizal associations in stress resistance of plants of the forest understory. In addition, not enough research effort has focused on understanding complex interactions, which take place when plants are affected by more than one stress factor. I have identified some of these issues to be especially important for plants growing in oil sands reclamation areas and addressed them in my thesis research. This study focused on

the selected tree (*Populus tremuloides*), shrub (*Vaccinium myrtilloides*), and herbaceous (*Agropyrum trachycaulum, Melilotus officinalis*) species, which are commonly used for oil sands reclamation. Three controlled-environment studies were carried out in this thesis. The first study assessed a possible redistribution of NaCl and water by the root system from lower (30-60 cm) soil layers, when the upper layer is subjected to drought. The second study, focused on the growth strategies of roots when NaCl and high pH were present in the lower (30-60 cm) soil profile. The third study, investigated the presence of ericoid (ERM) mycorrhizal associations in the boreal forest of northeastern Alberta, and the role of the different ERM fungi in the responses of velvetleaf blueberry (*Vaccinium myrtilloides*) plants to drought. The main objective of my thesis research was to contribute new knowledge concerning plant responses to the soil factors that can impact successful revegetation of oil sands mining areas. Specific objectives were to:

1) Examine the processes of salt and water redistribution in trembling aspen (*Populus tremuloides*) plants subjected to soil NaCl and water gradients.

2) Examine the growth and physiological responses of sweet yellow clover (*Agropyrum trachycaulum*) and slender wheatgrass (*Melilotus officinalis*) to NaCl when the roots of plants are exposed to high pH in the lower layer of soil.

3) Investigate the diversity of ericoid mycorrhizal (ERM) fungi in plants of the boreal forest in northeastern Alberta, and the role of different ERM fungi in drought resistance of the upland and lowland populations of velvetleaf blueberry (*Vaccinium myrtilloides*).

I examined the following hypotheses:

1) Roots of trembling aspen can redistribute and release water and NaCl to the soil through the process of hydraulic lift.

2) High soil pH in the deeper soil profile can aggravate NaCl stress and affect growth and physiological processes more compared to individual NaCl or high pH

treatment.

3) The ERM fungi can significantly improve the growth of velvetleaf blueberry under both drought and non-drought conditions and the effectiveness of ERM associations on plant drought resistance varies between the different ERM fungi.

4) ERM associations improve drought resistance of velvetleaf blueberry plants and their contribution to stress resistant is more significant for the upland, compared with lowland, population.

1.2 Thesis structure

1.2.1 Chapter 1 Introduction and literature review

This chapter provides an introduction to the thesis and an overview of oil sands mining and reclamation, the processes of hydraulic lift, and the effects of high pH, salinity, and drought on plants, as well as a short review of the biology of studied plant species.

1.2.2 Chapter 2 Water redistribution in trembling aspen (*Populus tremuloides*) exposed to salinity and drought

The second chapter presents the study in which trembling aspen plants were subjected to NaCl and reduced soil water content in the upper part of the soil to examine possible redistribution of NaCl and water by the roots.

1.2.3 Chapter 3 Growth and physiological responses of yellow sweet clover (*Melilotus officinalis*) and slender wheatgrass (*Elymus trachycaulus*) to the presence of high soil pH and salt below the root zone

In this chapter, I examined the effects of high pH present in the deeper soil profile on growth and physiological responses of two above herbaceous plant species to NaCl.

1.2.4 Chapter 4 Ericoid mycorrhizal associations alleviate drought stress in lowland and upland populations of velvetleaf blueberry (*Vaccinium myrtilloides*)

This chapter describes the study carried out to investigate the contribution of ericoid mycorrhizal (ERM) associations to drought resistance of upland and lowland velvetleaf blueberry populations.

1.2.5 Chapter 5 Conclusions and suggestions

Chapter 5 provides synthesis of the above research studies as well as general conclusions and suggestions for future research.

1.3 Literature review

1.3.1 Oil sands mining and reclamation

Oil sands deposits in Alberta, Canada, account for 97% of Canada's 172.5 billion barrels of proven oil reserves (Natural Resources Canada 2016). The expanding oil sands industry is widely regarded as one of the driving forces for the Canadian economy (Sauchyn et al. 2015). The oil sands deposits are a mixture of crude bitumen and rock

material together with other associated mineral substances (Alberta Energy Regulator 2018). These areas occupy about 142 000 square kilometers (54 000 square miles) in the northern boreal forest region of Alberta (Alberta Energy Regulator 2018). Presently, surface mining activity is carried out in approximately 500 square kilometers of these areas (Government of Alberta 2019).

1.3.1.1 Oil sands mining

Surface mining, also referred to as open-pit mining, is used to exploit oil sands deposits that are less than 75 meters below the surface. The area that requires bitumen to be recovered by this method constitutes about 3% of the total oil sands surface area or 20% of oil sands reserves. Before mining activities can commence, all vegetation of the boreal forest and the water-laden muskeg must be removed. The topsoil is separated and stockpiled for later reclamation use. The uncovered subsoil and the overburden are stripped, exposing the underlying oil sands layer, which is usually at the depth 40 to 60 m. For bitumen extraction, the oil sands ore is mixed with hot alkaline water and diluents (naphthenic and parafanic) to separate bitumen from the sand. The waste from bitumen extraction is then deposited in tailings ponds where the sand and particles settle (Government of Alberta 2019).

For the oil sands deposits below 75 meters (usually more than 350 below the surface), in-situ (thermal in-situ) recovery is used for the bitumen extraction (Government of Alberta 2019). In general, high-pressure steam is injected through the upper well to heat the bitumen to make it more fluid. The bitumen then flows to the lower well and is pumped to the surface (Dyer and Huot 2010). No tailings ponds are required for the in-situ methods of recovery.

After extraction, the diluted bitumen can be piped to an upgrader on site. For upgrading, hydrogen is added to bitumen and carbon is removed together with

contaminants such as heavy metals and salt to achieve a synthetic crude oil that undergoes further refinement (Government of Alberta 2019).

1.3.1.2 Oil sands reclamation

According to the policy of the Alberta Government (Alberta Environment 2010), all disturbed land must be returned to the state of natural productivity that existed before the start of the industrial activity. Different soil reclamation strategies have been used after the mining activity (Carrera-Herna´ndez et al. 2012, Zhang 2015). In general, the tailings of processed sand and sediment from tailings ponds are returned to the pit to fill the mining site. Saline-sodic overburden (soil and organic material) that was stored at the beginning of the operation is placed over the tailings layer. Finally, the stockpiled salvaged soil is placed on the surface. There are usually two main types of material used as a topsoil: peat-mineral mix and upland surface soil. The peat-mineral mix contains organic peat and mineral soil (Brown 2010). The upland surface soil is a mix of the LFH horizon with underlying A horizon, and the part of or entire B horizon (Singh 2007). The presence of the peat mineral mix and upland surface soil can greatly improve soil organic matter, nutrient availability and diversity of soil microorganisms (Brown 2010).

The oil sands tailings are generally stored in tailing ponds and may be combined with gypsum or other flocculants to facilitate the consolidation of solids (Zhang 2017). The tailings usually have high pH levels, high salinity and pollutants that include oil, alkaline, sulphates, phenols, and iron (Xu 2015). Leaking of tailings water from the tailings ponds into the surrounding soil and surface water through the groundwater system can also increase soil pH in the surrounding areas (Tenenbaum 2009, National Energy Board 2015). In many oil sands reclamation sites, elevated salinity and high pH persist in the deeper parts of the soil due to the presence of saline-sodic overburden materials (SSOB) (Kessler et al. 2010, Lazorko and Van Rees 2012). In the reclamation

areas, electrical conductivity (EC) values in the soil cover can range from 0.60 to 6.32 dS m^{-1}, while those in SSOB can range from 4.50 to 9.30 dS m^{-1} (Lazorko and Van Rees 2012). The pH of undisturbed soils in the boreal forests is typically below 6.0, while the soil pH in oil sands reclamation areas frequently exceeds 8.0 (Howat 2000). In addition to salinity and high soil pH, episodes of drought stress can make reclamation efforts more challenging. Drought has been identified as one of the main factors affecting tree mortality and forest dieback in Canada's boreal forests (Allen et al. 2010, Peng et al. 2011, Michaelian et al. 2011).

There have been extensive studies on the effects of salinity (Renault et al. 2001, Calvo-Polanco et al. 2009b, Calvo-Polanco et al. 2014), high pH (Calvo-Polanco et al. 2009a, Siemens and Zwiazek 2011, Zhang 2015), drought (Michaelian et al. 2011, Landhäusser et al. 2012), and mycorrhizal associations (Onwuchekwa et al. 2014, Hankin et al. 2015, Scott et al. 2019) on plant species native to the boreal forest in northeastern Alberta and used for oil sands reclamation. However, little is known about the combined effects of these factors on plants of the species native to the boreal forests.

1.3.2 Hydraulic lift in plants

Hydraulic lift, the term that was first defined by Richards and Caldwell (1987), is used to describe the phenomenon of water movement by passive translocation through roots from wetter, usually lower soil layers to drier, usually upper parts of the soil. Since then, numerous studies have focused on the processes of hydraulic lift (Dawson 1993, Ludwig et al. 2003). In addition to hydraulic lift (HL), Burgess et al (1998) and Schulze et al. (1998) found that water can move down along the taproot of plants from the wetter surface soil layers to the lower drier soil layers, which was called inverse hydraulic lift (IHL) or hydraulic descent (HD) (Hultine et al. 2003). Later, Brooks et al. (2002) provided evidence for both vertical and horizontal transfer of water by roots of two

conifer trees, which they referred to as lateral redistribution (LR). Overall, Prieto et al. (2012) pointed out that the passive movement of water in different soil parts by plant root systems, driven by water potential gradients, in the soil-plant interface should be referred to as hydraulic redistribution (HR).

In general, HR occurs mostly at night when the stomata are closed and transpiration is very low. At that time, water potential gradients between plant roots in moist soil and the drier parts of the soil provide driving forces for water flow from roots to these dry soil layers (Prieto et al. 2012). The hydraulic lift is thought to help plants maintain the viability of fine roots (Bauerle et al. 2008), preserve microbial activity (Querejeta et al. 2003), facilitate nutrient uptake (Caldwell and Richards 1989), and help shallow-rooted plants buffer against drought stress (Xu et al. 2006). By now, HR has been found in more than 120 plant species (Yu et al. 2015). However, less is known about the effect of soil salinity on redistribution of water by roots and whether salt is redistributed along with water during this process. Armas et al. (2010) pointed out that salt might be taken up by the roots of salt-tolerant plants (*Pistacia lentiscus*) from the deeper, moister, soil layers and be subsequently discharged to the upper, drier, soil layers. Consequently, salt may accumulate in the upper layers and, therefore, induce salt stress in the nearby plants. In addition, Bazihizina et al. (2017) pointed out that HR is very limited in plants in saline environments where differences in soil salinity result in external soil osmotic gradients. The limitations come from ion accumulation in plants, which causes the decreases of osmotic potential in the xylem sap of roots. This can result in large plant predawn disequilibrium between shoots and roots, which can reduce the driving force for water backflow from the roots to the saline soil (HR). Therefore, it is necessary to consider the effects of salt when studying HR in plants exposed to heterogeneous salinity in the soil.

To date, HR has been most commonly studied by measuring changes in soil moisture content of the drier soil layers using methods such as the Time Domain

Reflectometry (TDR) (Ryel et al. 2002, Oliveira et al. 2005, Sulis et al. 2019) and Frequency Domain Reflectometry (FDR) (Hawkins et al. 2009, Brooksbank et al. 2011). However, in field experiments or when changes of soil moisture are small, an alternative hydrogen isotope method can be used for more precise measurements (Prieto et al. 2014, Zhang and Zwiazek 2018).

1.3.3 Salt stress

Soil salinity is a major environmental factor affecting plants worldwide. The concentration of salts in seawater is about 3.5 % (~0.6 M), with NaCl as the main salt. Most of the terrestrial plants cannot tolerate even one-tenth of this salt concentration (Waisel 1972). In addition to coastal areas, about 954.8 million ha or 10% of the world's land area has been identified to be affected by salinity (Szabolics 1994). The presence of salt in the soil is one of the most stressful environmental factors to plants. When a plant is exposed to salt, osmotic stress instantly affects plant growth. When the salt levels reach a certain threshold, ion toxicity follows and creates ionic imbalance, which directly affects physiological processes in plants. As a consequence of these primary effects, oxidative damage and other secondary stresses often occur (Zhu 2001).

1.3.3.1 Effect of salt stress on plant growth and physiology

Like many other abiotic stresses, salt stress inhibits plant growth. Growth inhibition is also an adaptive feature that helps plants survive salt stress by diverting from growth multiple resources to combat stress (Yokoi et al. 2002). In most cases, salt stress causes a decrease of net photosynthetic rates due to stomatal closure and a resulting decrease of carbon dioxide uptake (Zhang et al. 2018, Mahlooji et al. 2018, Çiçek et al. 2018). In addition, salt stress can directly inhibit cell division and expansion (Zhu 2001).

In terms of salt tolerance, plants are divided into halophytes and glycophytes. Halophytes are remarkable plants that can complete the life cycle in a salt concentration equal to or higher than 200 mM NaCl (Flowers et al. 1986, Flowers and Colmer 2008). The remaining 99% of plants species belong to salt-sensitive glycophytes (Flowers and Colmer 2008). The growth of glycophytes can be inhibited by NaCl concentrations much lower than 200 mM, even when exposed for a short period of time (Acosta-Motos et al. 2017). It has been reported that the concentrations as low as 20 mM NaCl can inhibit growth in some glycophytes (Lutts et al. 1996, Evers et al. 1997, Renault et al. 2001, Flowers 2004, Saied et al. 2005). Exposure of roots to as little as 10-mM NaCl was reported to reduce root cell hydraulic conductivity in *Arabidopsis thaliana* within 30 min (Lee and Zwiazek 2015). However, glycophytes vary in salt tolerance levels. In the highly salt-sensitive milkflower (*Cotoneaster lacteus*), juniper-leaf grevillea (*Grevillea juniperina*) and white firethorn (*Pyracantha 'Harlequin'*), growth rates were significantly reduced by 10 mM NaCl concentration (Cassaniti et al. 2009) and in Siberian elm (*Ulmus pumila*), 0.3% w/v NaCl (about 51 mM) was not sufficiently high to affect plant growth in most of the examined tree clones (Mu et al. 2016).

Under high salinity conditions, roots are the first organs perceiving salt-induced osmotic stress. In the longer-term, salt stress produces ion toxicity due to NaCl build-up (Munns 2005). In woody tree species plants, salt stress was found to induce suberisation of the hypodermis and endodermis in roots and caused the formation of a well-developed Casparian strip closer to the root apex (Walker et al. 1984). In salt-sensitive plants, salt stress also reported decreasing the total root length in thin (≤ 0.5 mm) and medium-thickness (0.5-2.0 mm) roots (Gómez-Bellot et al. 2013). In addition, an increase of root to shoot ratio under salt stress had been reported for many plant species (Hsiao and Xu 2000). The greater proportion of roots under salt stress can increase the retention of Na in the roots, and delay the translocation to the aerial parts (Cassaniti et al. 2009). Overall

decreases in fresh weights, dry weights and relative growth rates under salt stress have been observed in many plant species (Rodríguez et al. 2005, Mu et al. 2016). Many studies have also reported alterations of leaf and cell structures (Acosta-Motos et al 2015a, Acosta-Motos et al. 2015b), decreases of the total leaf areas, and chlorophyll concentrations (Franco et al. 1997, Rodríguez et al. 2005, Stepien and Johnson 2009, Ashraf and Harris 2013). Decreases in photosynthetic rates, commonly reported for salt-treated plants, are largely due to the stomatal closure (gs), and/or non-stomatal limitations, such as the disturbance of the photosynthetic electron chain transport and/or the inhibition of the Calvin Cycle enzymes (Parida and Das 2005, Chaves et al. 2009). In the study on salinity effect on radish (*Raphanus sativus*) growth, 80% of the growth reduction (dry weights) of radish under higher salt stress (> 4 dS m^{-1}) was attributed to the decrease of leaf area expansion and the resulting reduction of light interception. The remaining 20% of growth reduction was explained by a decrease in stomatal conductance (Marcelis and Hooijdonk 1999). In the study of plant responses to the naturally saline sites in northern Alberta, the results indicate that the aspen basal area increment (BAI) decreased by 50% as salinity increased in the study area (Lilles et al. 2011). In red clover (*Trifolium pratense*), the salinity largely sharply decreased the growth and root elongation under 120 and 180 mM NaCl treatments (Asci 2011). In the study of tall wheatgrass (*Agropyron elongatum*) and basin wild rye (*Elymus cinereus*), the salinity (1.0, 10, and 20 dS m^{-1}) largely drastically decreased the growth of shoots and soil penetration of roots in both species (Roundy 1985). After remaining in 200 mM NaCl for two weeks, the sea wheatgrass (*Thinopyrum junceiforme*), the shoot and root growth rates were reduced by 45.2% and 50.8% compared to the untreated control. (Li et al. 2019).

At the functional level, the accumulation of salt in plants causes a reduction of tissue osmotic potentials, which leads to a decrease in plant water potential (Sánchez-Blanco et al. 2004, Franco et al. 2011). Under short-term (1 and 2 days) NaCl stress, the decrease of

leaf water potential, relative water content, water uptake, transpiration rates, stomatal conductance and water use efficiency were shown in *Corchorus capsularis* (Chaudhuri and Choudhuri, 1997). With an increase of salinity, water potential, osmotic potential, and stomatal conductance became progressively lower in *Urochondra setulosa*y (Gulzar et al. 2003).

In general, salt stress decreases the concentration of chlorophyll and carotenoids in leaves, which can lower photosynthetic rates (Parida and Das 2005). Usually, chlorosis starts in the oldest leaves and the leaves fall when salt stress continues (Hernandez et al. 1999, Agastian et al. 2000). Under salt stress, needle necrosis increased and the chlorophyll concentrations decreased with increasing NaCl concentrations in *Pinus leiophylla* (Jimenez-Casas and Zwiazek 2014). Leaf chlorophyll a and chlorophyll b concentrations in *Cornus stolonifera* seedlings treated with 100 mM Na_2SO_4 were 34% and 44%, respectively, lower than in the control plants (Renault et al. 2001). Salinity was also shown to trigger significant decreases in chlorophyll a, chlorophyll b and carotenoid concentrations in leaves of *Bruguiera parviflora* (Parida et al. 2004).

Under salinity stress, Na^+ uptake competes with the uptake of mineral nutrient ions, especially K^+, leading to K^+ deficiency (Mudgal et al. 2010). NaCl treatment induces the decrease in Ca^{2+}, K^+, and Mg^{2+} levels in many plants including *Haloxylon recurvum*, *Ceriops tagal* and *Bruguiera parviflora* (Khan et al. 2000, Aziz and Khan 2001, Parida et al. 2004). In salt-tolerant *Populus euphratica,* salt stress had little effect on the leaf Ca^{2+} and Mg^{2+} levels and both elements increased in roots. In contrast, Ca^{2+} and Mg^{2+} concentrations in roots and leaves of the relatively salt-sensitive *Populus talassica* were significantly reduced by salt stress (Chen et al. 2001). Decreases in tissue concentrations of essential elements have been commonly reported for other salt-sensitive species of woody plants. Treatments with NaCl and Na_2SO_4 that enhanced Na^+ contents in roots and

shoots of *Cornus stolonifera* also caused significant decreases of K^+ and Mg^{2+} (Renault et al. 2001). In *Pinus banksiana*, the shoot Na^+ and Cl^- concentrations significantly increased in both NaCl and Na_2SO_4 salt treatments and the K^+ concentrations decreased (Franklin and Zwiazek 2004).

1.3.3.2 Salt tolerance and adaptive physiological mechanisms in plants

Plants have evolved numerous physiological strategies to cope with salt stress. When salt stress signals are transmitted to the plants, multiple secondary signals are activated and intracellular Ca^{2+} level immediately increases. The secondary signals can trigger a phosphorylation cascade reaction and act on proteins involved in cell defense or transcription factors. As in consequence, stomatal closure, osmolyte accumulation, increased Na^+/H^+ antiporter activity and activation of reactive oxygen species (ROS) all occur in response to salt stress (Liang et al. 2018).

1) Osmotic stress

Osmotic stress, caused by reduced water availability and accumulation of osmotically active molecules (osmotic adjustment), is among the initial responses of plants to salt stress (Munns 2005). Through osmotic adjustment, plants can maintain cell turgor to maintain metabolic activity and growth (Sharp et al. 1990). Under salt stress, plants synthesize and accumulate proline, soluble sugars, glycine betaine, and other osmolytes to reduce osmotic potential (Garg et al. 2002, Taji et al. 2002).

Proline content in plants can be used as a physiological stress index. In addition to its role in osmotic adjustment, proline can scavenge free radicals, stabilize sub-cellular structures, and buffer cellular redox potential (Ashraf and Foolad 2007). Many studies have shown a link between leaf proline concentration and salt tolerance (Azza Mazher et al. 2007, Wutipraditkul et al. 2015). Soluble sugars can also stabilize cell membranes and

protoplast structures (Guo et al. 2015). The accumulation of soluble sugars can help plants lower osmotic potential to tolerate salt stress and, as a result, the content of soluble sugars can be used as a physiological indicator of salt tolerance (Mu et al. 2016). In the studies of *Cistus monspeliensis* and *Ulmus pumila*, high accumulation of soluble sugars was believed to be the main reason for plants to be able to withstand salt stress (Sánchez-Blanco et al. 2004, Mu et al. 2016). In the study of aspen (*Populus tremula*) plants, stable protein 1 (sp1) cDNA was found to accumulate in response to salt, cold, heat, and desiccation stresses (Wang et al. 2002). Under salt stress (50 and 150 mM NaCl), proline, spermine, sucrose, mannitol, and raffinose levels in aspen (*Populus tremula*) plants increased either temporarily or throughout the salt treatment (Jouve et al. 2004).

2) Ion toxicity

Salt stress causes ion toxicity in plants mainly due to the influx of Na^+ to the cells. The accumulation of Na^+ in plant cells causes intracellular ion and charge imbalance. Sodium in plant cells negatively affects plant nutrition, cytosolic enzyme activities, photosynthesis, and metabolism (Zhu 2001, Flowers 2004). Plants have evolved a variety of strategies to minimize the effects of ion toxicity. Ion uptake, efflux and compartmentalization are crucial for the plant to maintain ion balance in cells. Plants cannot tolerate large amounts of salt in the cytoplasm, therefore, they either restrict the excess of salts in the vacuole or compartmentalize them in different tissues to facilitate metabolic functions (Zhu, 2003). Several possible strategies in plants could be employed to avoid a damaging decrease in the K^+/Na^+ ratio: 1) reducing the entry of Na^+ into the cell, 2) removing Na^+ from the cell, or 3) compartmentalizing Na^+ into the vacuole where it cannot disrupt cellular functions (Katiyar-Agarwal et al. 2005). Na^+/H^+ antiporter is believed to be important in plant salt tolerance. In a study of the expression of the AgNHX1 gene (encoding Na^+/H^+ antiporter) in a yeast mutant (Hamada et al. 2001), the

lack of the vacuolar-type Na^+/H^+ antiporter gene (NHX1) was associated with poor viability after exposure to NaCl. In poplar plant (*Populus davidiana* × *Populus bolleana*), the expression of the AtNHX1 and AtNHX3, two tonoplast $Na^+(K^+)/H^+$ antiporter encoding genes from *Arabidopsis thaliana*, increased resistance to both salt and water-deficit stresses compared with wild type plants (Yang et al. 2017). In addition, the Salt Overly Sensitive (SOS) signaling pathway, composed of the SOS1, 2 and 3 proteins, play an important role in salt stress tolerance and ionic balance (Zhu 2003). The protein kinase complex consisting of the myristoylated calcium-binding protein SOS3 and the serine/threonine protein kinase SOS2 is activated by a salt-stress-elicited calcium signal. The protein kinase complex then phosphorylates and activates various ion transporters, such as the plasma membrane Na^+/H^+ antiporter SOS1 (Zhu et al. 1993). In a study of the expression of the PtSOS2 (PtSOS2TD) gene, the constitutive expression of PtSOS2TD significantly enhanced the salt tolerance of transgenic aspen hybrid (*Populus davidiana* × *Populus bolleana*) plants by maintaining optimal ion homeostasis and improving antioxidative capacity (Yang et al. 2015).

3) Scavenging of reactive oxygen species

Salt stress in plants can lead to oxidative stress by increasing reactive oxygen species (ROS), such as superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radicals, which can cause cellular damage in plants (Mudgal et al. 2010). In general, chloroplast, mitochondria, and peroxisomes are important sources of ROS in plants during abiotic stresses including salt stress (Takagi et al. 2016, Acosta-Motos et al. 2017). In chloroplasts, the over-reduction of the electron chain along with the lack of regeneration of the final electron acceptor in PSI ($NADP^+$) favor electron transfer from ferredoxin to oxygen to form O_2^- (Mehler Reaction), which undergoes dismutation to H_2O_2 and O_2 that is catalyzed by the superoxide dismutase (SOD) (Asada 1999). ROS accumulation in

mitochondria during salt stress is typically mediated via electron leakage from complex I and III to produce $O_2^{\cdot -}$, which can be converted to H_2O_2 by Mn-SOD (Quan et al. 2008, Huang et al. 2016). The production of ROS in peroxisomes under salt stress is mainly due to enhanced photorespiration resulting in the production of H_2O_2 by glycolate oxidase (Baishnab and Ralf 2012, Kerchev et al. 2016).

To overcome the oxidative stress induced by salinity, the ROS scavenging system, such as SOD, CAT, and POD is activated in defense against the oxidative stress (Zhang et al. 2011). Various ROS scavenging enzymes and pathways were found to mitigate the ROS in chloroplasts such as Fe-and CuZn-SODs and the Asada-Foyer-Halliwell pathway, as well as high concentrations of antioxidants such as ascorbic acid and GSH (Mittler et al. 2004). The ROS in mitochondria can be mitigated by alternative oxidase (AOX), type II NAD(P)H dehydrogenase and uncoupling proteins in the inner mitochondrial membranes (Noctor et al. 2007, Rasmusson and Wallstrom 2010). CAT was the main ROS scavenging system to cope with the production of H_2O_2 from peroxisomes under salt stress (Kerchev et al. 2016). However, a decrease in the ROS scavenging system occurs when salt concentration exceeds the threshold value (Song et al. 2006). In the study of centipede grass (*Eremochloa ophiuroides*), the salt-tolerant genotype had a higher ROS scavenging enzyme (POD, APX and CAT) activities than the sensitive genotype under salt stress (Maeda et al. 2011, Li et al. 2018). Similar results were also reported for salt-tolerant *Medicago sativa* plants under salt stress (Quan et al. 2016). In transgenic *Populus euphratica* plants, the PeHSF-transgenic lines exhibited an increased capacity to control ROS homeostasis. However, the transgenic plants did not show an enhanced capacity to retain ionic homeostasis under salt stress (Shen et al. 2013).

1.3.4 High pH and plants

Soil alkalization is one of the most major factors limiting plant growth. Over one-third of the world surface is covered by calcareous, saline, or sodic soils, which pH usually higher than 7.5 (Marschner 2012, Zhang 2015). Most plants favor slightly acidic soil with pH of pH 6.0 - 6.5 (Fageria and Baligar 2003). The effects of high pH may lead to a reduction in seed germination (Peng et al. 2008, Gao et al. 2014), direct damage to plant roots by the accumulation of [OH^-] (Shi and Zhao 1997, Kopittke and Menzies 2004), ion imbalance, and altered mineral nutrition in plants (Shi and Zhao 1997). High pH often causes the deficiency of micronutrients such as Fe and Mn (Fageria and Baligar 1999, Zhang et al. 2013).

1.3.4.1 Effects of high pH on root growth

Root growth inhibition by high pH has been reported for many plant species including *Capsicum annuum*, *Lupinus angustifolius, Pinus pinaster* and *Pisum sativum* (Stoffella et al. 1991, Tang et al. 1992, Tang et al. 1992, Tang et al. 1993b). In *Lupinus angustifolius*, pH ≥ 6.0 affected root elongation within one hour (Tang et al. 1993b). Arduini et al. (1998) also reported that the increase of pH from 5.5 to pH 6.5 inhibited root elongation rate in *Pinus pinaster* and decreased root thickness as well as lateral root growth after one-month of treatment. However, in some high pH tolerant species such as *Pisum sativum*, the growth of roots was little affected by high pH (Tang et al. 1992, Tang et al. 1993b). The proposed reasons for the decreased root elongation in *L. angustifolius* by high pH were failure to acidify the apoplast and impaired membrane permeability of the cortex cells (Tang et al. 1996).

1.3.4.2 Effect of high pH on nutrient balance and physiological processes

Soil pH plays an important role in nutrient uptake since it affects nutrient sorption and solubility in soil (Zhang 2015). Plant uptake of several essential elements, including P, Fe, Zn, and Mn, is reduced at high pH. Phosphorus deficiency can quickly develop since P is a macroelement and is required in relatively high concentrations (Ila'ava et al. 1999). In some calcareous soils, high concentrations of Ca in soil combine with P to precipitate calcium phosphate minerals and suppress P availability to plants (Gray and Schwab 1993).

Under natural conditions, Fe^{3+} oxides are the dominant forms of Fe in most soils. Fe^{3+} may form several oxide forms such as Fe_2O_3, $Fe(OH)_2^+$, and $Fe(OH)_3$ and precipitate under alkaline or calcareous soils (Xu 2017). Zinc deficiency has been reported to be the most widespread of all essential elements in plants growing in calcareous soils (Graham 2008). The availability of Mn is also reduced in alkaline and calcareous soils since it can combine with Ca and precipitate as insoluble manganese calcite ($MnCO_3$) under pH 6 to 8 (Hewitt et al. 1974, Jauregui and Reisenauer 1982). Another essential microelement that is sensitive to changes in soil pH is boron (B). The availability of B in the soil increases with the increase of soil pH from 4.7 to 6.7 and decreases when the soil pH ranges from 7.1 to 8.1 (Xu 2017).

In general, high soil pH can inhibit the water uptake and root water transport in plants. In the study of *Betula papyrifera*, pH 8 in the root zone caused a decrease of root hydraulic conductivity and stomatal conductance compared with pH 6 (Kamaluddin and Zwiazek 2004). Under high pH conditions, leaf water potentials and chlorophyll concentrations in *Lupinus angustifolius* plants decreased compared with the acidic soil conditions (Tang et al. 1993a, Tang and Turner 1999). In addition, altered nutrient balance caused by high pH, including Fe^{3+} and Mn deficiencies, can result in leaf

chlorosis and lower photosynthetic rates (Tang and Turner 1999, Kosegarten et al. 2001, Tang et al. 2006).

1.3.5 Drought stress

Drought stress is the leading environmental factor affecting the growth and productivity of plants worldwide. Water availability is among the most limiting factors to plant growth (Almeida-Rodriguez et al. 2010). In general, drought is classified into three types (Wilhite 2000, Dai 2011). 1) The most common type of drought is meteorological drought, which is a prolonged time period with low precipitation. Meteorological drought is often accompanied by above-normal temperatures and precedes and causes other types of droughts. 2) Agricultural drought is a period with dry soils (lower soil water potential) that results from below-average precipitation, intense but less frequent rain events, or above-normal evaporation, all of which lead to reduced crop production and plant growth. 3) Hydrological drought occurs when river streamflow and water storages in aquifers, lakes, or reservoirs fall below long-term mean levels. Hydrological drought develops more slowly because it involves stored water that is depleted but not replenished.

1.3.5.1 Effect of drought stress on plant growth

Drought stress has detrimental effects on plant growth and development at any growth stage. However, the effects vary depending on the severity of stress and the growth stage of plants (Farooq et al. 2012). Seeds may not germinate below a certain soil water potential, which for *Helianthus annus* was reported to be -1.2 MPa (Kaya et al. 2006). However, under the same level of water potential, seed germination and seedling growth may be affected more by drought compared with salinity (Okçu et al. 2005). Common consequences of drought stress include reduced growth (Kiani et al. 2007,

Hussain et al. 2009, Asrar and Elhindi 2011, Liu et al. 2011), decrease of biomass owing to reductions in tissue water potential, cell elongation and division, impaired enzyme activities, loss of turgor, and decreased energy supply (Farooq et al. 2009, Farooq et al. 2012). Production of the ramified root system and increased root to shoot ratio under drought stress are also important characteristics in plants (Jaleel et al. 2009). The increased root growth under water deficit was also reported for many plants including *Heliantus annuus* (Tahir et al. 2002) and *Catharanthus roseus* (Jaleel et al. 2008).

1.3.5.2 Effects of drought stress on plant physiology

Drought upsets plants water balance. Altered relative water contents, the changes of leaf water potentials, stomatal conductance, transpiration rates, and osmotic potentials are among the most commonly reported physiological parameters in plants exposed to drought (Farooq 2012, Jaleel et al. 2009). Water potential, osmotic potential and relative water contents decrease in response to drought at different times in different plant species (Tezara et al. 2002, Liu et al. 2004, Subramanian et al. 2006, Ozkur et al. 2009). To lower osmotic potential, plants accumulate different types of organic and inorganic solutes in the cytosol to maintain cell turgor (Rhodes and Samaras, 1994). Under drought stress, proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes are synthesised and accumulate in the cytoplasm. In addition to their role in maintaining turgor, the lowering water potential through the accumulation of osmotically-active molecules helps plants absorb water from the drying soil (Anjum et al. 2011b).

Under drought stress, a reduction of nutrient uptake is a general phenomenon observed in plants. Decreased soil water availability can affect the diffusion rate of many nutrients in the soil and reduce their availability to plants (Singh and Singh 2004, Hu and Schmidhalter 2005). In addition, since nutrients are dissolved in water, the rates of water and nutrient uptake may be linked. Under water deficit conditions, phosphorous contents

were reduced in roots and shoots of tomato (*Solanum lycopersicum*) seedlings (Subramanian et al 2006). On the other hand, low water availability also limits microbial activity in the soil, which may lead to a total inhibition of microbial metabolism depending on the intensity and duration of the drought event (Borken and Matzner 2009). In a study of carbon and nitrogen cycles, Beier et al. (2008) found that the decomposition of organic carbon was mainly sensitive to temperature, but ammonification depended only slightly on temperature and was strongly inhibited by reduced soil water availability. The amount of organic nitrogen dissolved in the soil increases during drought events, presumably due to dieback of the microbial biomass (Borken and Matzner 2009, Dannenmann et al. 2009).

Many studies have shown decreases of photosynthetic activity under drought stress (Del Blanco et al. 2000, Samarah et al. 2009, Anjum et al. 2011a). The decreases of photosynthesis may be due to stomatal or non-stomatal factors (Anjum et al. 2011a). Anjum et al. (2011b) pointed out that stomatal closure is one of the first responses to drought stress and can result in decreased photosynthesis rate. In addition to the stomatal factors, severe drought stress inhibits photosynthesis due to a decline in ribulose-1,5-bisphosphate carboxylase / oxygenase (RuBisCO) (Bota et al. 2004). In general, the efficiency of photosynthesis under drought stress mainly depends on ribulose -1,5 -bisphosphate (RuBP) regeneration and the activity of RuBisCO (Medrano et al. 1997, Lawlor 2002). Under drought stress, the activity of the photosynthetic electron transport chain is finely tuned to the availability of CO_2 in the chloroplast and function of photosystem II (Loreto et al. 1995). The activity of Rubisco is modulated *in vivo* either by reaction with CO_2 and Mg^{2+} to carbamylate a lysine residue in the catalytic site, or by binding inhibitors within the catalytic site. During the night, 2-carboxyarabinitol-1-phosphate is formed in many species, which binds tightly to Rubisco, inhibiting catalytic activity. Reduced tissue water contents also increase the activity of Rubisco-binding

inhibitors. As a consequence, the tight-binding inhibitors can decrease Rubisco activity in the light (Farooq et al 2009). In addition, the non-cyclic electron transport is down-regulated to match the reduced requirements of NADPH production and, thus, reduces the ATP synthesis (Farooq et al. 2009).

1.3.5.3 Plant drought resistance

Plants resist drought stress through various morphological, biochemical and physiological mechanisms (Farooq et al 2009). Morphologically, plants resist water deficit mainly through drought escape, drought avoidance and drought tolerance. Drought escape is accomplished through a shortened life cycle or growing season, allowing plants to complete their life cycle before the environment becomes dry. Drought escape occurs when the plants' phenological development periods are completed before terminal drought stress predominates (Araus et al. 2002).

Drought avoidance is the ability of plants to avoid reduce tissue water content despite reduced water content in the soils (Basu et al. 2016). Under transient periods of drought stress, drought avoidance occurs when plants increase water-use efficiency, lower stomatal conductance, changes in leaf area, limiting vegetative growth, changes in hydraulic conductivity, or increasing root growth to avoid the plants' dehydration (Kooyers 2015). Drought stress causes isohydric (drought-avoidant) plants control over water loss mainly through stomatal movements (Almeida-Rodriguez et al. 2010). Root characteristics such as increased length, density and depth are the main drought avoidance traits that contribute to plant growth under drought stress (Turner et al. 2001). Common strategies include reduction of transpiration area leaf shedding and production of smaller leaves to reduce the water loss, deposition of heavy cuticle layer on the epidermis, sunken stomata, and trichomes (Farooq et al 2009).

Drought tolerance is to endure low tissue water content through adaptive traits which involve maintenance of cell turgor through osmotic adjustment and cellular elasticity as well as increasing protoplasmic resistance (Morgan 1984).

Physiologically, osmotic adjustment, osmoprotection, antioxidation, and the scavenging defense system have been the most important bases responsible for drought tolerance (Farooq et al. 2012). Water deficit affects plant-water relations by lowering tissue water potential and turgor (Hussain et al. 2009). Under drought stress conditions, sugars and free amino acids have been reported to accumulate in many plant species (Manivannan et al. 2007, Jalil et al. 2007, Sankar et al. 2007). In addition to lowering water potential and increasing cell turgor by osmotic adjustment, compatible solutes can also protect enzymes from the damaging effects of ROS (Farooq et al. 2012). Similarly to salt stress described above, in response to ROS caused by drought stress, complex antioxidant enzyme systems such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) are triggered in plants (Vardharajula et al. 2011, Kaya et al. 2013). In addition to the high activity of antioxidant enzymes, some non-enzyme antioxidants such as glutathione (GSH), ascorbate (ASC) and carotenoids in plants also provide protection by quenching toxic ROS (Kubiś et al. 2014, Singh et al. 2015). The antioxidant enzymes may directly scavenge ROS or may produce non-enzymatic antioxidants, which can act protect the integrity of the photosynthetic membranes under oxidative stress (Anjum et al. 2011b). In the study of sweet sorghum (*Sorghum bicolor*), the drought-tolerant variety had significantly higher activities of the antioxidant enzymes than the drought-sensitive variety (Guo et al. 2018). Also, in soybean (*Glycine max*), drought stress triggered higher activities of CAT, POD, APX and glutathione reductase enzymes in the drought-tolerant variety compared with the drought-sensitive variety (Devi and Giridhar 2015). Similar results were also found in faba bean (*Vicia faba*), with the activities of antioxidant enzymes being higher in the drought-

tolerant genotype compared with the drought-sensitive plants (Abid et al. 2017). Therefore, the ability of antioxidant enzymes to scavenge ROS may be among important drought tolerance mechanisms in plants.

1.3.6 Ericoid mycorrhizal associations

1.3.6.1 Introduction

Mycorrhizas are symbiotic associations between plants and fungi that are present in most of the terrestrial vascular plants (Al-Karaki 2013). Based on the morphological characteristics and the host plants, there are four main types of mycorrhizas: arbuscular mycorrhizas (AM), ectomycorrhizas (ECM), ericoid mycorrhizas (ERM), and orchid mycorrhizas (ORM). According to the report by Brundrett and Tedersoo (2017), 72% of world vascular plant taxa form AM associations, approximately 2% of plants form ECM associations, 10% of plants form ORM associations, 1.5% are colonized by ERM fungi, and only 8% are completely nonmycorrhizal (NM). The main characteristic of AM is that the fungal hyphae penetrate the cortical cells and form tree-like structures inside the cells (Van Der Heijden et al. 2015). For the ECM, the fungal hyphae do not penetrate the cell walls and the highly branched hyphae form a Hartig net between the epidermal and cortical cells. The primary and secondary roots are often completely surrounded by a fungal mantle (Van Der Heijden et al. 2015). The hyphae of orchid mycorrhizas fungi usually penetrate the root cortex cells and form special structures called pelotons (Dearnaley et al. 2016). The characteristic of ERM is the formation of compact intracellular hyphal coils in enlarged epidermal hair-root cells, which function as the site of nutrient exchange (Perotto et al 2018). The mycorrhizal symbiosis plays a key role in the cycling of carbon (C), nitrogen (N), phosphorus (P), seed germination and stress resistance of the host plants (Al-Karaki 2013, Rasmussen and Rasmussen 2014, Van Der

Heijden et al. 2015).

1.3.6.2 Ericoid mycorrhizas (ERM)

Ericoid mycorrhizal associations are common to most ericaceous plant species, which are widely distributed across the world (Perotto et al. 1995). In boreal forests and arctic tundra areas, ground cover plants are often colonized with ERM, which help the plants adapt to the nutrient-poor and acidic soils (Michelsen et al. 1998). In ERM, the fungus colonizes fine roots (also referred to as hair roots) in large epidermal cells and forms hyphal coils since these fine roots lack cortical parenchyma (Selosse et al. 2007). In most cases, root hair cells are independently colonized by several different ERM fungi (Perotto et al. 1996, Bergero et al. 2000, Massicotte et al. 2005). The surface of the narrow-diameter hyphae may form appressorium-like structures, which penetrate the epidermal cell walls (Massicotte et al. 2005). There is an interface matrix between the fungal cell wall and the host plant hair root membrane through which the nutrients are transported, referred to as the interface matrix (Perotto et al. 1995).

Ericoid mycorrhizal fungi can benefit ericaceous plant species by promoting the absorption of organic nitrogen. In the studies of heather (*Calluna vulgaris*) and cranberry (*Vaccinium macrocarpon*), ERM facilitated the nitrate influx and increased the capacity for amino acid uptake to their host plants (Sokolovski et al. 2002, Kosola et al. 2007). In general, ericaceous plants grow in soils with low availability of phosphorus (P), which the ERM fungi provide to the host plants (Read 1996). In the study of *Vaccinium macrocarpon*, *Rhododendron ponticum*, and *Vaccinium macrocarpon*, organic P was utilized by the ERM including phosphomonoesters (Straker and Mitchell 1986) and phosphodiesters (Myers and Leake 1996). The reason for the enhancement of nutrient uptake by the ERM could be explained as : 1) ERM expansion of the contact area between roots and soil (Chen et al. 2003); 2) Conversion of the unavailable N into

another source that can be used by plants (Bougoure et al. 2006), and 3) The presence of hydrolytic and oxidizing enzymes in the ERM fungi, which can allow the fungi to decompose organic matter and release P and N in the form as that is available to plants (Shaw et al. 1989, Cairney et al. 1998). Additionally, ERM associations are thought to be important to the host plant survival in soils polluted by metals such as Zn, Cd, Al and Fe (Lacourt et al. 2000, Martino et al. 2000, Vallino et al. 2011). Enhanced resistance of ERM associations to water stress was reported for the ericaceous plants *Woollsia pungens* and *Epacris microphylla* (Chen et al. 2003).

1.3.7 Biology of the studied plant species

1.3.7.1 Trembling aspen (*Populus tremuloides* Michx.)

Trembling aspen is a deciduous fast-growing tree that is widely distributed in North America (Dayanandan et al. 1998). Trembling aspen trees produce separate male and female individuals, which can grow in a great variety of soils ranging from shallow and rocky to deep loamy sands and heavy clays (Perala 1990). The typical good aspen soils are well-drained, loamy, with high organic matter, calcium, magnesium, potassium, and nitrogen elements (Alban et al. 1978, Alban 1982). Trembling aspen can tolerate soil pH from 5.3 to 8.4 (Xu 2017). According to Perala (1990), trembling aspen grows in three major components of forest cover types: Aspen (Eastern Forest) (Society of American Foresters Type 16), Aspen (Western Forest) (Type 217), and White Spruce-Aspen (Type 251). The reproduction of trembling aspen can be accomplished by seeds or roots sprout (suckers). The flowers of trembling aspen bloom in April to May and the seeds mature in four to six weeks after the flowering. When the seedlings grow bigger (after more than one year), they are capable of reproducing by root sprouts (Zhang 2015, Xu 2017). Trembling aspen is a pioneer tree in disturbed sites and has an important role in nutrient

cycling due to its rapid growth and high nutrient demand (Perala 1990). Pure aspen stands gradually deteriorate and are replaced over time by slower growing, but more shade-tolerant conifers.

1.3.7.2 Velvetleaf blueberry (*Vaccinium myrtilloides* Michx.)

Velvetleaf blueberry is a perennial, shade-tolerant, small (10-50 cm in height) shrub that grows across Canada from central Labrador to British Columbia and the Northwest Territories (Tirmenstein 1990). Velvetleaf blueberry is usually found on acidic soils in bogs and rocky areas. The optimal soil pH conditions range from 4.0 to 5.5 in dry sandy loam areas under open coniferous trees, bogs and wooded hillsides (Smith 1962, Smith & David 1966, Xu 2017). Velvetleaf blueberry can be regenerate both by seed and via rhizomes, sprouts, and suckers (Xu 2017). It is also fire-tolerant and is often abundant after forest fires or clear-cut logging (Tirmenstein 1990).

1.3.7.3 Slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners ssp. *trachycaulus*)

Slender wheatgrass is s a relatively short-lived (3 to 5 years) perennial, tufted bunchgrass ranging in height from 0.3 m to 0.76 m (Ogle 2002). The slender wheatgrass is found in many plant communities and is native to Western North America. It can grow naturally in moist to dry sites receiving more than 250 mm annual precipitation. Slender wheatgrass grows well in loamy soils, and can well adapt to higher soil pH (up to pH 8.8) and moderately saline conditions (Acharya et al. 1992, Ogle 2002).

Slender wheatgrass starts growing in mid-spring, and seeds mature by August to September. The plant can reproduce by seeds and tillers. The forage value and erosion control values of slender wheatgrass are good. It is also used as a pioneer species in oil sand reclamation sites (Darroch and Acharya 1996).

1.3.7.4 Yellow sweet clover (*Melilotus officinalis* (L.) Lam.)

Yellow sweet clover can be an annual or biennial herbaceous plant that is native to Eurasia and introduced to North America in the 17th century as a forage crop (Meyer, 2005). The whole plant can reach 1.2 to 1.8 m in height at maturity (Meyer, 2005). Yellow sweet clover can adapt to all soil textures, but the optimal soil condition is medium-textured sandy to clayey soils with a pH of above 6.5 (Baldridge and Lohmiller, 1990). Yellow sweet clover plants can also tolerate high pH, low temperatures and drought (Ogle et al. 2008). Yellow sweet clover usually regenerates by seed from June through July but can germinate at any time when water is available and temperatures favorable (Ogle et al. 2008).

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Chapter 2

Water redistribution in trembling aspen (*Populus tremuloides*) exposed to salinity and drought

2.1 Introduction

Salinity affects plant growth, productivity, and survival in many natural ecosystems and areas affected by human activity (Munns and Tester 2008). Plants exposed to salinity suffer from a combination of osmotic stress, ion toxicity, and altered nutrient balance (Munns and Tester 2008). In many oil sands reclamation sites in Alberta, Canada, elevated salinity persists in lower parts of the soil due to the presence of saline-sodic overburden materials (SSOB) that are capped with peat and mineral soil layers (Kessler et al. 2010, Lazorko and Van Rees 2012). Salinity levels may fluctuate over time depending on variations in evapotranspiration and precipitation, upward water flux, and changes in the depth of the water table (Kessler et al. 2010, Carrera-Hernández et al. 2012). Soil electrical conductivity (EC) values in the oil sands reclamation sites can range from 0.60 to 6.32 dS m^{-1}, while those in SSOB can range from 4.50 to 9.30 dS m^{-1} (Lazorko and Van Rees 2012). Soil salinity can be challenging for the successful reestablishment of boreal forests in oil sands reclamation sites (Zhang and Zwiazek 2016). Most of the boreal woody plant species exhibit a relatively low level of salinity tolerance (Howat 2000). Salinity levels higher than 4 dS/m^{-1} in the topsoil (0-20 cm)

preclude an establishment of boreal forest stands (Alberta Environment 2010). For the declaration of commercial forest end land use, the threshold is set at 2 dS m^{-1}, since higher values are expected to reduce the overstory productivity (Alberta Environment 2010).

Drought stress has been reported to induce widespread forest dieback and tree mortality in Canadian boreal forests (Allen et al. 2010, Peng et al. 2011, Michaelian et al. 2011). Although plants share some similar responses to salinity and drought stresses, some of the morphological, physiological and molecular mechanisms vary between the salt- and drought-stressed plants (Munns 2002, Chaves et al. 2009, Miller et al. 2010). More importantly, the physiological and biochemical responses of plants to the combined drought and salinity stresses are considered to be unique and, therefore, cannot be directly extrapolated from the corresponding responses to each individual stress (Mittler 2006). However, despite important practical significance, only relatively few studies have been carried out to assess the combined effects of salt and drought stresses, and they were carried out mostly with agricultural crop species (Maggio et al. 2005, Katerji et al. 2009).

Many plant species have the ability to transport water from the wetter parts of the soil and release it to the drier, usually shallow, soil areas by their root system through the process known as hydraulic lift (Richards and Caldwell 1987). Hydraulic lift occurs

mostly at night when transpiration is at a minimum and the root xylem water potential increases. During water deficit periods, increased moisture content in the upper, drier soils by hydraulic lift helps plants maintain viability of their fine roots (Bauerle et al. 2008), preserve microbial activity (Querejeta et al. 2003), facilitate nutrient uptake (Caldwell and Richards 1989), and help shallow-rooted plants buffer against drought stress (Xu et al. 2006). However, it is also plausible that, as a consequence of hydraulic lift, salt could be taken up by the roots of salt-tolerant plants from the moist places in the soil and discharged drier, usually upper, soil layer. Consequently, salt may accumulate in the upper layer and, therefore, aggravate salinity concerns (Armas et al. 2010).

Trembling aspen (*Populus tremuloides*) is widely used for oil sands reclamation in northeastern Alberta, Canada, since it is one of the dominant tree species in the boreal mixedwood forest (Huang et al. 2010). However, it does not tolerate soil salinity well and it also often suffers from drought stress (Khasa et al. 2002, Hogg et al. 2002). Upland forests in Alberta, Canada, have commonly limited water supply in the summer, which results in extensive tree dieback (Hogg et al. 2002). Although earlier studies examined the effects of salt (Piatt and Krause 1974, Kamaluddin and Zwiazek 2002, Yi et al. 2008) and drought stresses (Siemens and Zwiazek 2003, Siemens and Zwiazek 2004, Voicu and Zwiazek 2011) on trembling aspen, there is little information concerning the effects of combined salinity and drought stress and potential contribution of hydraulic lift to salt

redistribution during drought events. The objectives of the present study were to determine 1) whether hydraulic lift occurs in trembling aspen, 2) to what extend salt is redistributed by the root system through hydraulic lift, and 3) what the combined effects of salinity and drought are on the physiology of trembling aspen. I hypothesized that hydraulic lift occurs in trembling aspen and this process can redistribute salt from lower parts of the soil when water is discharged in the upper soil layer.

2.2 Materials and Methods

2.2.1 Experimental set-up

Plants were grown in polyvinyl chloride (PVC) tubes (10-cm in diameter) divided into two parts. The upper part of the tube was 30-cm long, and the lower part was 35-cm long (Fig. 2.1). Holes were drilled at 2 cm from the end of the lower tube and iron wires were knitted into the holes to form a web supporting a 0.5-cm-thick Styrofoam board. The lower PVC tube was filled with the horticultural soil mix (Sunshine Professional Growing Mix 2.8CU FT SS LA4, Sun Gro Horticulture, Seba Beach, Alberta), that consisted of sphagnum peat moss, coarse perlite, dolomitic limestone, and a long-lasting wetting agent. The tube was topped with a 3-cm-thick layer of polystyrene beads (3-mm in diameter), connected with an adhesive tape to the upper tube that was filled with the horticultural soil mix. The set-up prevented upward movement of NaCl and water

through the polysterene beads and allowed for an easy root penetration from the upper into the lower layer.

2.2.2 Plant material

This study was conducted with trembling aspen seedlings (*Populus tremuloides* Michx.) one-year-old seedlings (obtained from Coast to Coast Reforestation Inc. Edmonton, AB, Canada). Roots of the dormant plants were placed in the upper PVC tubes (described above) filled with horticultural soil and grown for three months in the controlled-environment growth chamber, at 25/18°C (day/night) temperature, 30±5 % relative humidity, and 16-h photoperiod (6:00 to 22:00) with 350 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the top of the seedlings provided by the full spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada). The plants were watered with distilled water every second day and fertilized with 14-14-14 Scotts Osmocote slow-release fertilizers (ICL Group, Dublin, OH, USA).

2.2.3 Drought and salt treatment

After three months of growth, roots were distributed in both the upper and lower layers. Plants of a similar size (approximately 70 cm in height) were selected and used for the drought and salt treatments. Half of the plants were subjected to drought by withholding watering until 80% of the plants showed signs of wilting. The other half of

plants were watered every two days until the start of NaCl treatment and served as the drought control. Soil relative water content (volumetric water content) was monitored over time using the time-domain reflectometry (TDR) probes inserted into the soil (Robinson et al. 2003). After eight days, drought treatment reached the desired level and the lower 8-10 cm parts of the tubes with both drought-stressed and drough control plants were immersed in 0, 30, or 60 mM NaCl solutions for two weeks. During the salt treatment period, no water was supplied to the upper layer. There were 12 plants for each of the six (NaCl × drought) treatment combinations.

2.2.4 Measurements

2.2.4.1 Gas exchange

Net photosynthesis (Pn) and, transpiration (E) rates were measured daily in six plants per treatment combination using a portable open-flow photosynthesis system equipped with a red/blue LED light source (LI-6400XT, LI-COR, Inc., Nebraska, USA). Photosynthetic photon flux density was set at 400 μmol·m^{-2} s^{-1}, leaf temperature was kept at 28 °C and reference CO_2 concentration was maintained at 400 μmol·mol^{-1} using the 6400-01 CO_2 mixer. All measurements were carried out between 09:00 AM and 12:00 PM on the uppermost fully-expanded leaves. When readings were stable, data were logged every 10 to 20 s during a one-min period. The average of 3 to 4 measurements from each plant was used for further data analysis. For the leaf gas exchange, the same

leaf from each plant was used for repeated measurements over the three-week period.

2.2.4.2 Leaf chlorophyll concentrations

Chlorophyll concentrations were determined in fully-expanded leaves (after 14 days of salt treatments) harvested from six randomly-selected seedlings per treatment (n = 6). Leaves were freeze-dried and ground with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from pulverized leaf samples (10 mg dry weight) with 8 mL dimethyl sulfoxide (DMSO) at 65 °C for 22 h. Chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden), at 648 nm for chlorophyll-a and 665 nm for chlorophyll-b. Total chlorophyll concentrations were calculated using the Arnon's equation (Sestak et al. 1971).

2.2.4.3 Predawn and midday stem water potential

Pre-dawn (4:00 to 6:00 h) and midday (13:00 to 15:00 h) stem water potential (ψ_w) measurements were conducted using a Scholander-type pressure chamber (PMS instruments, Corvallis, OR, USA). Stem water potential was measured in leaves that were enclosed *in situ* in plastic Ziploc® bags lined with aluminum foil for a minimum of 1h in order to stop transpiration and approach equilibrium with the stem water potential (Lu et al. 2010). Only one mature leaf was enclosed in one individual plant for midday stem

water potential measurement. Bagged leaves were then excised and immediately placed in the pressure chamber. Leaves were chosen from the lower middle part of the seedlings. Four different seedlings were taken for the measurements.

2.2.4.4 Soil water content

Soil water content was measured with a 1502C Metallic Time-Domain Reflectometer (TDR) (Tektronix, Inc., Beaverton, OR, USA). A three-rod (1.5 mm in diameter, 15-cm long) TDR stainless steel probe was vertically inserted into the soil. For each treatment, 6 samples of soil water content were measured every morning between 8:30 and 9:30 h. A calibration curve (R^2=0.9885) oforwater and soil volumes was made using 15 points ranging from 0% to 100% before the measurements.

2.2.4.5 Sodium concentrations in plant tissues and soil

Soil samples were taken from six PVC tubes per treatment combination and plants were harvested at the end of the NaCl treatment to assess Na^+ concentrations. Soil and root samples were collected from the upper and lower PVC tubes. Root samples for the upper tube were taken at 15 to 25 cm depth and root samples for the lower tube were taken at 45 to 55 cm depth. All soil samples were taken near the corresponding root samples. Plant stems, root (rinsed by tap water for 5 to 10 seconds) and soil samples were dried in an oven at 70°C for 72 h. Dried (0.2g) pulverized samples were digested with 10

ml 70% HNO_3 at 185°C for 10 min in a microwave oven (Mars 5 Microwave Accelerated Reaction System, CEM, Matthews, NC, USA) and diluted with Milli-Q water to 40 ml. Sodium concentration was then determined with the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Radiogenic Isotope Facility of the University of Alberta (Zarcinas et al. 1987).

2.2.4.6 Data analysis.

All measurements were carried out in 6 randomly selected plants (n = 6) per treatment combination. The data were analyzed by One-way ANOVA in Figures 2.3 (water potential in different salt or pH treatments), 2.6 (chlorophyll concentration in different salt or pH treatments), 2.7(soil sodium concentration in different salt or pH treatments), 2.8 (plant tissue sodium concentration in different salt or pH treatments) and Table 2.1(soil water content in different salt treatment); Two-way ANOVA was used for analysis of drought and NaCl effect on leaf chlorophyll and stem sodium concentrations, Three-way ANOVA was used for drought, NaCl, and time (Pre-drawn and middle-day) comparisons on stem water potential as well as drought, NaCl, and location (upper *vs* lower layer) root Na^+ concentration using IBM SPSS Statistics (version 21, IBM Corporation, Armonk, New York, US) and separation of means was conducted based on Duncan's multiple range test at the 0.05 significance level. Data were transformed by $\log_{10}$ when the original data did not meet the normality and homoscedastic postulates.

2.3 Results

2.3.1 Soil water content (upper tube)

In well-watered treatment, in which the upper tube was watered every two days, the soil water content was maintained above 70% before the NaCl solution was added (Fig 2.2). For the drought treatment, soil water content in the upper tube steadily decreased from about 70% to 38.6% during the eight days in which watering was suspended (Fig 2.2).

When the lower parts of the tubes were immersed in NaCl solutions, soil water content in the upper part of the drought-stress treatment significantly increased after one day from 39.7%, 37.6 % and 38.4% to 42.2%, 42.2% and 43.4% in 0 mM, 30 mM and 60 mM NaCl treatments, respectively (Table 2.1, Fig 2.2). The mean soil water content remained stable at 44% for the rest of the treatment period (16 days) and was about 13% higher than the soil water content measured on the 8^{th} day of treatment. In contrast, immersing the pipes with non-droughted plants in the NaCl solutions led to a progressive decrease in the soil water content in the upper layer, reaching 45% after 6 days and then remaining stable in all NaCl concentration treatments for the remaining treatment duration (Fig 2.2).

2.3.2 Stem water potential

After 6 days of drought and two weeks of NaCl treatments, stem predawn water potentials were significantly lower in both well-watered and drought treatments compared with control (0 mM NaCl) (Fig 2.3 A). A similar tendency was also observed for the midday water potential. The stem midday water potentials decreased with the increase in NaCl treatment concentration in both drought and well-watered treatments (Fig 2.3 B).

The predawn water potentials were significantly higher than the midday water potentials and no significant differences were found between the drought and well-watered treatments after two weeks of treatments with NaCl (by two-way ANOVA analysis, Table 2.2). No significant interactions were found for the stem water potential between the three variables (Table 2.2).

2.3.3 Gas exchange

2.3.3.1 Net photosynthesis (Pn)

Net photosynthesis (Pn) in well-watered plants fluctuated before the NaCl treatments (Fig. 2.4). After the plants were immersed in NaCl solutions, Pn in well-watered plants decreased until day 11 of the salt treatment (three days after the start of NaCl treatments) (Fig. 2.4). The Pn increased after 13 days in 0 mM NaCl well-watered

treatment and was maintained at a similar level for the next several days (Fig. 2.4). In well-watered plants treated with 30 mM NaCl, Pn increased in the following four days until day 15 of treatment and then it decreased to the similar lower level after 11 days of treatment (three days after NaCl treatments) in the following 8 days. The Pn continued decreasing after 11 days of treatment with 60 mM NaCl in well-watered treatment until the end of the experiment. (Fig. 2.4).

The Pn in drought-stressed plants decreased sharply before the 9^{th} day of treatment (Fig. 2.4). After immersing the pipes with the drought-treated plants in 0 mM, 30 mM and 60 mM NaCl solution, Pn increased quickly on day 11 of the treatment (Fig. 2.4). After 8 days of treatment, Pn of the plants treated with 0 mM NaCl increased and remained steady for the next several days. The Pn in plants subjected to 30 mM and 60 mM NaCl solutions, decreased after 13 days of treatment and the decrease continued for the remaining measurement days (Fig. 2.4).

2.3.3.2 Transpiration rates (E)

Transpiration rates (E) show a similar trend to Pn (Fig. 2.5). In well-watered plants, E increased for the initial two days before the tubes with plants were immersed in the different NaCl treatment solutions. In drought-stressed plants, E decreased dramatically after drought was imposed and before the onset of NaCl treatments. One day after NaCl treatments, E in both drought-stressed and well-watered plants increased when subjected

to 0 mM NaCl and remained relatively high in the following days (Fig. 2.5). In plants subjected to 30 and 60 mM NaCl, E initially increased after one day of treatment in both drought-stressed and well-watered plants and then decreased again over time (Fig. 2.5).

2.3.4 Leaf chlorophyll concentration

Chlorophyll concentrations in leaves decreased with the increasing NaCl treatment concentration in both drought and well-watered treatments (Fig 2.6). Overall, the concentration of leaf chlorophyll in the well-watered group was significantly higher than in the drought-stressed plants (Table 2.3). Chlorophyll concentrations significantly declined in plants subjected to 30 and 60 mM NaCl (Table 2.4). No statistical interactions were found for the chlorophyll concentrations between drought and well-watered conditions and between 30 and 60 mM NaCl) treatments (Table 2.3).

2.3.5 Soil Na^+ concentrations

Sodium concentration in the lower soil layer (lower tube) significantly increased with the increasing NaCl treatment concentrations in both drought and well-watered treatments (Fig 2.7 A & B). Soil Na^+ concentration increased from about 1.1 g kg^{-1} in 0 mM NaCl treatment to 9.4 g kg^{-1} in the 30 mM NaCl drought treatment and 8.6 g kg^{-1} in the 30 mM NaCl well-watered treatment. In the 60 mM NaCl treatment, Na^+ concentration was approximately 13.5 g kg^{-1} in the well-watered lower soil layer (lower

tube) and 14.6 g kg^{-1} in the drought-stressed treatment. There were no significant differences in Na^+ concentrations between the drought and well-watered treatments in the upper soil layer in each NaCl concentration (Fig 2.7 A & B). For both 30 and 60 mM NaCl treatments, the Na^+ soil concentrations were significantly higher in than the upper compared with the lower tube (Fig 2.7).

The three-way ANOVA results showed a significant increase of soil Na^+ in the lower tube (Table 2.6). Na^+ concentration increased significantly with the increased NaCl concentration treatment (Table 2.6 and 2.7). No significant differences were present between the drought and well-watered treatments (Table 2.6). Statistical interactions were found between NaCl treatment and different soil layers (Table 2.6).

2.3.6. Tissue Na^+ concentrations

No significant differences were found between stem Na^+ concentrations for each NaCl concentration treatment in both drought-stressed and well-watered plants (Fig. 2.8 A, B). In plant roots from the upper tube in the drought treatment, Na^+ concentration was significantly higher in the 60 mM NaCl treatment compared with the 0 and 30 mM NaCl treatments (Fig. 2.8A). In roots from the lower tube subjected to drought, Na^+ concentrations significantly increased with the increasing NaCl treatment concentration (Fig. 2.8 A). Sodium concentrations in roots from the lower tube were almost 11 times higher in the 30 mM NaCl treatment and almost 18 times higher in the 60 mM NaCl

treatment compared with the 0 mM NaCl treatment (NaCl control) (Fig. 2.8A). A similar tendency was found in roots from the upper tube in well-watered plants with significant differences between the 60 mM NaCl treatment compared with the 0 and 30 mM NaCl treatments (Fig 2.8 B). In the lower tube (Fig 2.8 B), root Na^+ concentrations significantly increased with the increasing NaCl treatment concentration. Sodium concentrations in roots from the lower tube were almost 15 times higher in the 30 mM NaCl treatment and 23 times higher in the 60 mM NaCl treatment compared with the 0 mM NaCl treatment (Fig. 2.8).

Overall, three-way ANOVA showed a significant increase of Na^+ in the roots present in the lower tube (Table 2.8). Root Na^+ concentration significantly increased in plants treated with NaCl (Tables 2.8, 2.9). No significant differences were found between the drought and well-watered treatments (Table 2.8). Statistical interaction was found between NaCl treatments and roots from the upper and lower tubes (Table 2.8).

2.4 Discussion

I demonstrated water redistribution by roots of trembling aspen (*Populus tremuloides*) seedlings. When the lower part of the root system of drought-stressed seedlings was immersed in water and NaCl solutions, the upper soil layer, that was separated from the lower layer by the polysterene beads, showed significant increases in water content (Table 2.1). In the next two weeks (Fig 2.2), soil water content further

increased in the upper layer and reached about 44% of the soil volumetric water content, which was 13% higher than the soil water content measured in the soil of the drought-treated plants. This substantial increase demonstrated that trembling aspen could potentially alter soil water dynamics under drought conditions. Hydraulic redistribution could be also observed in well-watered plants. After withholding watering from the upper soil layer, the decrease in soil moisture of the soil in the upper tube was due to evapotranspiration. The declining trend in soil water content continued for four days until it reached approximately 45%, which was similar to the level in the drought stress treatment. This indicates that without water release from the parts of the root system, the soil water content in the upper tube would have decreased to about 37% (the same level as in the drought treatment) (Fig 2.2) or lower.

The process of hydraulic redistribution, was initially thought to be present mostly in deep-rooted plants (Prieto et al. 2012). However, it is presently believed to be a widespread phenomenon and has been reported for herbaceous and woody plants growing in various ecosystems, including poplars (Zapater et al. 2011). The present study demonstrates hydraulic redistribution in *Populus tremuloides* and suggests that this process may play an important role in the growth and survival of trembling aspen, and may potentially affect neighboring plants under fluctuating soil moisture conditions.

Salinity inhibits plant growth through a combination of osmotic, ionic, and nutrition

effects (Zhu 2001, Parvaiz and Satyawati, 2008). High salt concentrations in the soil inhibit water uptake, disrupt metabolic processes and reduce the efficiency of photosynthesis (Munns and Tester 2008, Sohrabi et al. 2017). For most woody perennials, the majority of Na^+ taken up by roots is retained in the woody roots and stems (Tester and Davenport 2003, Muuns and Tester 2008). Tree seedlings, including jack pine (*Pinus banksiana*), red-osier dogwood (*Cornus stolonifera*), white spruce (*Picea glauca*), oak (*Quercus rober*) and paper birch (*Betula papyrifera*), have an ability to restrict Na^+ accumulation in roots and limit translocation to shoots (Alaoui-Sossé et al. 1998, Renault et al. 2001, Muhsin and Zwiazek 2002, Franklin and Zwiazek 2004, Nguyen et al. 2006, Yi et al. 2008). My results demonstrated that the lower part of the root system accumulated more Na^+ compared with the upper part. This may partly be due to the contact of the lower part of the root system with the NaCl solution resulting in a greater salt uptake through the roots from the lower soil layer. The soil Na^+ concentrations in the upper tube were not affected by the NaCl treatments applied to the soil in the lower tube suggesting that the release of water by the roots through hydraulic lift was not accompanied by a significant release of Na^+. In the field study of the coastal habitat, the *Pistacia lentiscus* shrubs supplied water to nearby *Juniperus phoenicea* plants growing in a saline soil through hydraulic lift, however, this process did not appear to affect the growth and physiology of juniper plants (Armas et al. 2010). Armas et al. (2010)

suggested that salt may be taken up and discharged by the hydraulic lift. However, Bazihizina et al. (2017) pointed out that hydraulic redistribution is very limited in plants growing in saline environments where differences in soil salinity result in external osmotic gradients. In addition, during the night or under lower transpirational conditions, the asymmetric distribution of influx and efflux carriers within the root and the Casparian strip at the endodermis may prevent backflow of ions from the stele to the apoplast of the cortex (Robbins et al. 2014, Bazihizina et al. 2017). Therefore, the limitation of influx and efflux carriers and the Casparian strip at the endodermis in roots could be the reason for no significant release of Na^+ in aspen seedlings by hydraulic lift that I observed in the present study.

Leaf transpiration and photosynthetic rates can reflect the plant's hydraulic properties (Meinzer 2002, Mencuccini 2003, Brodribb and Field 2000). In the present study, plant gas exchange followed a similar pattern to the soil water content (Fig 2.4, 2.5). In the well-watered treatment, Pn and E declined after withholding watering from the upper soil layer. The decreases of Pn and E in well-watered plants may be due to a water decrease in the upper soil layer in the first few days after withholding soil water supply to the upper tube (the lower tube was immersed in NaCl solutions), which caused some degree of water deficiency in plants. It has been demonstrated that some woody plants prefer to use water from the sub-surface layers of the soil where the majority of

fine roots are present (Weltzin and McPherson 1997, Moreira et al. 2000). In response to the drought treatment, plant Pn and E quickly decreased and started recovering two days after immersing the lower tubes in treatment solutions. It took several more days for the plants to fully recover pointing to the importance of the upper part of the root system for water uptake. The Pn and E increased and recovered to the-pre-drought level only after the plants absorbed more water from the lower soil layer. In plants placed in the NaCl solutions, the Pn and E values remained low due to salt stress (Yi et al. 2008). The quick increase and decrease of Pn and E in 30 mM and 60 mM NaCl solutions under drought treatment indicated that water deficiency in plants was an overriding factor for their gas exchange responses. Even under NaCl stress, a transient increase of Pn and E three days after the drought stressed plants were subjected to the NaCl treatments applied to the lower tube (Fig. 2.4,2.5). It is highly possible that the transient increase of gas exchange may due to the alleviation of osmotic stress that was triggered by the drought treatment, and then, after three days of NaCl stress, ion toxicity (Munns 2005, Franco et al. 2011) and NaCl-induced osmotic stress (Gulzar et al. 2003) led to the decreases of Pn and E.

Changes in chlorophyll concentrations have been used to monitor the impact of environmental stresses on plants (Lagriffoul et al. 1998, Xiao et al. 2008, Keyvan 2010). Similarly to other studies (Xu et al. 2014, Meng et al. 2016a, Otgonsuren et al. 2016), NaCl treatments caused a significant decrease in leaf chlorophyll concentrations in well-

watered trembling aspen plants. In drought-treated plants, leaf chlorophyll concentrations also significantly decreased since trembling aspen is sensitive to drought (Meng et al. 2016b, Chen et al. 2017). However, there was no additive effect of NaCl treatments and drought on leaf chlorophyll concentrations.

Salinity affects plants by reducing water availability and increasing tissue ion concentrations, which contribute to a decrease in plant water potentials (Renault et al. 1998). In the present study, both predawn and midday stem water potentials significantly decreased in plants subjected to NaCl treatments. Significantly higher predawn stem water potential than the midday stem water potential (Table 2.2) may be due to root hydraulic lift during the night time (Prieto et al. 2010).

Hydraulic lift in plants is widely accepted to play an important role in maintaining plant water balance innatural ecosystems (Ludwig et al. 2003, Moreira et al. 2003, Anderegg et al. 2018). Also in agro-ecosystems, hydraulic lift under drought stress can benefit both shrubs and neighboring annual plants in water-limited environments (Hirota et al. 2004, Kizito et al. 2012). However, hydraulic lift could also potentially benefit plants in challenging reclamation areas, such as those affected by surface mining. The oil sands mining in northeastern Alberta, Canada, severely disturbs large areas of the boreal forests, which need to be restored following mine closure (Huang et al. 2015). The processes of bitumen extraction and backfill of soil may result in heterogeneous soil

salinity in reclamation areas (Lazorko and Van Rees 2012). Additionally, abrupt changes in soil texture (textural interface or discontinuity) in the oil sands reclamation areas can largely restrict water and nutrient movement among the textural interface (Li and Liu, 2011, Peng et al. 2011, Jung et al. 2014). This can produce water deficit in the soil cover layer and result in water-deficit stress in shallow-rooted plants in reclamation areas during the periods of low precipitation. My results show that hydraulic lift increased the volumetric water content in the upper soil layer by 13% (Table 2.1, Fig 2.2). However, this increase was not accompanied by an increase of Na^+ in the upper soil layer when the lower soil layer (lower tube) was immersed in NaCl solutions (Fig 2.7). This demonstrates that in oil sands areas affected by salinity, trembling aspen could improve soil water conditions during drought without having a significant effect on salt redistribution in the soil. This could not only help aspen trees survive drought stress conditions, but also improve growth and survival of other reclamation plants, especially those with shallow root systems.

In summary, the results indicate that trembling aspen seedlings can hydraulically redistribute water through the root system and this process may also potentially have important consequences for the survival and growth of neighboring plants. The results also demonstrate that hydraulic redistribution by trembling aspen is not likely to be accompanied by Na^+ discharge into the soil by roots.. Therefore, in sites affected by soil

salinity and drought, trembling aspen could improve soil water dynamics and benefit neighbouring plants during the periods of drought. However, since hydraulic lift may be affected by soil texture (Hultine et al. 2006, Siqueira et al. 2008, Wang et al. 2009, Prieto et al. 2010), more studies will be required to examine the effects of different soil types on hydraulic lift in trembling aspen trees.

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2.6 Tables

Table 2.1 Soil water content in the upper tube following drought and NaCl stress treatments

	Soil water content (V/V)		
Day of Treatment	0 mM	30 mM	60 mM
6	44.30% ±1.2% b	43.29% ±0.6% b	46.22% ±1.3% b
7	43.80% ±0.7% b	42.46% ±0.3% b	44.38% ±1.1% b
8	39.74% ±0.5% a	37.61% ±1.5% a	38.36% ±1.8% a
9	42.21% ±0.8% ab	42.21% ±0.8% b	43.38% ±1.1% b
10	42.71% ±1.0% b	42.37% ±1.0% b	43.13% ±0.7% b

Day 6 represents two days before the lower tube was immersed in treatment solutions Data are means ± SE (n = 6); Different letters indicate significant differences in each column, One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

Table 2.2 Three-way ANOVA analysis of water (drought and well-watered), time (predawn and middle-day) and salt (0, 30 and 60 mM NaCl) effect on stem water potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.842[a]	11	.077	12.849	.000
Intercept	34.929	1	34.929	5861.993	.000
Salt	.481	2	.240	40.323	.000[b]
Water	.017	1	.017	2.823	.098
Time	.287	1	.287	48.137	.000
Salt * Water	.004	2	.002	.333	.718
Salt * Time	.030	2	.015	2.525	.089
Water * Time	.022	1	.022	3.763	.057
Salt * Water * Time	.002	2	.001	.128	.880
Error	.358	60	.006		
Total	36.129	72			
Corrected Total	1.200	71			

a. R Squared = .702 (Adjusted R Squared = .647). b. Duncan Post Hoc Test see Table 2.3.

Table 2.3 Post Hoc Tests of water, time and salt effect on stem water potential

Salt concentration	N	Subset	
		1	2
0 mM	24	.581822	
30 mM	24		.741779
60 mM	24		.765935
Sig.		1.000	.283

Based on observed means (Square root transformation). The error term is Mean Square (Error) = .006. Duncan: Uses Harmonic Mean Sample Size = 24.000. Alpha = .05.

Table 2.4 Two -way ANOVA analysis of water and NaCl effects on leaf chlorophyll concentrations

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	47.196[a]	5	9.439	3.123	.022
Intercept	1671.045	1	1671.045	552.820	.000
Salt	21.024	2	10.512	3.478	.044[b]
Water	22.154	1	22.154	7.329	.011
Salt * Water	4.018	2	2.009	.665	.522
Error	90.683	30	3.023		
Total	1808.925	36			
Corrected Total	137.879	35			

a. R Squared = .342 (Adjusted R Squared = .233). b. Duncan Post Hoc Test see Table 2.4

Water means: well-watered and drought treatments.

Table 2.5 Post Hoc Tests of water and salt effect on leaf chlorophyll concentrations

Salt	N	Subset	
		1	2
60	12	6.234966	
30	12	6.311329	
0	12		7.892917
Sig.		.915	1.000

Based on observed means. The error term is Mean Square(Error) = 3.023. Duncan: Uses Harmonic Mean Sample Size = 12.000. Alpha = .05.

Table 2.6 Three-way ANOVA analysis of water, location (upper layer and lower layer) and salt effect on soil sodium concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.149[a]	11	.104	203.635	.000
Intercept	241.969	1	241.969	471683.098	.000
Salt	.306	2	.153	297.940	.000[b]
Water	6.310E-005	1	6.310E-005	.123	.727
Location	.534	1	.534	1040.840	.000
Salt * Water	.000	2	.000	.361	.699
Salt * Location	.308	2	.154	300.093	.000
Water * Location	.001	1	.001	1.949	.168
Salt * Water * Location	.000	2	7.307E-005	.142	.868
Error	.031	60	.001		
Total	243.149	72			
Corrected Total	1.180	71			

a. R Squared = .974 (Adjusted R Squared = .969). b. Duncan Post Hoc Test see Table 2.7

Water means: well-watered and drought treatments; Location means: sodium in upper soil layer and lower soil layer.

Table 2.7 Post Hoc Tests of salt effect on soil Na^+ concentration

Salt	N	Subset		
		1	2	3
0	24	1.7418		
30	24		1.8693	
60	24			1.8886
Sig.		1.000	1.000	1.000

Based on observed means. ($\sqrt{Lg_{10}}$ transformation) The error term is Mean Square (Error) = .001. Duncan: Uses Harmonic Mean Sample Size = 24.000. Alpha = .05.

Table 2.8 Three-way ANOVA analysis of water, location and NaCl effects on root Na^+ concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1420942373.049[a]	11	129176579.368	43.004	.000
Intercept	821450750.127	1	821450750.127	273.470	.000
Salt	395406551.504	2	197703275.752	65.818	.000[b]
Water	124812.516	1	124812.516	.042	.839
Location	686075332.212	1	686075332.212	228.402	.000
Salt * Water	479692.307	2	239846.154	.080	.923
Salt * Location	336403345.882	2	168201672.941	55.996	.000
Water * Location	1453358.926	1	1453358.926	.484	.489
Salt * Water * Location	999279.703	2	499639.851	.166	.847
Error	180228125.360	60	3003802.089		
Total	2422621248.536	72			
Corrected Total	1601170498.409	71			

a. R Squared = .887 (Adjusted R Squared = .867) b. Duncan Post Hoc Test see Table 2.9

Water: well-watered and drought treatments; Location means: sodium in upper soil layer and lower soil layer.

Table 2.9 Post Hoc Tests of salt effect on root sodium concentration

Salt	N	Subset		
		1	2	3
0 mM	24	322.311398		
30 mM	24		3793.695235	
60 mM	24			6017.173705
Sig.		1.000	1.000	1.000

Based on observed means. The error term is Mean Square (Error) = 3003802.089. Duncan: Uses Harmonic Mean Sample Size = 24.000 Alpha = .05.

Table 2.10 Two -way ANOVA analysis of water and salt effect on stem sodium concentration

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	555.747[a]	5	111.149	.779	.573
Intercept	25479.569	1	25479.569	178.505	.000
Salt	81.097	2	40.549	.284	.755
Drought	229.659	1	229.659	1.609	.214
Salt * Drought	244.991	2	122.495	.858	.434
Error	4282.153	30	142.738		
Total	30317.470	36			
Corrected Total	4837.901	35			

a. R Squared = .115 (Adjusted R Squared = -.033).

2.7 Figures

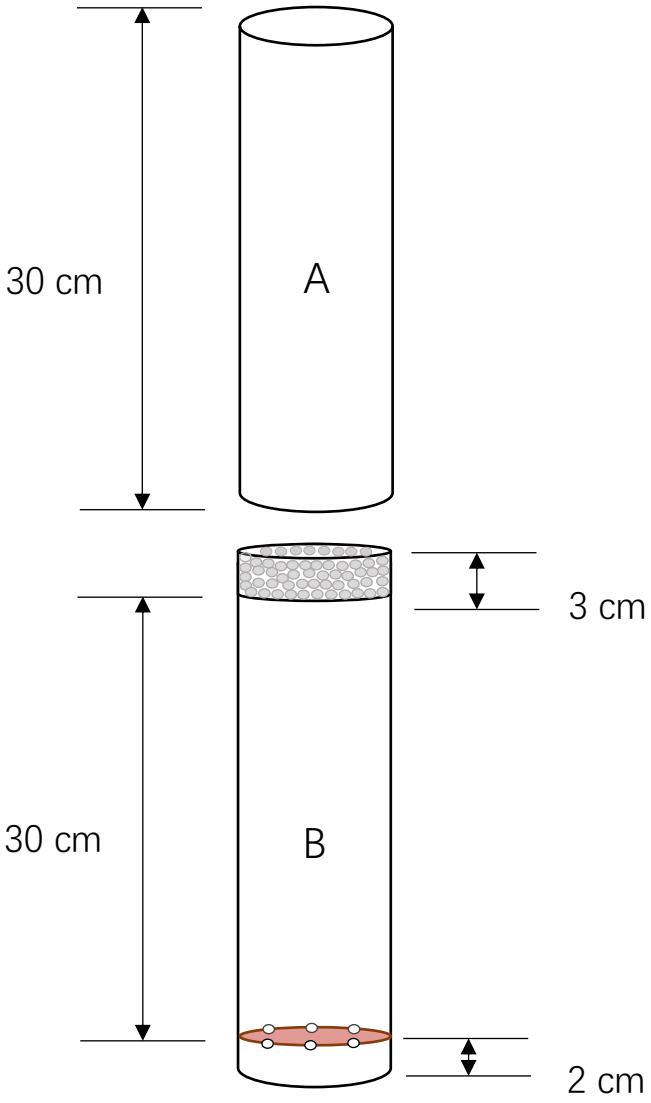

Figure 2.1: Schematic illustration of plant growth system. The root growth system was constructed in two-parts. Part A (upper tube): made by the 30-cm-long (10 -m in diameter) PVC tube. Part B (lower tube): made by the 35-cm-long (10-cm in diameter) PVC tube. Holes were drilled 2 cm above the base of the lower tube and iron wires were knitted to from a net supporting a 0.5 cm thick styrofoam board with the soil on the top. The growing mix in the upper and lower tube was separated by 3 cm thick polystyrene beads to prevent upward water movement in the soil from the lower layer.

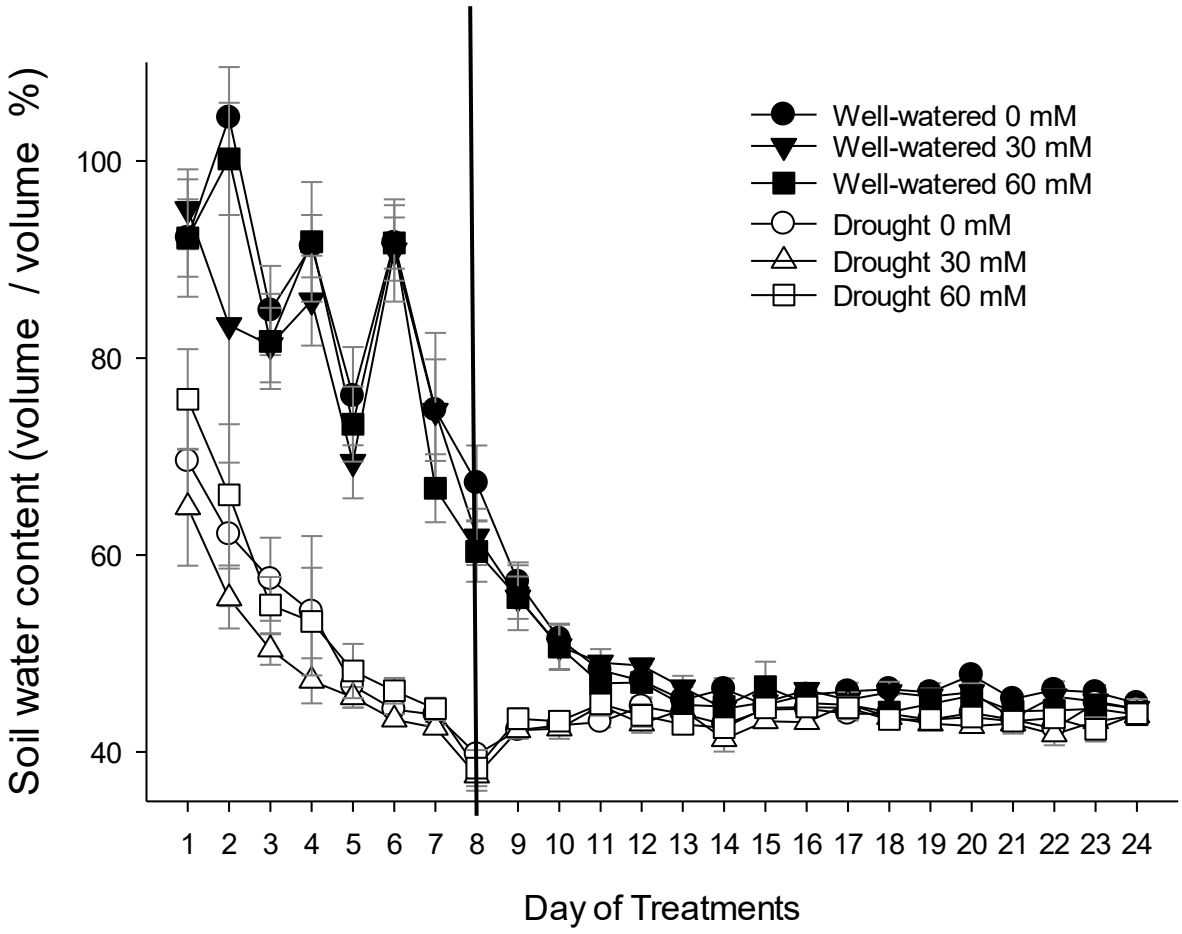

Figure 2.2 Daily change of the soil water content in the upper layer under different NaCl and watering treatments. Data are means (n = 6) ±SE. Water treatments were applied in the left of the vertical line, salt treatments were applied in the right of vertical line.

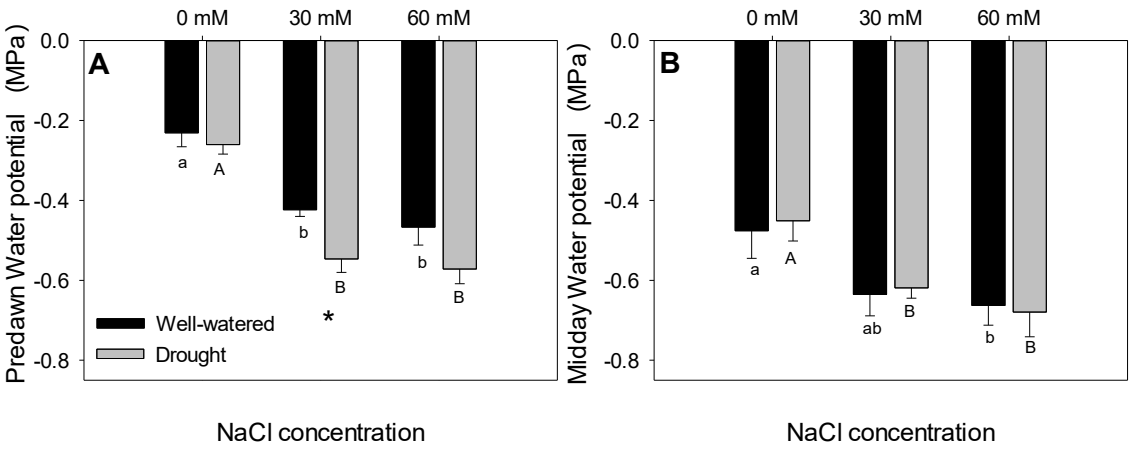

Figure 2.3: Predawn and midday stem water potentials in different watering and NaCl treatments. Data are means (n = 6) ±SE. Different lowercase and uppercase letters indicate significant differences in Well-watered and Drought plants in different NaCl treatments; * indicates significant differences between non-Drought and Drought treatment in same NaCl treatment.

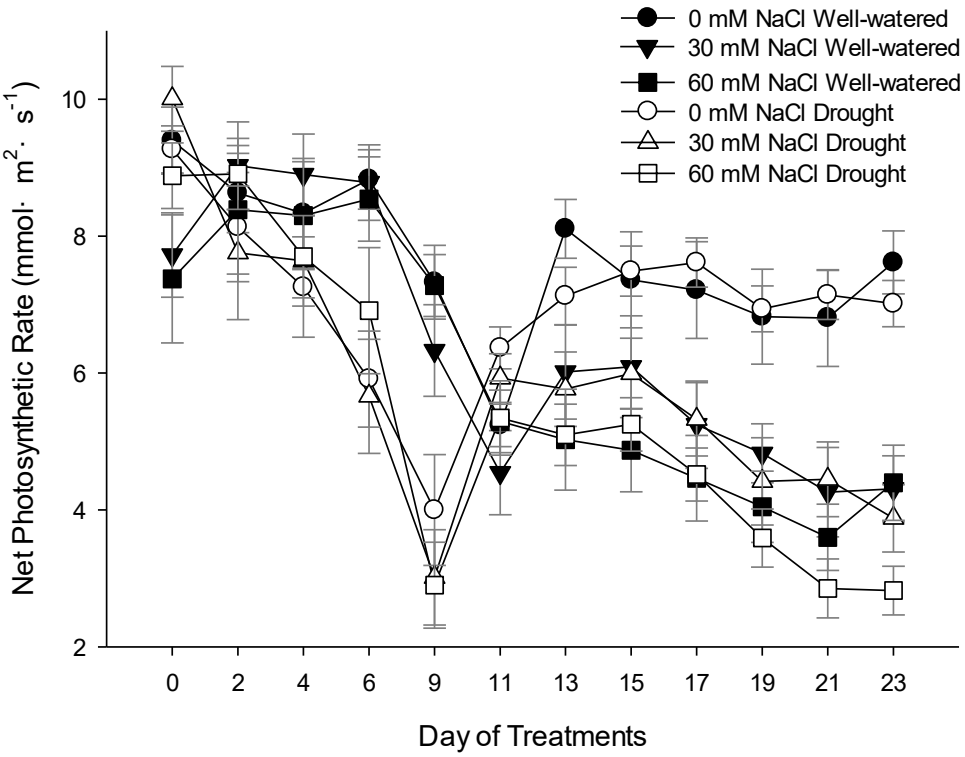

Figure 2.4: Changes of net photosynthetic rate (Pn) under different NaCl and watering treatment. Data are means (n = 6) ±SE. NaCl treatments started on the 8th day of treatment.

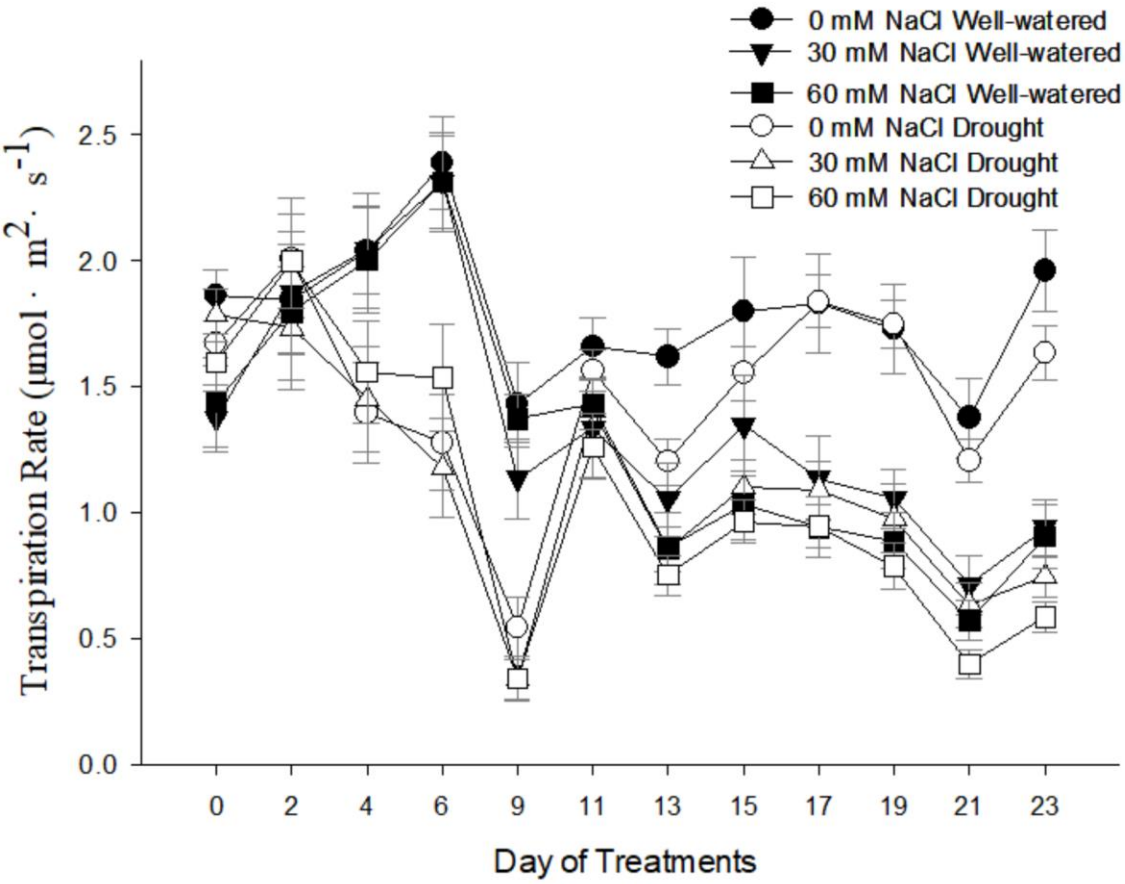

Figure 2.5 Changes of transpiration rate (E) under different NaCl and watering treatment. Data are means (n = 6) ±SE.

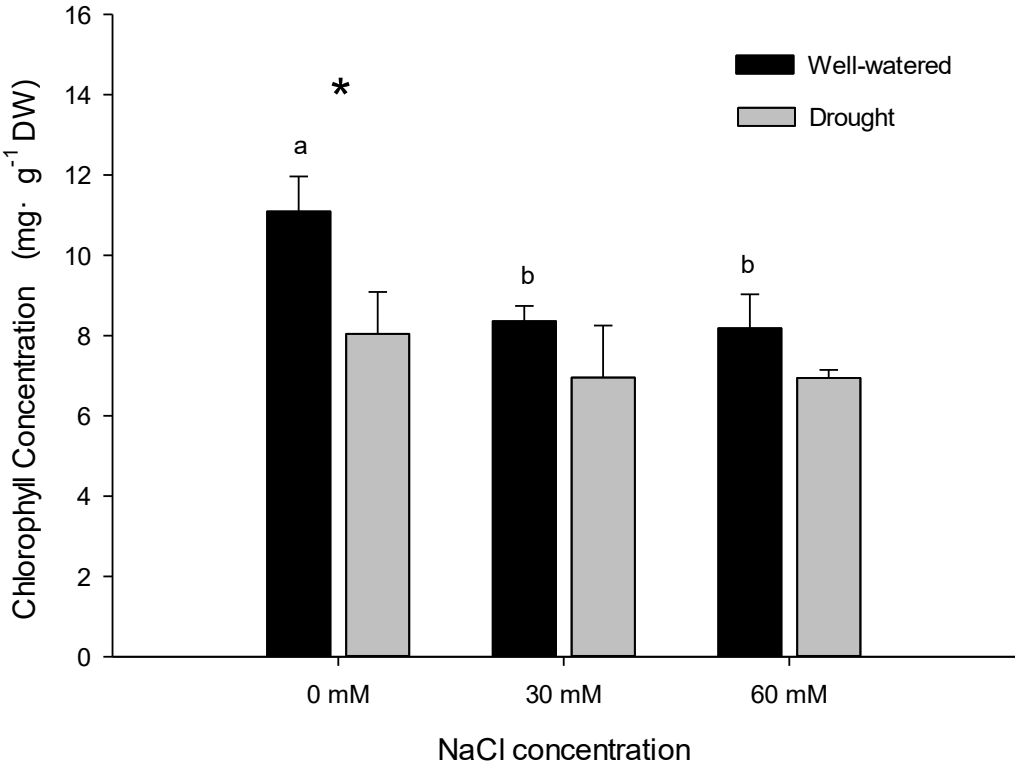

Figure 2.6: Chlorophyll concentration under different NaCl and water treatments. Data are means (n = 6) ±SE. Different lowercase letters indicate significant differences in Well-watered plants in different salt treatment; * Indicates significant differences between well-watered and drought stress treatments.

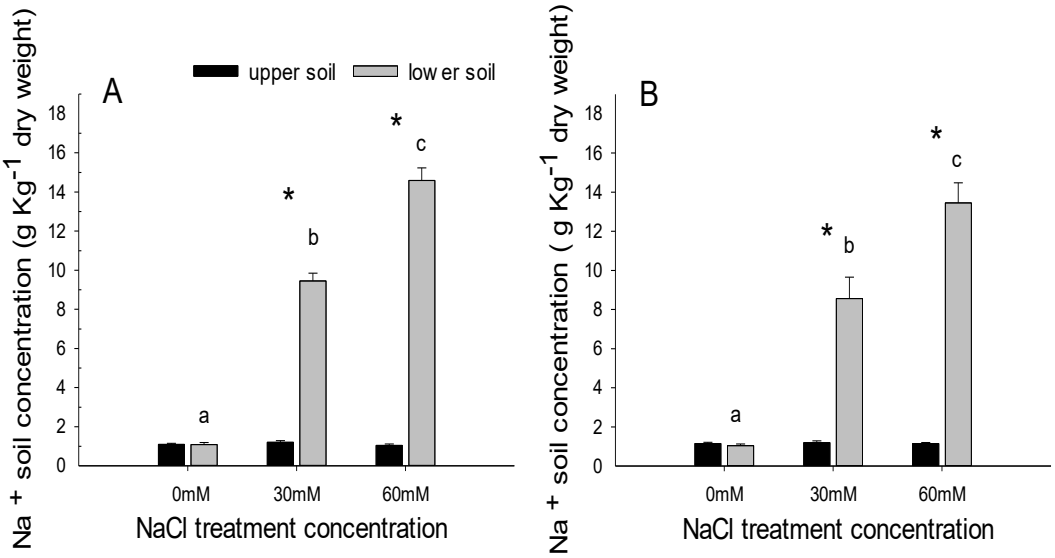

Figure 2.7: Soil Na^+ concentrations in the upper and lower tubes under deferent NaCl treatments (A: soil Na^+ concentration in drought treatment； B: soil Na^+ concentration in well-watered treatment). Data are means (n = 6) ±SE. Different lowercase letters indicate significant differences in lower layer of soil in different salt treatment; * Indicates significant differences between upper and lower tubes.

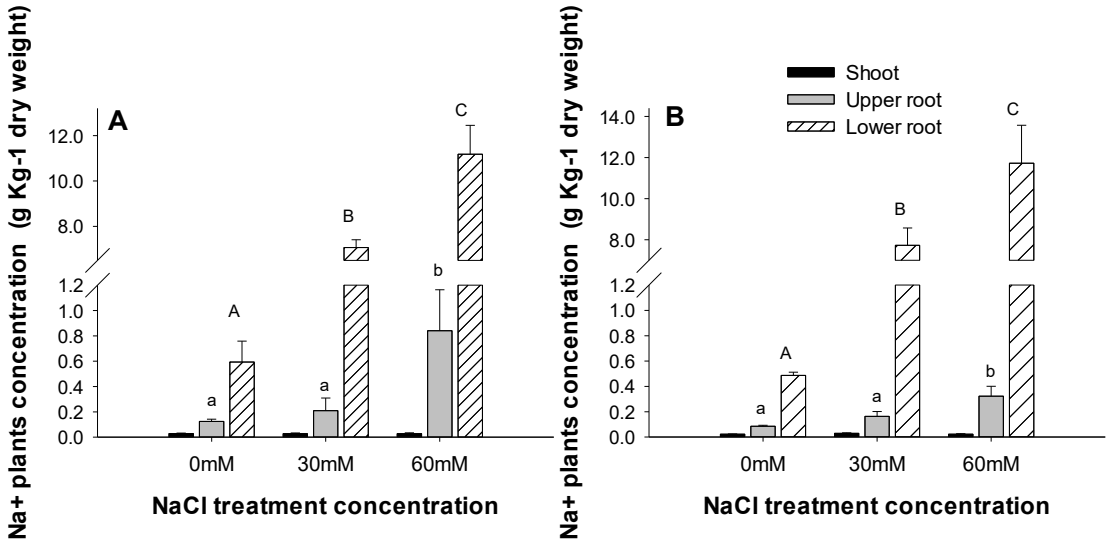

Figure 2.8 Sodium concentration in the different parts of the plant under deferent NaCl treatments (A: plant Na^+ concentration in Drought treatment； B: plant Na^+ concentration in Well-watered treatment soil). Data are means (n = 6) ±SE. Different lowercase and uppercase letters indicate significant differences in upper root and lower root in different salt treatment.

Chapter 3

Growth and physiological responses of yellow sweet clover (*Melilotus officinalis*) and slender wheatgrass (*Elymus trachycaulus*) to the presence of high soil pH and salt below the root zone

3.1 Introduction

The oil sands deposits in northeastern Alberta have been estimated to contain about 1.7 trillion barrels of bitumen (Fung and Macyk 2000). Oil sands mining is preceded by the removal of vegetation and stripping the soil and subsoil layers in the boreal mixedwood forests where many of the oil sands deposits are located. Following mine closure, these areas must be reclaimed to restore self-sustainable boreal ecosystems. In the oil sands reclamation sites, soil salinity has been identified among the most challenging revegetation concerns (Howatt 2000). Electrical conductivity values in the reclamation sites were reported to range from 0.60 to 6.32 dS m^{-1} in the soil cover and 4.50 to 9.30 dS m^{-1} in the overburden (Lazorko and Van Rees 2012). Heterogeneous salt distribution along the soil profile is common in these reclamation sites (Kessler et al 2010). A similar pattern has also been found in natural saline sites in the mixedwood forests in northern Alberta (Purdy et al 2005, Lilles et al 2010). In these sites, soil EC values were found to be below 4 dS m^{-1} in the top layer (0-20 cm) and from 4 to 23 dS m^{-1} in the lower subsurface layer (50-100 cm). In addition, the chemistry of oil sands mine tailings may alter soil chemistry in some of the reclamation sites and further elevate salinity (Lilles et al 2010).

The elevated soil salt levels in reclamation sites are often accompanied by high pH

(Allen 2008, Lilles et al. 2012). The pH of undisturbed soils in the boreal forests near Fort McMurray is typically below 6.0, while the soil pH in the oil sands reclamation areas frequently exceeds 8.0 (Howat 2000). The combination of salinity and high pH poses challenges to the survival and growth of plants in the oil sands reclamation areas.

Salinity reduces plant growth and survival due to the direct ionic effects, indirect osmotic effects, and altered mineral nutrition (Tester and Davenport 2003). The degree of sensitivity to salt also varies between different species of plants since the plants can use different strategies to resist salt stress. Stress avoidance is one of the most effective salt resistance strategy (Li and Zhang 2008). Numerous studies have examined the effects of salt stress and salt resistance strategies in plants, including woody perennials (Muhsin and Zwiazek 2002, Beritognolo et al 2007, Plett and Møller 2010, Calvo-Polanco et al 2014, Jimenez-Casas and Zwiazek 2014). However, relatively little research has been conducted concerning the effects of salinity on plant growth and physiology in the presence of other confounding environmental factors (Renault et al. 1999, Kopittke and Menzies 2005, Li et al. 2010). Several studies have shown that high soil pH can severely aggravate salinity problems in plants. Salt uptake can decrease the apoplastic pH in plants leading to growth inhibition (Pitann et al. 2011). Several studies have also reported the effects of soil pH on plant Na^+ uptake. In rice (*Oryza sativa*), shoot Na^+ uptake was increased by high pH (Ochiai and Matoh 2004). Similar effects of high pH on Na^+ uptake have been also reported for alfalfa (*Medicago sativa*) (Ruili et al. 2010) and American sweet flag (*Acorus americanus*) (Calvo-Polanco et al. 2014). Most of the studied boreal plant species are sensitive to high pH and suffer from nutrient imbalance and reduced growth (Zhang et al. 2013, Calvo-Polanco et al. 2017).

In both the natural areas affected by salinity and in reclamation sites, about 80% of plant roots were found in the upper 30 cm of the soil profile (Purdy et al. 2005, Lazorko and Van Rees 2012, Lilles et al. 2012). A smaller fraction of roots was present in the

saline-sodic overburden (Lazorko and Van Rees 2012) and in the lower subsurface layers in natural saline sites (Lilles et al. 2012). It appears that successful survival of plants in the sites with elevated salinity may be related to their ability to take advantage of heterogeneous salt distribution in the soil and distribute their roots in the low salinity areas (Purdy et al 2005). It is not clear if this is part of the adaptive processes present in local plant populations or represents a wider and more general salt-avoidance mechanism. Little is also known about the rooting responses of different plant species to salt and about the rooting patterns that plants develop when salt is unevenly distributed in the soil.

In the present study, I examined the growth and physiology of plants respond to NaCl and different pH levels whene these factors affect only the lower part of the root system. For this study, I selected two plant species that are commonly used for revegetation of oil sands areas: yellow sweet clover (*Melilotus officinalis*) and slender wheatgrass (*Elymus trachycaulus*). Yellow sweet clover was first introduced into Canadian grassland communities in the mid-1800s (Susan et al. 2008) and is commonly found in oil sands reclamation sites (Carey 2008). Slender wheatgrass is a native species that has also been recommended for the reclamation areas in Alberta (Renault et al. 2004). As pioneer herbal species, these plants are also known to be relatively salt tolerant (Pearen et al. 1996, Ghaderi-Far et al. 2010, Luo et al. 2016).

The objectives of the study were to determine 1) growth patterns and growth allocation in plants in which only part of the root system is in contact with the NaCl-affected soil substrate, 2) plant responses to heterogeneous soil pH conditions, 3) how plants respond to a combination of salt and high pH that are present only in the deeper soil layer, and 4) whether the two plant species adopt similar strategies to cope with heterogeneous salt and pH soil conditions. I examined the hypothesis that both plant species have similar strategies to avoid new root growth into the soil layers with elevated pH and salt.

3.2 Materials and methods

3.2.1 Experimental set-up

The experimental set-up consisted of two polyvinyl chlorides (PVC) tubes (10 cm in diameter). The upper tube was 30-cm long, and the lower one was 35-cm long (Fig. 2.1). Holes were drilled at 2 cm from the end of the lower tube and iron wires were knitted in those holes to form a net supporting a 0.5-cm-thick Styrofoam board. The lower PVC tube was filled with a 30-cm-thick commercial growing mix (sunshine professional growing mix 2.8CU FT SS LA4, Sun Gro Horticulture, Seba Beach, Alberta), that consisted of Canadian sphagnum peat moss, coarse perlite, dolomitic limestone, and long-lasting wetting agent. The top 3-cm of the lower tube was filled with polystyrene beads (3 mm in diameter). The upper and lower tubes were connected, sealed with an adhesive tape and the upper tube was filled with the growing mix to the top. The polystyrene beads (3-mm in diameter) were used to prevent an upward movement of salt and water, but to allow for root penetration into the lower layer.

3.2.2 Plant material and treatments

Slender wheatgrass (*Agropyrum trachycaulum* (Link) Gould ex Shinners ssp. *trachycaulus*), and yellow sweet clover (*Melilotus officinalis* (L.) Lam.) were used in the study. The experiment was carried out in the controlled-environment growth room at 25/18°C (day/night) temperature, 30 ± 5 % relative humidity, and 16-h photoperiod (6:00 to 22:00) with 350 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the top of the seedlings provided by the full spectrum fluorescent bulbs (Philips high output, F96T8/TL835/HO, Markham, ON, Canada).

Slender wheatgrass and yellow sweet clover seeds were surface-sterilized with 1.6%

sodium hypochlorite and germinated in trays on moist paper in the growth room. After three weeks, plants of similar size were transplanted into the upper PVC tube containing the soil adjusted with 10% HCl to pH 5 (soil pH was 5.2 to 5.6 when watered with the deionized water; for the pH 5 treatment, one to three drops of 10% HCl were mixed with 1 L of deionized water and added to the soil), while the soil pH in the lower layer was adjusted to either pH 5 or pH 8 by immersing the lower part of the tube in 10% HCl (the same treatment as above) or 0.4% KOH, respectively and 0, 30 or 60 mM NaCl. The lower part of tube (without seedlings) were immersed into high pH solution for a week prior to salt treatment. Once a week, the soil was flushed with water to prevent salt build up after the salt treatment started. The upper part was watered daily with a small amount of water to prevent runoff (for the first two months, the upper part of tube was not connect with the lower part of tube since the root were not growth out of the upper part). In the first 75 days, 250 to 500 ml of water was added every morning to the upper soil layer and in the following 75 days, 300 to 500 ml of water was added twice a day since the plants were larger. Hoagland's nutrient solution (25%) was used twice a week (for about two months) before the salt treatment. The relative soil water content was monitored using the time-domain reflectometry (TDR, Robinson et al 2003) to control water supply. One plant was grown in each PVC tube and 12 replicates were used for each plant species per treatment for the total of 72 plants. The plants were treated for 3 months.

3.2.3 Measurements

3.2.3.1 Plant dry weights

Plants were harvested after three months of treatments and their stems, leaves, and roots were separated. Each PVC tube was cut into four 15-cm sections and from each

section roots were collected. Leaf areas were measured using the Sigmascan Pro 5.0 computer software (Systat Software, San Jose, CA) following computer scanning. The stems and roots were dried in an oven at 70°C for 72 h and the leaves were dried in the freeze-drier for 72 h and weighed. Weights of the leaves and stems from each plant were combined to determine the shoot dry weights and the weights of the four root sections were combined to obtain the total root dry weights. The root percentage in each section was calculated by dividing the dry weights of roots by the total root dry weight.

3.2.3.2 Gas exchange

Net photosynthetic (Pn) and transpiration (E) rates were measured in six plants from each treatment (n = 6) using a portable open-flow photosynthesis system equipped with a red/blue LED light source LI-6400XT (LI-COR, Inc., Nebraska, USA). The PPFD was set at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, leaf temperature at 28°C, and reference CO_2 concentration at 400 $\mu mol \cdot mol^{-1}$. All measurements were carried out on the fully expanded leaves between 4 and 6 hours after start of the photoperiod. When the readings were stable, data were logged every 10 to 20 s during a one-min period. The average of 3 to 4 measurements from each plant was used for further data analysis. The leaf areas were calculated following computer scanning using the Sigma scan Pro 5.0 computer software (Systat Software, San Jose, CA).

3.2.3.3 Leaf chlorophyll concentrations

Chlorophyll concentrations were determined in fully-expanded leaves harvested from six randomly selected seedlings per treatment (n = 6). Leaves were freeze-dried and ground with a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from pulverized leaf samples (10 mg dry weight) with 8 mL dimethyl sulfoxide (DMSO) at 65°C for 22 h. Chlorophyll concentrations were measured in DMSO extracts

with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden), at 648 nm for chlorophyll-a and 665 nm for chlorophyll-b. Total chlorophyll concentrations were calculated using the Arnon's equation (Sestak et al. 1971).

3.2.3.4 Leaf water potential

Leaf water potential (Ψ_L) measurements were conducted with a Scholander-type pressure chamber (PMS instruments, Corvallis, OR, USA). The fully expanded leaves were taken for the leaf water potential measurements. Water potentials were measured 4 to 6 hours after the onset of photoperiod in 6 plants per treatment (n = 6).

3.2.4 Data analysis

The data were analyzed by one-way ANOVA followed by the Duncan's test to determine statistically significant differences between treatments. Two-way ANOVA was used for comparisons between the effects of two factors (salt x pH) using IBM SPSS statistical package (version 21, IBM Corporation, Armonk, New York, US) and separation of means was conducted based on Duncan's multiple range test at the 0.05 significance level. Data were transformed by $\log_{10}$ when the original data did not meet the normality and homoscedastic postulates.

3.3 Results

3.3.1 Effects of salt and pH on plant dry weights and leaf areas

3.3.1.1 Shoot dry weights

Both 30 and 60 mM NaCl treatments resulted in significant decreases in shoot dry weights in slender wheatgrass at both pH 5 and 8 compared to the control plants that were

treated with 0 mM NaCl (Fig. 3.1 A). A significant decrease in shoot dry weights was also recorded in control plants exposed to pH 8 compared with pH 5 (Fig. 3.1 A).

There was no statistically significant interactions between NaCl and pH treatments (P = 0.23) (Table 3.1).

At pH 8, shoot dry weights of yellow sweet clover slightly decreased in the 30 mM and 60 mM NaCl treatments and were 3% and 8% lower compared with NaCl control (Fig 3.1 B). At pH 5, shoot dry weights increased in the 30 mM NaCl treatment and decreased in the 60 mM NaCl treatment compared to control plants at the same pH (Fig 3.1 B). The pH 8 treatment resulted in lower shoot dry weights in yellow sweet clover compared with pH 5 treatment in all NaCl treatments (Fig 3.1 B).

There was no statistically significant interactions between NaCl and pH treatments (P = 0.38) (Table 3.2).

3.3.1.2 Root dry weights

Root dry weights of slender wheatgrass decreased in response to NaCl treatments at both pH 5 and pH 8 compared with the control (0 mM NaCl) treatment (Fig 3.1 C). Significant decreases of root dry weights were shown in plants subjected to the 60 mM NaCl compared with the 0 mM and 30 mM NaCl treatments at both pH 5 and pH 8 (Fig 3.1 C). Root dry weights were higher by36%, 20% and 2% at pH 5 compared with pH 8 in the 0, 30 and 60 mM NaCl treatments, respectively (Fig 3.1 C).

There was no statistically significant interactions between the NaCl and pH treatments (P= 0.208) (Table 3.1).

Root dry weights of yellow sweet clover decreased with the increase in NaCl treatment concentration at pH 5 (Fig. 3.1 D). At pH 8, the root dry weights were 15% higher in the 30 mM NaCl treatment and 22% lower in the 60 mM NaCl treatment compared with the 0 mM NaCl treatment (Fig 3. 1 D). The root dry weights were higher

by 66%, 15% and 66% higher at pH 5 than pH 8 in the 0, 30 and 60 mM NaCl treatments, respectively (Fig 3.2 A).

There was no statistically significant interactions between the NaCl and pH treatments (P = 0.48) (Table 3.2).

3.3.1.3 Total dry weights

The total dry weights of slender wheat plants were significantly lower in response to the 30 mM and 60 mM NaCl treatments at both pH 5 and 8 compared with the respective controls (0 mM NaCl at pH 5 and 8) (Fig 3.1 E). A significant decrease in dry weights was shown at pH 8 compared with pH 5 in all NaCl treatments (Fig 3.1 E).

There was no statistically significant interactions between the NaCl and pH treatments (P= 0.175) (Table 3.1).

The total dry weights of yellow sweet clover decreased slightly in the 30 mM and 60 mM NaCl treatments at pH 5, with 10% and 19% lower values compared with control (Fig 3.1 F). The total dry weights increased in the 30 mM NaCl treatment and decreased in the 60 mM NaCl treatment compared with 0 mM NaCl control plants at pH 8 (Fig 3.1 F). The total dry weights were lower at pH 8 compared with pH 5 in all NaCl treatments (Fig 3.1 E, F).

There was no statistically significant interactions between the NaCl and pH treatments (P = 0.63) (Table 3.2).

3.3.2 Root distribution

3.3.2.1 Effects of NaCl and pH on the slender wheatgrass root dry weight distribution in different soil layers

In control (0 mM NaCl) slender wheatgrass plants, nearly 80% of roots were

distributed in the upper (0 - 30 cm) soil layer and 20% of the roots were found in the lower (30 - 60cm) soil layer at both pH 5 and 8 (Table 3.3). However, when the slender wheatgrass plants were subjected to 30 and 60 mM NaCl, nearly 90% of the root dry biomass was present in the upper soil layer and only 10% of the root dry biomass was present in the lower soil layer (lower tube) at both pH levels (Table 3.1). No significant difference ($P = 0.3$) was shown for the root distribution in the lower soil layers between the pH levels and no significant interactions were found between NaCl and pH treatments in both upper ($P = 0.34$) and lower ($P = 0.50$) soil layers.

Root dry weights of slender wheatgrass in the 0 - 15 cm and 15 - 30 cm soil layers were significantly higher in the 0 mM and 30 mM NaCl treatments compared with the 60 mM NaCl treatment at pH 5 (Fig 3.2 A). In the 30 - 45 cm soil layer, the root dry weights were significantly lower in 30 mM and 60 mM treatments by 61% and 75%, respectively, compared with the 0 mM NaCl control treatment at pH 5 (Fig 3.2 A). In the deepest (45 - 60 cm) soil layer, dry weights of slender wheatgrass roots were 56% and 79% lower in the 30 mM and 60 mM treatments, respectively, compared with the 0 mM NaCl treatment at pH 5 (Fig 3.2).

At pH 8, the dry weights of slender wheatgrass roots were 14% and 9% greater in the 30 mM and 25% and 40% lower in the 60 mM NaCl treatments compared with control in the 0 -15 cm and 15 - 30 cm soil layers, respectively (Fig 3.2 B). In the lower soil layers (30 - 45 and 45 - 60 cm), the root dry weights of slender wheatgrass were significantly higher in the 0 mM NaCl treatment compared with the 30 and 60 mM NaCl treatments at pH 8 (Fig 3.2 B).

3.3.2.2 Effects of NaCl and pH on the yellow sweet clover root dry weight distribution in different soil layers

In yellow sweet clover, over 85% of the root dry biomass was found in the upper (0

- 30cm) soil layer in all treatments (Table 3.3). In the upper soil layer, 74% of root dry mass was found in the 0 - 15 cm layer at both pH 5 and 8 (Fig 3.2 C D). In both the upper (0 - 30 cm) and lower (30 - 60 cm) soil layers separated by the middle Styrofoam beans layer, there was no significant effect of NaCl treatments on root distribution (Table 3.7). The pH level had a significant ($P = 0.01$) effect on the root dry weight distribution of yellow sweet clover in the upper root layers. There were no statistical interactions between pH and NaCl treatments on the root distribution in both the upper ($P = 0.44$) and lower ($P = 0.38$) soil layers.

The root dry weights in the 0 - 15 cm layer were lower by 24% and 16% in the 30 mM and 60 mM NaCl treatments, respectively, compared with the 0 mM NaCl treatment at pH 5 (Fig 3.2 C). In the 15 - 30 cm soil layer at pH 5, root dry weights decreased with the increase in NaCl treatment concentration (Fig 3.2 C). In the 30 - 45 cm layer at pH 5, root dry weights of yellow sweet clover were 47% higher in the 30 mM NaCl treatment and 35% lower in the 60 mM NaCl treatment compared with the control (Fig 3.2 C). Significantly lower root dry weights were found in the 45 - 60 cm soil layer of the 60 mM NaCl treatment compared with control (Fig 3.2 C).

At pH 8, root dry weights were higher in the 30 mM and lower in the 60 mM NaCl treatments compared with control in both the 0 - 15 and 15 - 30 cm soil layers (Fig 3.2 D). However, in the 30 - 45 cm soil layer at pH 8, the root dry weights of yellow sweet clover were 81% and 39% higher in the 30 and 60 mM NaCl treatments, respectively, compared with the 0 mM NaCl control (Fig 3.2 D). In contrast, the dry weights of roots in the 45 - 60 cm soil layer at pH 8 were 66% and 80% lower in the 30 mM and 60 mM NaCl treatment, respectively, compared with control (Fig 3.2 D).

3.3.3 Leaf water potentials (Ψ_L)

The NaCl and pH treatments decreased leaf water potentials in both slender

wheatgrass and yellow sweet clover (Fig 3.3).

In wheatgrass, the Ψ_L decreased significantly in plants treated with 30 and 60 mM NaCl at both pH 5 and pH 8 compared with the respective 0 mM NaCl controls (Fig 3.3 A). A significant decrease in Ψ_L was shown in pH 8 treatment compared with pH 5 in plants treated with 0 mM 60 mM NaCl (Fig 3.3 A). No significant interaction effect was present between the NaCl and pH treatments (P = 0.98) (Table 3.1).

In sweet yellow clover at pH 5, Ψ_L was significantly lower in 60 mM NaCl compared with the 0 and 30 mM NaCl treatments (Fig 3.3 B). However, at pH 8, shoot water potential significantly decreased in plants treated with 30 and 60 mM NaCl compared with 0 mM NaCl (Fig 3.3 B). The Ψ_L was lower at pH 8 compared with pH 5 in all NaCl treatments (Fig 3.3 B).

There was no significant interactions between NaCl and pH treatments on Ψ_L (P = 0.32) (Table 3.2).

3.3.4 Net photosynthesis (Pn) and leaf transpiration (E) rates.

In slender wheatgrass, Pn decreased with the increase in NaCl treatment concentration at pH 5 and 8 (Fig 3.4 A). Compared with 0 mM NaCl controls, Pn in plants treated with 30 mM NaCl was lower by 11% and 10% at pH 8 and 5, receptively (Fig 3.4 A). A significant decrease of Pn was found at pH 8 compared with pH 5 in plants treated with 60 mM NaCl (Fig 3.4 A).

A similar trend of treatment effects on Pn as for slender wheatgrass was also observed in yellow sweet clover with Pn decreasing as a result of NaCl treatments at both pH 5 and 8 (Fig 3.4 B). Compared with pH 5, leaf Pn significantly decreased at pH 8 in all NaCl treatments (Fig 3.4 B).

In slender wheatgrass, a significant decrease of E was shown with the increase in NaCl treatment concentration at both pH 5 and 8 soil (Fig 3.5 A). No significant changes

in Pn were found between pH 5 and 8 in plants treated with 0 and 30 mM NaCl (Fig 3.5 A).

In yellow sweet clover, a significant decrease of E was shown with the increase in NaCl treatment concentration at pH 8 (Fig 3.5B). A significant decrease in leaf E was found in plants treated with 60 mM NaCl compared with control at pH 5 (Fig 3.5 B). An increase of pH from 5 to 8 resulted in significant decreases of E in the 0 and 60 mM NaCl treatments (Fig 3.5 B).

There were no statistically significant interactions between NaCl and pH treatments for Pn and E in both slender wheatgrass and yellow sweet clover (Tables 3.1,3.2).

3.3.5 Leaf chlorophyll concentrations

Leaf chlorophyll concentrations in slender wheatgrass treated with 30 and 60 mM NaCl were lower by 5 and 9% at pH 5 and 0.4 and 3% at pH 8, respectively, compared with the 0 mM NaCl treatment (Fig 3.6 A).

Similar changes in leaf chlorophyll concentration to those in slender wheatgrass were also shown in yellow sweet clover with 7% and 10% decreases in the 30 mM 60 mM NaCl treatments at pH 5 compared with control (Fig 3.6 B). At pH 8, the leaf chlorophyll concentrations were lower by 6% and 10% in the 30 and 60 mM NaCl treatments compared to plants treated with 0 mM NaCl (Fig 3.6 B).

3.4 Discussion

In my study, the two examined plant species showed different strategies to cope with soil salinity and high pH that affected the lower parts of their root systems. The shoot and root growth of slender wheatgrass was sharply inhibited when the lower soil layer (lower tube) was exposed to NaCl and/or high pH, but was relatively less affected in yellow

sweet clover.

Salinity induces osmotic stress, ionic stress and nutritional disturbances in plants (Zhu 2001, Parvaiz and Satyawati, 2008). In salt-tolerant species, plant growth is only moderately inhibited, or even stimulated by low salt concentrations (Renault et al. 1998, Siemens and Zwiazek 2003, Mu et al. 2016, Vaziriyeganeh et al. 2018). The shoot and root dry weights as well as the total dry biomass and leaf areas in slender wheatgrass were significantly decreased after a relatively small part of the system was subjected to the 30 and 60 mM NaCl treatments. Contrary to other studies that reported slender wheatgrass to be a salt-tolerant species (Pearen et al. 1996), my results demonstrate that the growth of these plants was affected even by relatively low NaCl levels. In contrast, yellow sweet clover did not show significant decreases in dry biomass and leaf areas when 30 and 60 mM NaCl were applied to the lower soil layer (30 - 60 cm). Surprisingly, a slight increase in shoot dry weights was found in plants treated with 30 mM NaCl (Fig 3.1 B). These results suggest that yellow sweet clover is more salt-tolerant compared with slender wheatgrass and could be a better choice for the reclamation of salt-affected sites. A related plant species *Melilotus indicus* was also reported to exhibit relatively high tolerance to salt stress (Sherif 2009).

High soil pH affects soil structure, plant nutrient uptake (Yang et al. 2007, Calvo-Polanco et al. 2017), and inhibits growth (Tang et al. 1992, Tang et al. 1993, Yang et al. 2009, Xu et al. 2019). In most studies, a combination of high pH and salinity stress was more deleterious to plants than each of these stresses alone (Shi and Sheng 2005). The survival rate of *Aneurolepidium chinense* was reported to be 100% when NaCl treatment concentration was below 125 mM or pH was below 8.8. However, when NaCl concentration was over 125 mM and pH was above 8.8, the survival rates sharply declined by increasing either NaCl concentration or pH (Shi and Wang 2005). In my study, high pH (0 mM NaCl at pH 8) significantly decreased total dry weights compared

with the low pH (0 mM NaCl and pH 5) in both slender wheatgrass and yellow sweet clover. Decreases in biomass were reported for several species of boreal woody plants exposed to high root zone pH (≥8) including red-osier dogwood (*Cornus sericea*), paper birch (*Betula papyrifera*), trembling aspen (*Populus tremuloides*), tamarack (*Larix laricina*), and jack pine (*Pinus banksiana*) (Zhang et al. 2013, Zhang et al. 2015, Zhang and Zwiazek 2016, Calvo-Polanco et al. 2017, Xu et al. 2019) .

Leaf transpiration (E) and net photosynthetic rates (Pn) have been frequently reported to be inhibited by high pH and salt stress (Wang et al. 2011, Zhang et al. 2015, Zhang and Zwiazek 2016, Calvo-Polanco et al. 2017, Xu et al. 2019). The responses vary between the different plant species. In barley (*Hordeum vulgare*), salt and alkalinity caused sharp decreases in plant E and Pn (Yang et al. 2009). In alfalfa (*Medicago sativa*), Li et al. (2010) found only a slight decrease of Pn and E under salt stress but gas exchange was sharply decreased by high soil pH. In my study, both NaCl and high pH treatments of the lower soil layer (lower tube) caused a significant decrease in Pn in slender wheatgrass and yellow sweet clover plants. The combined NaCl and high pH reduced Pn more than a single factor alone. However, NaCl stress significantly decreased E in yellow sweet clover and there was little effect of pH on E in 0 mM and 30 mM NaCl treatments.

Transpiration and net photosynthesis rapidly reflect the plant's osmotic imbalance and hydraulic properties such as tissue water potential and root hydraulic conductance (Brodribb and Field 2000, Meinzer 2002, Mencuccini 2003, Koyro 2006). Similarly to the gas exchange, Ψ_L significantly decreased in response to NaCl treatments in both plant species. High pH also resulted in a decrease of Ψ_L, but the change was not significant in yellow sweet clover. This may due to a better adaptation of yellow sweet clover to relatively higher pH as earlier reported (Baldridge and Lohmiller, 1990). However, the physiological mechanisms underlying the differences in Ψ_L responses of these two plant

species to high pH require further studies.

Although nutritional effects of high pH on plants cannot be ignored, in the shorter term, growth decreases of several studied boreal tree species exposed to high pH were found to be largely due to the decreases in gas exchange and root hydraulic conductivity, which reduced the rate of water transport to the leaves (Zhang et al. 2013, Zhang et al. 2014, Calvo-Polanco et al. 2017). In my study, both slender wheatgrass and yellow sweet clover showed reductions in gas exchange parameters and Ψ_L at pH 8, which likely contributed to the decrease of biomass.

No significant decrease was found in leaf chlorophyll concentrations as a result of NaCl and high pH treatments in the two examined plant species. This may because of the root system that was not exposed to high pH and NaCl and sufficient nutrients were supplied to the leaves to maintain chlorophyll synthesis. Leaf chlorophyll concentrations commonly decrease as a result of environmental stresses, largely due to the stress-induced nutrient deficiencies (Lagriffoul et al. 1998, Xiao et al. 2008, Keyvan 2010). Leaf chlorophyll concentrations are relatively sensitive to salt and high pH conditions (Suriyan et al. 2009, Li et al 2010). However, the decreases in leaf water potentials and E in plants exposed to NaCl and high pH point to the importance of the relatively minor (less than 20%) lower part of the root system in maintaining plant water balance since only this part was exposed to treatments. It is also plausible that high pH stress in the lower soil layer (lower tube) plays a role as a stress signal (Wilkinson 1999) or functions as the secondary messenger in plant cells (Kader and Lindberg 2010) and triggered a series of different physiological responses in the studied plant species.

In general, about 80% of plant roots are commonly found in the upper 30 cm of the soil profile (Purdy et al. 2005, Lazorko and Van Rees 2012, Lilles et al. 2012). Plants have the ability to change root distribution to avoid soil stress factors (Benjamin and Nielsen 2006). Many plant species, such as soybean (*Glycine max*) and common bean

(*Phaseolus vulgaris*), were reported to have the greatest root growth in the drier years and the least root growth in the wetter years (Merrill et al. 2002). It was reported that only a small fraction of plant roots grow into the saline-sodic overburden in oil sands reclamation areas (Lazorko and Van Rees 2012). Very few roots were also found in the lower subsurface layers in the natural saline sites (Lilles et al. 2012). In my study, about 80% of wheatgrass roots were distributed in the upper 30 cm soil layer in the absence of NaCl stress (Table 3.7). This value increased to about 90% in plants subjected to NaCl treatments. No significant difference in root distribution was found between pH 5 and 8 in wheatgrass ($P = 0.90$). This demonstrates that in slender wheatgrass, salt stress was the main reason for the inhibition of root growth and that by decreasing the proportion of the root system that is in contact with NaCl in the lower part of the soil, plants may be better able to avoid salt stress and survive in the sites with heterogeneous soil salinity. The results also indicated that in slender wheatgrass, NaCl was the dominant stress factor compared with high pH when applied to the lower part of the root system. In contrast, no significant effect on root growth was found in yellow sweet clover when the lower part of the soil was subjected to 30 mM and 60 mM NaCl treatments. High pH in the lower soil layer (lower tube) caused a significant decrease of yellow sweet clover root growth in the upper soil layer (Table 3.3). This indicates that the high pH applied to the lower part of the root system of yellow sweet clover could stimulate the distribution of roots in the deeper soil profile.

In summary, this chapter reports the responses of slender wheatgrass and yellow sweet clover to a combined NaCl stress and high pH when only the lower part of the root system was exposed to these conditions. The two examined plant species differently responded to the applied treatments. In slender wheatgrass, both NaCl and pH stress caused a significant decrease of dry biomass when only a small part (less than 20%) of roots were distributed in the lower saline and akaline soil. The effects of high pH and

NaCl stress included reductions in transpiration rates, decreased leaf water potentials and net photosynthetic rates. The results also demonstrated that salt stress, rather than high pH, was the main factor responsible for the decreased root distribution in the lower soil layer. In yellow sweet clover, high pH applied to the lower soil layer (lower tube)caused a significant decrease of dry biomass while NaCl applied to the lower soil layer (lower tube)only slightly affected root and shoot dry weights. High pH did not aggravate the effects of NaCl on growth in yellow sweet clover plants. The results also demonstrate that high pH stimulated root distribution in the lower soil layer (lower tube)when the yellow sweet clover was subjected to a high (7.7-8.3) pH soil conditions. This study simulated the heterogeneous soil salinity and pH conditions that could be expected in oil sands reclamation areas in northern Alberta. My results demonstrated that in the areas with heterogeneous soil pH and salinity, both of the studied herbaceous plants could potentially grow their roots into the deeper soil profile with high pH and elevated salinity. In slender wheatgrass, NaCl was the key factor responsible for the reduction of root growth in the lower part of the soil affected by high pH and NaCl. Exposure of less than 10% of the total root dry mass to NaCl and high pH stress caused significant decrease ingrowth and the examined physiological parameters in slender wheatgrass. In contrast, in yellow sweet clover, high pH stimulated root distribution in the lower part of the soil (with or without of NaCl stress) with only slight effects on the growth of aboveground parts (shoot dry weights and leaf areas). Therefore, it can be concluded that yellow sweet clover is more tolerant compared with the slender wheatgrass when salt and high pH affect some parts of the soil. However, other environmental conditions, including periodic or extreme drought events, could also potentially affect plant responses to salinity and high pH and pH should be also considered in future studies.

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3.6 Tables

Table 3.1 P values of Two-way ANOVA analysis results in slender wheatgrass

Source	Sig. (P value)					
	shoot dry weight	root dry weight	biomass	water potential	Pn	E
salt	＜0.001	＜0.001	＜0.001	＜0.001	＜0.001	＜0.001
pH	0.001	0.022	0.001	0.01	0.008	0.055
salt * pH	0.228	0.208	0.175	0.979	0.678	0.059

Table 3.2 P values of Two-way ANOVA analysis results in yellow sweet clover

Source	Sig. (P value)					
	shoot dry weight	root dry weight	biomass	water potential	Pn	E
salt	0.168	0.351	0.126	＜0.001	＜0.001	＜0.001
pH	0.013	0.013	0.001	0.085	＜0.001	＜0.001
salt * pH	0.377	0.481	0.633	0.324	0.489	0.104

Table 3.3 Distribution of root dry weight (% total) in the upper and lower soil layers

Salt	pH	Slender Wheatgrass		Yellow Sweet Clover	
		0-30 cm	30-60 cm	0-30 cm	30-60 cm
0 mM	5	80.7%±3% a	19.3%±3% a	91.0%±3% a	9.0%±3% a
	8	79.9%±3%	20.1%±3%	90.2%±2%	9.8%±2%
30 mM	5	91.1%±2% b	8.9%±2% b	87.1%±1% a	12.9%±1% a
	8	92.0%±2%	8.0%±2%	86.6%±0%	13.4%±0%
60 mM	5	88.7%±3% b	11.3%±3% b	93.6%±1% a	6.4%±1% a
	8	87.7%±3%	12.3%±3%	84.9%±3%	15.1%±3%

30-60 cm is the lower layer of the soil with pH and NaCl treatments. Percentage = (root DW in each section) / (total root DW). Data are means (n = 5) ± SE. Different lowercase letters indicate significant differences in different salt treatment analyzed by two-way ANOVA.

3.7 Figures

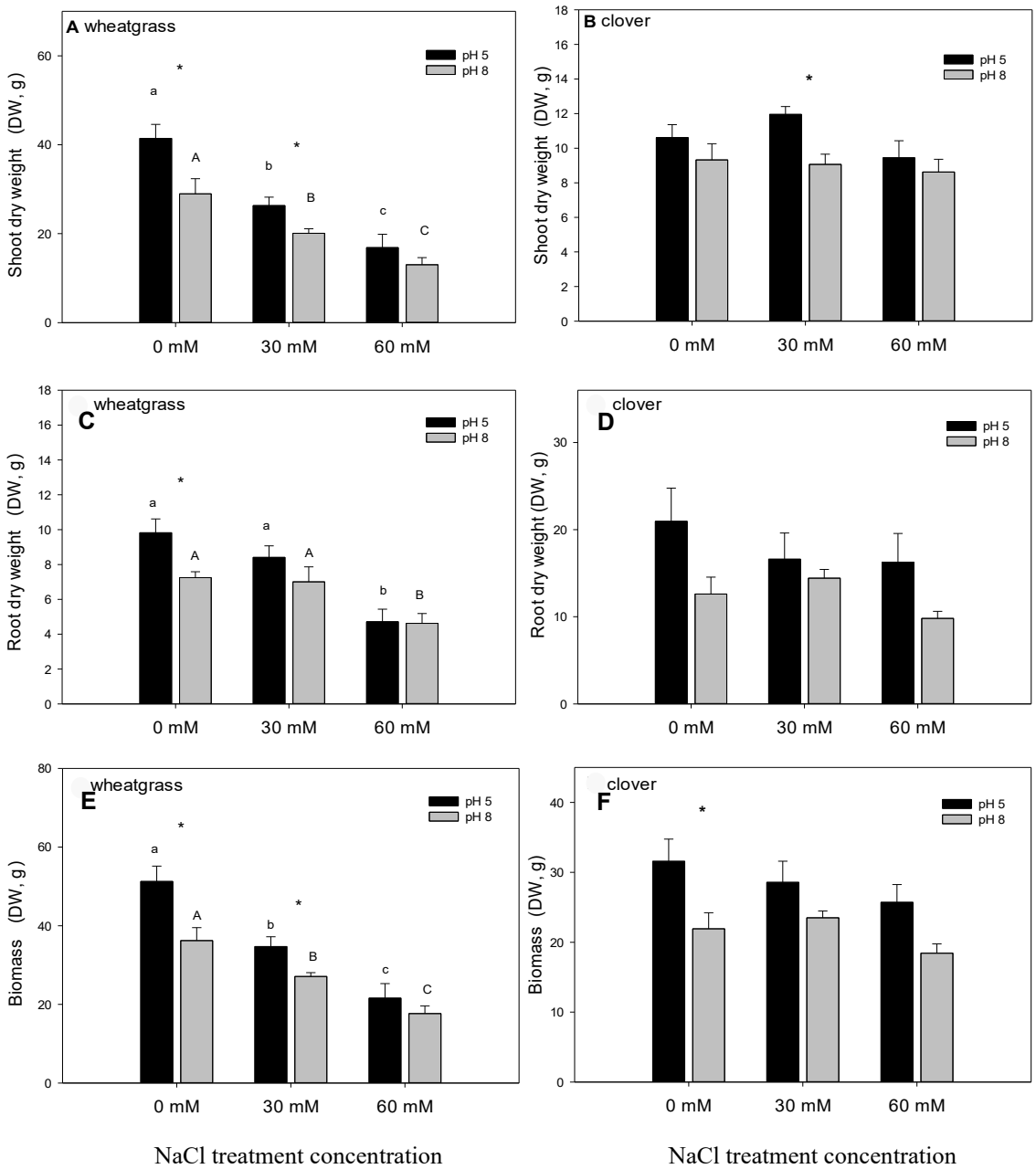

Figure 3.1 Shoot, root and total dry weights of slender wheatgrass and yellow sweet clover subjected to different NaCl and pH treatments in the lower soil layer. Different upper- and lowercase letters indicate significant differences between NaCl treatments for the same pH, * indicates significant difference between pH 5 and pH 8 treatment. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

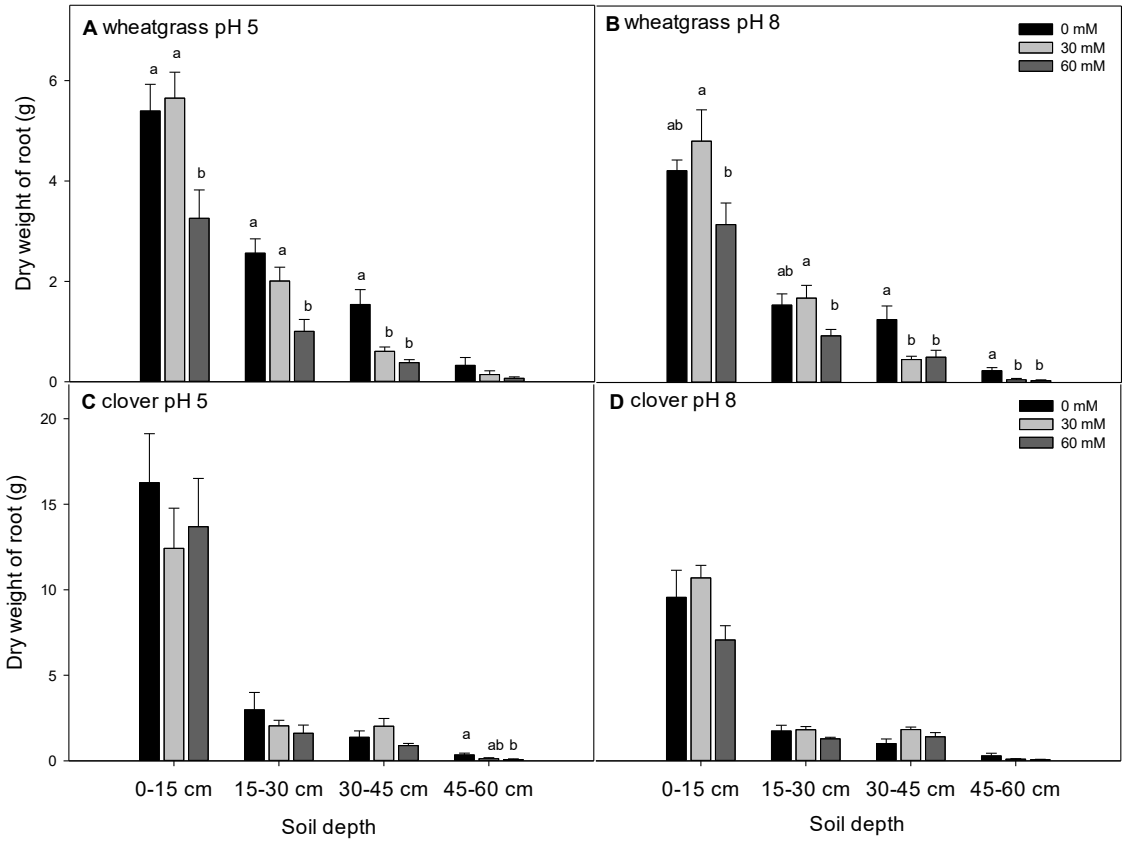

Figure 3.2 The dry weights of roots in different soil layers in wheatgrass and yellow sweet clover exposed to different salt and pH treatments in the lower soil layer (30 – 60 cm). Different upper- and lowercase letters indicate significant differences in different salt concentrations. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

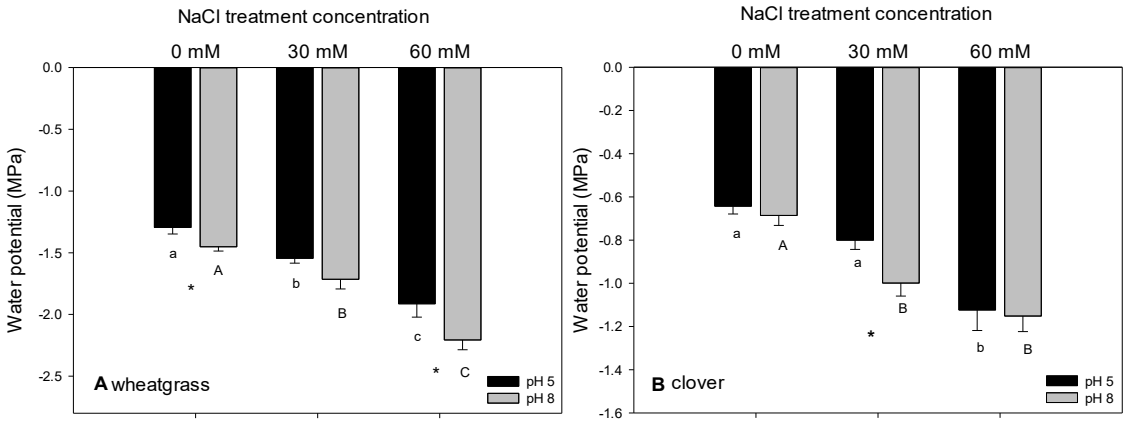

Figure 3.3 Leaf water potentials of wheatgrass and yellow sweet clover under different NaCl and pH treatments. Different upper- and lowercase letters indicate significant differences between different NaCl concentrations, at the same pH level. * indicates significant difference between pH 5 and pH 8 treatment. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

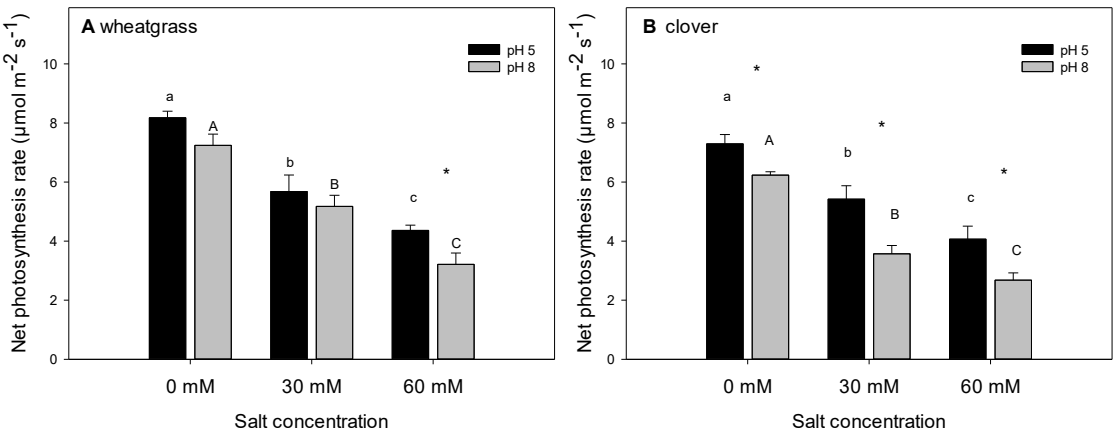

Figure 3.4 Net photosynthetic rates of slender wheatgrass and yellow sweet clover under different NaCl and pH treatments. Different upper- and lowercase letters indicate significant differences between different NaCl concentrations, at the same pH level. * indicates significant difference between pH 5 and pH 8 treatment. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

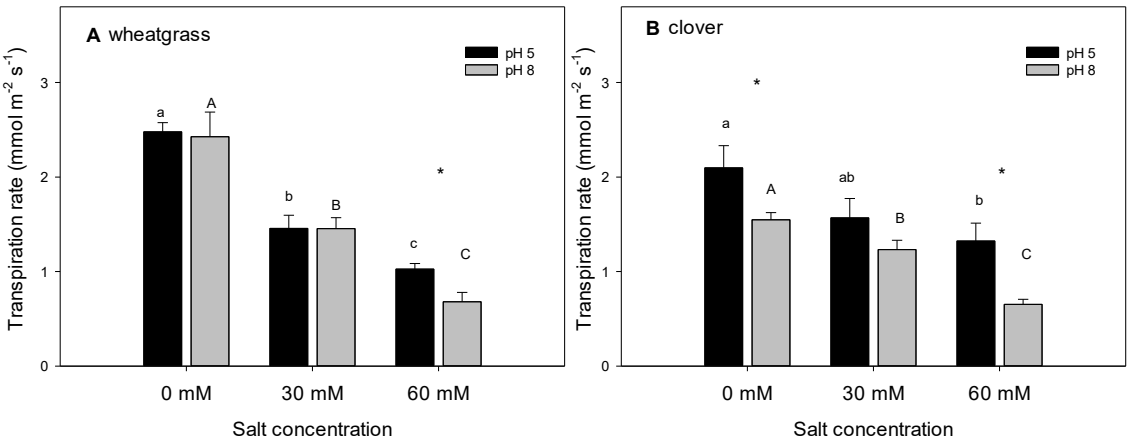

Figure 3.5 Transpiration rates of slender wheatgrass and yellow sweet clover under different NaCl and pH treatments. Different upper- and lowercase letters indicate significant differences between different NaCl concentrations, at the same pH level. * indicates significant difference between pH 5 and pH 8 treatment. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

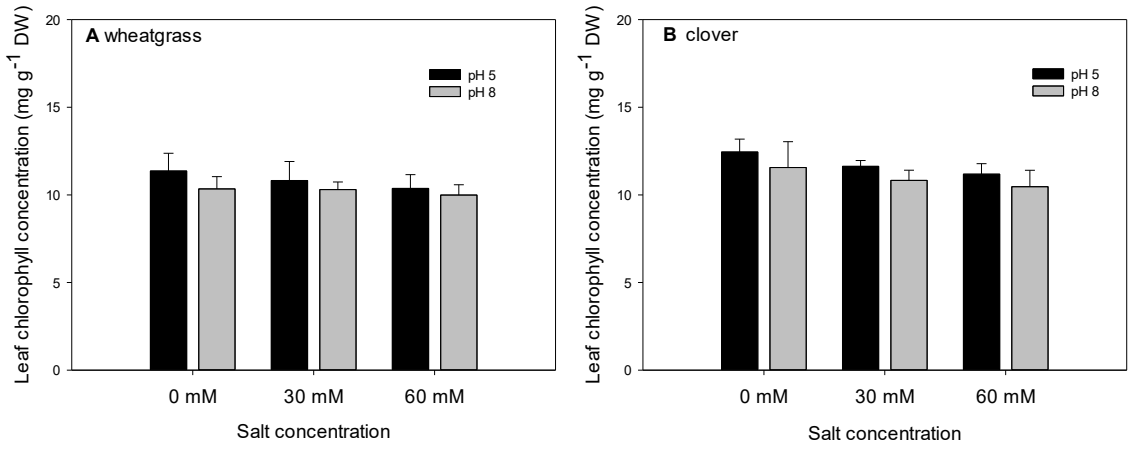

Figure 3.6 Leaf chlorophyll concentrations of slender wheatgrass and yellow sweet clover under different NaCl and pH treatments.

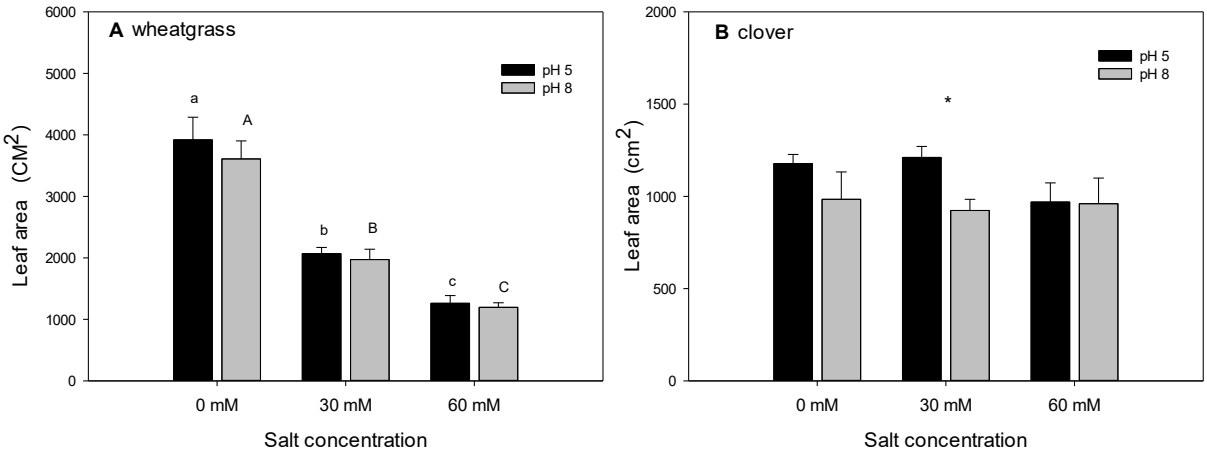

Figure 3.7 Total leaf areas of slender wheatgrass and yellow sweet clover under different NaCl and pH treatments. Different upper- and lowercase letters indicate significant differences between different NaCl concentrations, at the same pH level. * indicates significant difference between pH 5 and pH 8 treatment. Data are means (n = 5) ± SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

Chapter 4

Ericoid mycorrhizal associations alleviate drought stress in lowland and upland populations of velvetleaf blueberry (*Vaccinium myrtilloides*)

4.1 Introduction

Velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) of the Ericaceae family is a common plant species found in the understory of the Canadian boreal forests. Blueberry is regarded as a cultural keystone species (Garibaldi and Straker 2009) and its reestablishment following oil sands reclamation in northeastern Alberta, Canada is of high priority. Under natural conditions, ericaceous plants form symbiotic associations with ericoid mycorrhizal (ERM) fungi. ERM association is characterized by the formation of intracellular hyphal coils in the root epidermis and hyphal extensions of up to 1 cm from the root surface (Read 1984). Although the taxonomic status of ericoid mycorrhizal fungi has received some attention, far less effort has been focused on determining the diversity of ericoid fungi within the root systems at a single field site or on the same fungal taxon at different field sites (Sharples et al. 2000, Perotto et al. 2018). Even less is known about the effects of ERM on stress resistance of the host plants. Physiological studies of the symbiosis between ERM fungi and their ericaceous hosts have been largely based on *Rhizoscyphus ericae* (formerly *Hymenoscyphus ericae*). In the past several years, a deeper insight into ERM diversity has been obtained through the application of molecular techniques, which have revealed a much higher diversity of ERM fungi than previously thought (Allen et al. 2003, Bougoure et al. 2009). Small genetic differences among closely related ERM fungi have been shown to translate into

functional differences (e.g. N uptake) in both pure cultures and in symbiosis with different ericaceous hosts (Grelet et al. 2008). Furthermore, the growth habitats of ericaceous plants range from dry to wet. Morphologically distinct populations of *V. myrtilloides* can be found in both dry sandy areas (later referred to as upland blueberry) and wet hummocky areas in bogs (later referred to as lowland blueberry), suggesting possible genetic adaptations to soil moisture conditions (Barnes and Wagner 1981). These differences need to be recognized for genotype selection prior to planting in oil sands reclamation areas. However, it is also possible that other factors, including differences in the mycorrhizal taxa associated with the roots, may contribute to the apparent differences in soil moisture levels tolerated by the plants. The physiology of these ericaceous plants has been poorly studied and little is known about their water relations and drought resistance mechanisms. This important gap in knowledge needs to be filled to make further progress with the revegetation efforts.

The existing literature concerning Ericaceae and certain ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) associations strongly points to their importance in overcoming various environmental stresses, which often hamper reclamation efforts in the oil sands areas. The alleviation of adverse soil factors by AM (Bárzana et al. 2014) and ECM (Lehto and Zwiazek 2011) associations has been often explained by the enhancement of root water transport (Muhsin and Zwiazek 2002a,b, Marjanović et al. 2005, Xu et al. 2015). This effect of mycorrhizal associations is thought to be due to the stimulation of root (Marjanović et al. 2005, Xu et al. 2015) and fungal (Xu et al. 2015) aquaporin expression and the resulting increases in cell and root hydraulic conductivities (Lee et al. 2010). However, these effects vary between plant and fungal species (Siemens and Zwiazek 2011) and have not been investigated in ERM plants. Other effects of mycorrhizas, which can improve plant growth and survival in reclamation sites, include the enhancement of nutrient uptake, especially P and N, and alleviation of heavy metal

toxicity (Marschner 1986, Jones et al. 2004). Based on the information gathered from the AM and ECM mycorrhizas, it can be hypothesized that proper development of suitable ERM associations is essential to the survival and sustained growth of ericaceous plants in oil sands reclamation sites. Therefore, understanding the role that the ERM associations play in conferring stress resistance to velvetleaf blueberry may be crucial for improving the revegetation success.

The main objective of the study was to contribute new knowledge concerning the physiological processes in velvetleaf blueberry colonized by the ERM fungi. Specific objectives were to 1) identify ericoid mycorrhizas in the root of upland and lowland velvetleaf blueberry plants in the natural boreal forest sites and in oil sands reclamation areas, 2) examine whether ERM associations enhance drought tolerance in upland and lowland velvetleaf blueberry populations, and 3) examine the effects of ERM associations on gas exchange and water relations in the upland and lowland velvetleaf blueberry populations. I hypothesized that 1) different ERM fungi are present in the roots of upland and lowland velvetleaf blueberry populations, 2) the ERM associations enhance drought tolerance in both upland and lowland velvetleaf blueberry populations, and 3) compared with the lowland velvetleaf blueberry population, the upland velvetleaf blueberry population more strongly depends on ERM fungi for drought protection.

4.2 Materials and Methods

4.2.1 Isolation and identification of ERM fungi

Ericaceous plants root samples were collected from velvetleaf blueberry (*Vaccinium myrtilloides* Michx.) and labrador tea (*Rhododendron groenlandicum*) plants growing in six boreal forest sites in the proximity to the oil sands mining areas near Fort McMurray, AB, Canada. The velvetleaf blueberry and labrador tea roots were collected from one

population of plants (three for labrador tea) growing in wet hummocky areas in bogs (referred to as the lowland or lowland velvetleaf blueberry) and three populations of plants growing in dry sandy areas (upland blueberry). A total of 10 roots per species per site were collected.

Roots samples were washed and cut into 5- to 7-cm-long segments. To isolate the ERM fungi, the root segments were placed on modified Melin-Norkans medium (MN) and Potato Dextrose Agar medium (PDA). Fungal colonies were grown on plates and subcultured for fungal identification. ERM fungi present in the roots were identified by the molecular analysis (Henrion et al. 1994, Onwuchekwa et al. 2014) after extracting total genomic DNA using Sigma Extract-N-Amp Tissue Kit, following the manufacturer's protocol (Sigma-Aldrich, St. Louis, MO, USA). To identify the ERM fungi present in roots, a total of 114 DNA extracts were obtained from fungal colonies grown on plates from the root tips. The internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA) was amplified using the ITS1F and ITS4 primers (Gardes and Bruns 1993; White et al. 1990). All PCR products were visualized on a 1% agarose gel and purified using ExoSAP-IT (USB, Cleveland, OH, USA) and, when necessary, by gel incision following the manufacturer's protocol (Qiagen, Toronto, ON, Canada). Sanger sequencing was carried out in one direction using the Big Dye Terminator Sequencing Mix (v. 3.1, Life Technologies Corporation, Carlsbad, CA, USA) with the same PCR forward primer at a final concentration of 0.1 μM. The resulting products were precipitated using EDTA/ethanol following the manufacturer's instructions. The sequence was then searching in the database by Basic Local Alignment Search Tool on NCBI website (Altschul et al. 1990).

4.2.2 Plant material

The velvetleaf blueberry (*Vaccinium myrtilloides*) seeds were collected from four

boreal forest sites in the proximity of oil sands mining areas near Fort McMurray, AB, Canada in 2015. Seeds were collected from one population of velvetleaf blueberry plants growing in wet hummocky areas in bogs (lowland blueberry) and from three area of plants growing in dry sandy areas (upland blueberry). The seeds were surface-sterilized with 5% sodium hypochlorite (Siemens and Zwiazek 2011) and germinated in containers (72 holes Traditional 1206 Sheet Inserts, T.O. Plastics, Inc. Clearwater, MN, US) with autoclaved (twice, each time for 2 h) sand and peat mixture (2:1, by volume). The pH was adjusted to 4 - 4.5 with 20% H_2SO_4 and the soil was maintained moist by adding deionized water as required. After about four months, the seeds germinated and the germinants were transferred to individual square pots (9 x 9 x 9 cm) containing the same growth medium as described above. The seedlings were fertilized weekly with commercial Miracle-Gro acid fertilizer (28-10-10, The Scotts Company LLC, Marysville, OH, US, pH 4.5) until two weeks prior to inoculation.

The seedlings were grown in the controlled-environment growth room at the 18-h photoperiod, 400 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), and day/night temperature of 22/18℃ for 5 months.

4.2.3 Fungal culture

Four strains of ERM fungal cultures that had been obtained from the roots and identified, *Pezicula ericae* (isolated from root of upland blueberry and labrador Tea), *Pezoloma ericae* (isolated from root of lowland blueberry), *Meliniomyces variabilis* (isolated from root of both lowland and upland labrador tea) and *Oidiodendron maius* (isolated from root of lowland labrador tea). The isolated fungal were subcultured and grown in the potato dextrose agar (PDA) solid medium. Liquid MN (Modified Melin-Norkans medium) medium without agar was placed in 1 L glass media bottles and autoclaved for 20 min. The fungal cultures from the PDA solid medium were cut into 20

to 30 sections (1 x 1 cm) and placed in each bottle in liquid medium. The bottle was then placed on a shaker at 120 RPM for two weeks in the dark at room temperature.

4.2.4 Root inoculation and drought treatment

After two weeks of growth in liquid medium, the fungi were filtered and washed with autoclaved deionized water. The fungi were then homogenized in a blender and suspended in autoclaved water to the mycelial concentration of 40 (±5) g L^{-1}. Fungal inoculum was added to the soil with two-month-old seedlings that were 2-3 cm tall at the time of inoculation. One day before the inoculation, all seedlings were watered to keep the soil moist. Four holes were made in the soil around the roots and 5 to 7 ml of liquid inoculum was injected into each hole with a pipette. The non-inoculated seedlings were provided with the same amount of autoclaved deionized water to serve as non-inoculated control.

The following inocula were applied: 1) 20 ml *Pezicula ericae* (43 mg mL^{-1}), 2) 25 ml *Pezoloma ericae* (36 mg mL^{-1}), 3) 21 ml *Meliniomyces variabilis* (42 mg mL^{-1}), 4) 25 ml *Oidiodendron maius* (35 mg mL^{-1}), and 5) 20 ml autoclaved deionized water (non-inoculated control). There were 36 velvetleaf blueberry plants per population (upland and lowland) per inoculation treatment for a total of 360 plants. Three months after inoculation, the plants were divided into two groups. The first group was subjected to three cycles of drought treatment. In each drought cycle, watering was withheld for 10 days before re-watering (Zwiazek 1991) leaf gas exchange rate were measured daily except one day before re-watering (due to really low and unstable readings). After each 10-day cycle, the plants were watered and all measurements were carried out at the end of the third drought cycle. Non-stressed control seedlings were watered every two days. Soil moisture content in each of the pots was monitored daily with the time-domain reflectometry (TDR) probes (Arango et al. 2011). There were 18 plants from each

velvetleaf blueberry population (upland and lowland population) and each inoculation treatment (4 mycorrhizal + 1 control) subjected to the drought treatment and remaining 18 plants with the same treatments and served as well-watered control.

4.2.5 Measurements

4.2.5.1 Growth measurements

Plants were harvested at the end of the drought treatments, their roots separated from stems and leaves and weighed to determine fresh weights (n =6). The stems and roots were dried in an oven at 70°C and the leaves were freeze-dried for 72 h and weighed. The weights of leaves and stems from each plant were combined to calculate shoot dry weights.

4.2.5.2 ERM fungal colonization

Fine roots from 5 plants per treatment (n = 6) were harvested at the end of the treatments and fixed in FAA (formaldehyde: ethanol: acetic acid: water, 10%:50%:5%:35%). Root colonization by the ERM fungi was examined with the light microscope according to Trouvelot et al. (1986). For the microscopic examination, the roots were rinsed twice in distilled water to remove the FAA solution. The roots were then clarified with 10% KOH at 60°C for 1 hour and washed twice with distilled water and then with 5% acetic acid. The cleared roots were stained with black ink (Sheaffer Skrip Ink Bottle, CT, US) plus 5% acetic acid at 60°C for 20 minutes and rinsed with distilled water.

The fine roots were cut into approximately one-cm-long segments and 10 randomly selected segments from each seedling were mounted on microscope slides and examined. There were in total 1200 fine root segments from 120 seedlings examined for root

colonization by counting the coils in the roots under the microscope. The root samples were rated from 0 to 5 (Trouvelot et al. 1986) according to the ericoid mycorrhizal intensity in root sample (Fig. 4.1).

The intensity of the mycorrhizal colonization in the fine root system colonization intensity (M%) was calculated for all treatments using the following equation (Trouvelot et al. 1986).

Colonization intensity: $M\% = (95n5+70n4+30n3+5n2+n1)/(nb\ total)$

Where n5 = number of fragments (root segments) rated 5 (intensity of each fragment ＞90%); n4 = number of fragments rated 4 (intensity of each fragment ＞50%); n3 = number of fragments rated 3 (intensity of each fragment ＜50%); n2 = number of fragments rated 2 (intensity of each fragment ＜10%); n1 = number of fragments rated 1 (intensity of each fragment ＜1%); n0 = number of fragments rated 0 (intensity of each fragment = 0%).

4.2.5.3 Soil water content

Soil water content was measured with the 1502C Metallic Time-Domain Reflectometer (TDR) instrument (Tektronix, Inc., Beaverton, OR, USA). A three-rod (1.5 mm in diameter, 10-cm long) TDR stainless steel probe was vertically inserted into the soil (10 cm in depth near the main root) of 6 pots per treatment. The measurements were carried out daily between 9:30 and 11:30 AM. The soil relative water content was determined based on the below equation calibrated for the water volume and soil mixture volume (sand and peat mixture, 2:1, by volume) ratio used in our experiment. The correlation curve (R^2=0.99) was measured by 8 points range from 0% to 70% (0%, 10%,

20%, 30%, 40%, 50%, 60%, 70%,) (Arango et al. 2011).

Soil relative water content (V/V)×100% =3.5922×(d_2-d_1)-0.1727

Where d_1 is the first peak generated by the voltage change in the wave signal due to the connection between the coaxial cable and the rod of the probe. d_2 is the second peak generated when the wave signal reaches the end of the rods in the soil encountering an open circuit.

4.2.5.4 Gas exchange

Net photosynthetic (Pn) and transpiration (E) rates were measured daily in six plants per treatment combination (n = 6) using the LI-6400XT portable open-flow photosynthesis system equipped with a red/blue LED light source (LI-COR, Inc., Nebraska, USA). Photosynthetic photon flux density was set at 400 μmol·m^{-2}·s^{-1}, leaf temperature was kept at 28°C and reference CO_2 concentration was maintained at 400 μmol·mol^{-1} using the 6400-01 CO_2 mixer. All measurements were carried out between 09:00 and 13:00 h on the upper fully expanded leaves. The same leaf was used for repeated measurements over the three-week period. Leaf areas were calculated following computer scanning using the Sigmascan Pro 5.0 computer software (Systat Software, San Jose, CA).

4.2.5.5 Leaf chlorophyll concentrations

Chlorophyll concentrations were determined in fully expanded leaves harvested

from six randomly selected seedlings per treatment (n = 6). Leaves were freeze-dried and ground in a Thomas Wiley Mini-Mill (Thomas Scientific, NJ, USA). Chlorophyll was extracted from pulverized leaf samples (10 mg dry weight) with 8 mL dimethyl sulfoxide (DMSO) at 65°C for 22 h. Chlorophyll concentrations were measured in DMSO extracts with a spectrophotometer (Ultrospec, Pharmacia LKB, Uppsala, Sweden), at 648 nm for chlorophyll-a and 665 nm for chlorophyll-b. Total chlorophyll concentration was calculated using the Arnon's equation (Sestak et al. 1971).

4.2.5.6 Shoot water potential

Shoot water potential (ψ_w) measurements were conducted using a Scholander-type pressure chamber (PMS instruments, Corvallis, OR, USA). Shoot water potential was measured between 5.5 to 7.5 hours in randomly selected shoots following the onset of photoperiod. The shoot was cut and immediately placed in the pressure chamber for the measurements.

4.2.6 Data analysis

All measurements were carried out in six randomly selected plants (n = 6) per treatments combination. The data for shoot, root, and total dry weights, shoot/root ratio, Pn, E, shoot water potential, and chlorophyll concentrations in two velvetleaf blueberry populations were analyzed by one-way ANOVA. The three-way ANOVA was used for the analysis of multiple factors such as water (drought vs. no drought), ERM fungus (*Pezicula ericae, Pezoloma ericae, Meliniomyces variabilis*, and *Oidiodendron maius*) and population (lowland and upland velvetleaf blueberry) comparisons using IBM SPSS Statistics (version 21, IBM Corporation, Armonk, New York, US). Separation of means was conducted based on Duncan's multiple range test at the 0.05 significance level. Data were transformed with $\log_{10}$ transformation when they did not meet the normality and

homoscedastic postulates.

4.3 Results

4.3.1 Isolation and identification of ERM fungi

A total of 114 fungal isolates were obtained and cultured from the 130 ericaceous root samples. BLAST queries of the 114 amplified ITS sequences indicated that 13 isolates were ERM Ascomycota fungi of the six different species (Table 4.1). The remaining isolates were pathogenic, saprotrophic and cosmopolitan soil fungi including *Fusarium tricinctum* and *Gymnopus subnudus*.

Four out of 13 ERM fungal isolates were selected for the experiment and sub-cultured. Amplified ITS sequences from the four ERM fungi were 536 to 547 bp long. The isolates had the closest affinities to the previously identified ERM fungi *Pezicula ericae* (synonymy: *Cryptosporiopsis ericae*) (isolate #38), *Pezoloma ericae* (synonymy: *Rhizoscyphus ericae*) (isolate #50), *Meliniomyces variabilis* (isolate #81), and *Oidiodendron maius* (isolate #96) listed in the GenBank. (Table 4.2).

4.3.2 Soil water content

Soil volumetric water content (V/V, %) measured daily between the 7th and 21st days of treatment (Fig. 4.2). In well-watered pots, soil water content fluctuated between 15 and 38% on different days and was similar in upland and lowland populations and in mycorrhizal and non-mycorrhizal treatments (Fig. 4.2 A,B). After the second and the third cycles of drought stress, the soil water content in upland (Fig. 4.2 A) and lowland velvetleaf blueberry (Fig. 4.2 B) population was lower by approximately 50% compared with well-watered control after the first, 40% after second and the third cycle of drought stress (Fig. 4.2 A,B).

4.3.3 Root colonization

Hyphal coils were observed in the epidermal of velvetleaf blueberry plants. The colonization intensity (M) in the four ERM inoculation treatments ranged from 50% to 80% depending on the fungal species and watering treatment (Table 4.5). In the non-inoculated control plants, M was approximately 17% (Table 4.5).

4.3.4 Plant mortality

Approximately one-third of the non-inoculated lowland velvetleaf blueberry and 22% of the non-inoculated upland velvetleaf blueberry seedlings did not survive the three cycles of drought stress (Table 4.3). Plants inoculated with the ERM fungi had lower mortality rate compared with non-inoculated plants. In the lowland velvetleaf blueberry population, plants inoculated with *Pezicula ericae* and *Pezoloma ericae* had the lowest mortality rate (5.5%). In the drought-stressed upland velvetleaf blueberry, seedlings inoculated with *Meliniomyces variabilis* had the lowest mortality rate (5.5%). There was no plant mortality in the well-watered inoculated and non-inoculated groups.

4.3.5 Plant dry weights

4.3.5.1 Shoot dry weights

Shoot dry weights of upland and lowland velvetleaf blueberry seedlings inoculated with the ERM fungi were greater compared with non-inoculated control in both drought and well-watered treatments (Fig. 4.3 A & B).

In well-watered non-inoculated plants, shoot dry weights were significantly higher in the upland compared with lowland velvetleaf blueberry (Fig. 4.3 A). There was no significant difference in shoot dry weights between upland and lowland velvetleaf

blueberry inoculated with the different ERM fungi (Fig. 4.3 A). Well-watered upland and lowland plants inoculated with *Pezicula ericae* had several fold higher shoot dry weights compared with non-inoculated seedlings (Fig. 4.3 A). Significant increases in shoot dry weights were also measured in well-watered lowland plants inoculated with *Pezoloma ericae* and *Meliniomyces variabilis* (Fig. 4.3 A).

In drought stressed plants, there was no significant difference in shoot dry weights between upland and lowland velvetleaf blueberry seedlings in any of the inoculation treatments (Fig. 4.3 B). The drought stressed upland and lowland plants inoculated with *Pezicula ericae* had shoot dry weights higher by over 150% and 80% compared with the drought-stressed non-inoculated plants (Fig. 4.3 B). There were on significant differences in shoot dry weights between drought stressed non-inoculated upland and lowland plants and the drought stressed plants inoculated with *Pezoloma ericae*, *Meliniomyces variabilis*, and *Oidiodendron maius* (Fig. 4.3 B).

The three-way ANOVA analysis demonstrated significant increases of shoot dry weights in velvetleaf blueberry seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* and *Pezoloma ericae*. There were no significant differences in shoot dry weights between upland and lowland velvetleaf blueberry population (Table 4.4).

4.3.5.2 Root dry weights

Root dry weights were greater in plants inoculated with the ERM fungi compared with the non-inoculated plants in the lowland velvetleaf blueberry populations under well-watered treatments. Seedlings inoculated with *Pezicula ericae* had greater root dry weights than the non-inoculated upland and lowland velvetleaf blueberry populations in both well-watered and drought treatments (Fig. 4.4 A & B).

In well-watered non-inoculated plants, root dry weights were significantly higher in the upland compared with lowland velvetleaf blueberry (Fig. 4.4A). In general, root dry

weights of lowland velvetleaf blueberry seedlings inoculated with ERM fungi were significantly higher than the non-inoculated control (Fig 4.4 A). In well-watered upland velvetleaf blueberry, root dry weights of plants inoculated with *Pezicula ericae, Pezoloma ericae* and *Meliniomyces variabilis* were higher by about 200%, 80% and 80% compared with non-inoculated plants (Fig. 4.4 A). The root dry weights of both lowland and upland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* were higher than the non-inoculated controls (Fig 4.4 A).

In drought stressed plants, root dry weights in upland and lowland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* subjected to drought stress were about 250% and 150% higher compared with the non-inoculated controls respectively (Fig 4.4 B). Root dry weights of drought-stressed upland velvetleaf blueberry seedlings inoculated with *Pezoloma ericae* and *Oidiodendron maius* were slightly higher than the non-inoculated plants. However, root dry weights of the drought-stressed upland velvetleaf blueberry seedlings inoculated with *Meliniomyces variabilis* were slightly lower than the non-inoculated plants (Fig 4.4 B). In lowland velvetleaf blueberry inoculated with *Pezoloma ericae*, *Meliniomyces variabilis*, and *Oidiodendron maius*, root dry weights were slightly higher than the non-inoculated drought-stressed plants (Fig 4.4 B).

The results of three-way ANOVA analysis demonstrated significant increases of root dry weights in seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* and *Pezoloma ericae*. There were no significant differences in root dry weights between upland and lowland velvetleaf blueberry population (Table 4.4).

4.3.5.3 Total dry weights

The total dry weights of upland and lowland velvetleaf blueberry seedlings inoculated with the four ERM fungi were greater compared with non-inoculated controls

under drought and well-watered conditions (Fig. 4.5 A ,B).

In the well-watered treatment, the total dry weights of upland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* were significantly higher compared with non-inoculated control (Fig. 4.5 A). The total dry weights of upland velvetleaf blueberry seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* and *Oidiodendron maius* were 109%, 64% and 19% greater than the non-inoculated control, respectively (Fig. 4.5 A). In general, the total dry weights of lowland velvetleaf blueberry seedlings inoculated with *Pezicula ericae*, *Pezoloma ericae* and *Meliniomyces variabilis* were significantly higher than the non-inoculated control (Fig 4.5 A). Total dry weights of non-inoculated control seedlings from the upland velvetleaf blueberry population were significantly higher compared with the lowland population (Fig 4.5 A).

In drought stressed plants, the total dry weights of velvetleaf blueberry seedlings were significantly higher than the non-inoculated controls in both upland and lowland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* (Fig. 4.5 B). The total dry weights of both upland and lowland velvetleaf blueberry seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* and *Oidiodendron maius* were slightly greater than the non-inoculated control, respectively (Fig 4.5 B).

The three-way ANOVA analysis demonstrated significant increases of total dry weights in seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* and *Pezoloma ericae*. There were no significant differences in total dry weights between upland and lowland velvetleaf blueberry population (Table 4.4).

4.3.5.4 Shoot to Root Ratios (S/R)

In general, no significant differences were found in the shoot to root ratios between inoculated seedlings and non-inoculated controls (Fig. 4.6 A & B).

In well-watered plants of the lowland population, S/R was slightly lower (about 16%

on average) compared with the non-inoculated control inoculated with the four ERM fungi (Fig. 4.6 A). However, the S/R was about 15% higher on average in upland velvetleaf blueberry seedlings inoculated with *Pezicula ericae*, *Pezoloma ericae* and *Oidiodendron maius* compared with the non-inoculated control (Fig. 4.6 A). In general, in well-watered plants, S/R was higher in the upland velvetleaf blueberry compared with the lowland velvetleaf blueberry population (Fig 4.6 A).

In drought-stressed velvetleaf blueberry seedlings inoculated with *Pezicula ericae*, S/R was about 20% lower compared with non-inoculated controls in both upland and lowland populations (Fig. 4.6 B). The S/R in upland velvetleaf blueberry seedlings inoculated with *Pezoloma ericae*, and *Meliniomyces variabilis* was about 50% higher on average compared with the non-inoculated control (Fig. 4.6 B). In lowland velvetleaf blueberry population, S/R ratio was slightly greater than in the non-inoculated control under drought stress in the seedlings inoculated with *Pezoloma ericae* (Fig. 4.6 B). However, in seedlings inoculated with *Meliniomyces variabilis* and *Oidiodendron maius*, S/R was 20% lower compared with the non-inoculated control (Fig. 4.6 B).

The results of three-way ANOVA analysis showed that S/R in the upland velvetleaf blueberry population was significantly higher than in the lowland velvetleaf blueberry population (Table 4.4). The S/R ratio significantly increased as a result of drought (Table 4.4). There were no significant differences in S/R ratio for the effect of the four ERM treatments (Table 4.4).

4.3.5.5 Tissue water content

In general, no significant differences were found in the plant tissue water content between inoculated seedlings and non-inoculated controls (Fig. 4.7 A & B).

In well-watered plants of the lowland population, seedlings tissue water content inoculated with four ERM fungi were slightly (about 6%) higher than the non-inoculated

lowland population (Fig. 4.7 A). The tissue water content in well-watered treated upland velvetleaf blueberry inoculated with *Pezicula ericae*, *Pezoloma ericae* and *Meliniomyces variabilis* were slightly (about 3%) greater than the non-inoculated upland population (Fig. 4.7 A).

In drought-stressed plants, the tissue water content of upland velvetleaf blueberry seedlings inoculated four ERM fungi was about 10% higher than the non-inoculated controls (Fig. 4.8 B). For lowland velvetleaf blueberry, the tissue water content in seedlings inoculated with *Pezicula ericae* and *Oidiodendron maius* was about 12% higher than the control (Fig. 4.7 B).

According to the three-way ANOVA analysis there were no significant difference in plant tissue water content between upland and lowland velvetleaf blueberry populations and no significant differences between plants inoculated with different ERM fungi and control (Table 4.4).

4.3.6 Total leaf areas

In the well-watered treatment group, ERM inoculation resulted in greater total leaf areas compared with the non-inoculated plants (Fig. 4.8 A). In upland velvetleaf blueberry, the total leaf areas of seedlings inoculated with *Pezicula ericae* and *Pezoloma ericae* were significantly greater than the non-inoculated control (Fig. 4.8 A). In lowland velvetleaf blueberry, the total leaf areas were significantly greater in seedlings inoculated with *Pezoloma ericae* than the non-inoculated control (Fig. 4.8 A).

In the absence of water, total leaf areas were similar in inoculated and non-inoculated velvetleaf blueberry seedlings (Fig. 4.8 B). The total leaf areas were greater in lowland velvetleaf blueberry seedlings inoculated with the four ERM fungi compared with the non-inoculated control (Fig. 4.8 B). In upland velvetleaf blueberry seedlings, the total leaf areas were greater in seedlings inoculated with *Pezicula ericae* and *Pezoloma*

ericae compared with non-inoculated plants (Fig. 4.8 B).

The three-way ANOVA analysis showed no significant differences in the total leaf areas between upland and lowland velvetleaf blueberry populations (Table 4.4). However, the total leaf areas were significantly higher in well-watered seedlings compared with those stressed with drought (Table 4.4). The total leaf areas of velvetleaf blueberry seedlings inoculated with *Pezicula ericae* and *Pezoloma ericae* were significantly higher compared with the non-inoculated plants (Table 4.4).

4.3.7 Shoot water potential

Shoot water potentials were similar in the inoculated and non-inoculated well-watered upland velvetleaf blueberry plants (Fig. 4.9 A). In lowland velvetleaf blueberry, shoot water potentials of seedlings inoculated with *Pezicula ericae*, *Pezoloma ericae* and *Oidiodendron maius* were significantly higher compared with the non-inoculated control (Fig. 4.9 A).

When exposed to drought stress, shoot water potentials were significantly higher in the upland velvetleaf blueberry inoculated with the four ERM fungi compared to the non-inoculated plants (Fig. 4.9 B). In drought-stressed lowland velvetleaf blueberry, shoot water potentials were significantly higher in seedlings inoculated with *Pezicula ericae* and *Oidiodendron maius* compared with the non-inoculated control (Fig. 4.9 B).

The three-way ANOVA analysis demonstrated that shoot water potentials of velvetleaf blueberry seedlings inoculated with the four ERM fungi were significantly higher compared with the non-inoculated plants (Table 4.4). No significant difference between upland and lowland population in water potential was found (Table 4.4).

4.3.8 Leaf chlorophyll concentrations

Overall, no significant differences were found in the leaf chlorophyll concentrations

between inoculated seedlings and non-inoculated controls (Fig. 4.10 A & B).

In well-watered plants of the upland population, leaf chlorophyll concentration in seedlings inoculated with four ERM fungi was about 30% higher than the non-inoculated upland control seedlings (Fig. 4.10 A). In lowland population, the leaf chlorophyll concentration in seedlings inoculated with *Pezicula ericae*, *Pezoloma ericae* and *Meliniomyces variabilis* was slightly higher (about 13% on average) than the non-inoculated lowland control seedlings (Fig. 4.10 A).

In drought-stressed plants, the leaf chlorophyll concentration in both upland and lowland velvetleaf blueberry seedlings inoculated with the four ERM fungi were higher compared with the non-inoculated plants in both the drought and well-watered stresses treatments (Fig. 4.10 B).

The results of three-way ANOVA analysis demonstrated that leaf chlorophyll concentration of velvetleaf blueberry seedlings inoculated with the *Pezicula ericae* and *Meliniomyces variabilis* were significantly higher compared with the non-inoculated plants (Table 4.4). There was no significant difference between upland and lowland population in leaf chlorophyll concentration (Table 4.4).

4.3.9 Gas exchange

4.3.9.1 Net photosynthesis (Pn) change during drought stress

The Pn of well-watered upland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* and *Oidiodendron maius* was significantly higher compared with the non-inoculated plants (Fig. 4.11 A). The Pn in well-watered lowland velvetleaf blueberry seedlings inoculated with *Pezicula ericae*, *Meliniomyces variabilis* and *Oidiodendron maius* was also significantly higher compared with the non-inoculated plants (Fig. 4.11 A).

The Pn of drought-stressed velvetleaf blueberry seedlings inoculated with *Pezicula ericae* and *Pezoloma ericae* was significantly higher compared with the non-inoculated plants in both upland and lowland populations after three weeks of treatments (Fig. 4.11 B). The Pn in upland velvetleaf blueberry seedlings inoculated with ERM fungi *Meliniomyces variabilis* and *Oidiodendron maius* was 86% and 100% higher, respectively, compared with the non-inoculated plants (Fig. 4.11 B). In lowland velvetleaf blueberry inoculated with *Meliniomyces variabilis* and *Oidiodendron maius*, Pn was 94% and 74% higher, respectively, than in the non-inoculated seedlings (Fig. 4.11 B).

The three-way ANOVA analysis demonstrated that Pn was significantly higher in well-watered seedlings compared with the drought-stressed plants, however, there was no significant difference between the upland and lowland populations (Table 4.4).

4.3.9.2 Net photosynthesis (Pn) after drought stress

The Pn was measured in seedlings over time after the imposition of drought stress treatment. During the treatment, Pn was maintained at a higher level in well-watered compared with drought-stressed plants (Fig 4.12). In general, Pn was higher in the ERM-inoculated seedlings compared with the non-inoculated plants on most treatment days (Fig. 4.12 A B). Following the onset of drought stress treatment, Pn declined more in the non-inoculated compared with inoculated plants (Fig. 4.12 C D). The Pn increased to the pre-treatment level after two to four days following re-watering in both inoculated and non-inoculated plants (Fig. 4.12 C D).

4.3.9.3 Transpiration rate (E)

In well-watered stress treatment, lowland velvetleaf blueberry seedlings inoculated with *Meliniomyces variabilis* had significantly higher E than the non-inoculated plants after 21days of treatment (Fig. 4.13 A). There was no significant difference in E between

well-watered inoculated and non-inoculated plants (Fig 4.13 A).

Transpiration rates declined as a result of drought stress treatment (Fig.4.13B). However, after three weeks of the drought treatment, velvetleaf blueberry seedlings inoculated with the ERM fungi had higher E compared with the non-inoculated plants (Fig. 4.13 B). No significant differences in E were found between the inoculated lowland seedlings and non-inoculated plants following the drought stress treatment (Fig 4.13 B). In drought-stressed upland velvetleaf blueberry seedlings inoculated with *Pezicula ericae* and *Pezoloma ericae*, E was significantly higher compared with the non-inoculated control (Fig. 4.13 B).

The three-way ANOVA analysis showed no significant differences in E between the upland and lowland velvetleaf blueberry plants. However, E was significantly higher in well-watered seedlings compared with the drought-stressed plants (Table 4.4). Velvetleaf blueberry seedlings inoculated with *Pezicula ericae* had significantly higher E compared with the non-inoculated plants (Table 4.4).

4.4 Discussion

In my study, the effects of ERM fungi, *Pezicula ericae*, *Pezoloma ericae*, *Meliniomyces variabilis*, and *Oidiodendron maius* on drought resistance were examined in velvetleaf blueberry plants. The fungi were isolated from the roots of velvetleaf blueberry plants growing in the undisturbed forest in the north-eastern Alberta, Canada, near the oil sands mining areas (Table 4.2). *Oidiodendron maius* (Douglas et al. 1989, Schulz et al. 2006, Baba et al. 2016), *Meliniomyces variabilis* (Hambleton and Sigler 2005, Vohnik et al. 2013, Perotto et al. 2018), *Pezicula ericae* (Baral and Krieglsteiner 2006, Bruzone et al. 2017) and *Pezicula ericae* (Sigler et al. 2005, Scagel 2005, Gorzelak et al. 2011) have been reported to be commonly associated with *Vaccinium myrtilloides*

and are considered to be distributed worldwide (Kohout 2017). In our study, the root colonization intensity in four ERM fungi treatment ranged from 50% to 80% which are significant higher than the non-inoculated control plants. The root colonization intensity in non-inoculated control plants was 17%, likely due to of *Leohumicola verrucosa*. This heat-resistant ERM fungus is commonly found in a variety of soils (Hambleton et al. 2005) and in peat that was used in the present study (Fadaei 2019), and can survive autoclaving (Fadaei 2019, Fadaei et al. 2020).

4.4.1 Effect of ERM fungi on plant survival of drought

Population adaptations to soil moisture conditions have been observed in various plant species. Although *V. myrtilloides* is commonly found in sandy soils in relatively dry areas, it can occasionally grow in the moister, lowland areas. In the present study, I hypothesized that upland velvetleaf blueberry plants would more strongly rely on ERM fungi for drought protection. Upland populations of rice (*Oryza sativa*) and switchgrass (*Panicum virgatum*) were reported to be more drought resistant than the lowland populations, which was attributed to the drought adaptations that developed in these populations over time (Yu and Nguyen 1993, Stroup et al. 2003, Bernier et al. 2008). In my study, plants of the non-inoculated lowland velvetleaf blueberry population had a higher mortality rate than the non-inoculated upland velvetleaf blueberry population when exposed to the drought treatment. The results also showed that the four ERM fungi increased drought survival of both upland and lowland velvetleaf blueberry plants and that the survival rate was slightly higher in plants inoculated with *Pezicula ericae* and *Pezoloma ericae* in lowland velvetleaf blueberry compared with the upland velvetleaf blueberry (Table 4.3). Increased drought resistance and reduced mortality have been commonly reported for different mycorrhizal associations including ectomycorrhizas (Morte et al. 2000), vesicular arbuscular mycorrhizas (Sun *et al*. 2017) as well as in the

Rhododendron spp. plants colonized by the ERM fungus of *Oidiodendron sp*. isolated from *Vaccinium myrtillus* (Vosätka et al. 1999). The effect of mycorrhizal associations on drought resistance of the host plants is thought to involve increased water uptake by extending the root water absorption area and enhancing the aquaporin-mediated root water transport (Lehto and Zwiazek 2011). Although consistent decreases in drought-induced mortality rates were observed in the present study in plants inoculated with the ERM fungi, there was no clear difference between the inoculated upland and lowland plants in terms of their drought survival.

4.4.2 Effect of ERM fungi on growth

In most cases, mycorrhizal associations increase growth rates of the host plants, also under drought stress conditions (Augé 2001). In my study, the shoot, root, and total dry weights were significantly higher in lowland and upland velvetleaf blueberry seedlings inoculated with the ERM fungi compared with the non-inoculated plants under both adequate soil moisture and drought stress conditions. The effects varied between the ERM fungi with *Oidiodendron maius* having relatively more modest effects on plant dry weights and *Pezicula ericae* increasing the total dry weights of well-watered plants by over three-fold compared with the non-inoculated plants and by about 25% in the drought-stressed plants. It is interesting that the root, shoot, and total dry weights of non-inoculated upland velvetleaf blueberry were greater compared with the non-inoculated lowland velvetleaf blueberry population. However, there were no differences in plant dry weights between the upland and lowland blueberries inoculated with the ERM fungi suggesting an especially strong dependence of the lowland velvetleaf blueberry on mycorrhizal associations to support growth processes. Although the plants were fertilized during the experiment, the reasons for growth enhancement in lowland population could be partly due to stronger assimilation for nutrients as the root dry weights of the lowland

velvetleaf blueberry population were greater in well-watered plants and similar in drought stressed plants when inoculated with the ERM fungi.

Shoot/root ratios are often used to demonstrate changes in plant biomass allocation strategies in response to various environmental factors (Poorter et al. 2012). Drought stress, mycorrhiza symbiosis and several other factors could affect the distribution of carbohydrates between shoots and roots may change in shoot/root ratio and influence biomass allocation (Veresoglou et al. 2011, Xu et al. 2015b). The effect of mycorrhizal association on shoot/root ratio varies between different plant species (Rabie 2005, Zandavalli et al. 2004). In the present study, the shoot/root ratios significantly increased as a result of drought stress and they were higher in the upland velvetleaf blueberry population compared with the lowland population. The upland population appeared to be more balanced in the allocation of the resources as evidenced by the higher shoot/root ratios compared with the lowland population. It is plausible that the roots of the upland blueberries, which normally grow in relatively dry, sandy soils, are better adapted to withstand drought conditions compared with the lowland blueberries which grow in wet hummocky areas in bogs. The drought adaptations could help the upland velvetleaf blueberry more effectively accessing the water resources and sustain a higher shoot biomass in both dry and moist soils (Calvo-Polanco et al. 2019).

The plant water content considered as one of the indices of water deficit stress intensity (Todorov et al. 1998, Masand and Yadav 2015, Caser et al. 2017). Similarly to the shoot/root ratios, the plant tissue water content decreased in both plant populations in response to drought stress, reflecting a decrease in plant water content as a result of drought. In our study, there was no significant change of water content as a result of fungal inoculation. In non-inoculated control seedlings, the lowland population had a higher water content than the upland population under well-watered condition. The opposite was observed when seedlings were subjected to drought stress, with a higher

water content in the upland compared with lowland velvetleaf blueberry plants reflecting a more effective water conservation by the upland population.

4.4.3 Effect of ERM fungi on physiological processes

In the present study, shoot water potentials decreased in both inoculated and non-inoculated plants exposed to drought stress. However, compared with non-inoculated seedlings, shoot water potentials were higher in drought-stressed seedlings inoculated with the ERM fungi. Despite the differences in dry weight to fresh weight (DW/FW) ratios, no significant differences were found between the two populations. The maintenance of higher shoot water potentials in inoculated plants could be due to decreased water loss or increased root water uptake and transport through the direct contribution of fungal hyphae or indirect effects on the root water transport properties (Lehto and Zwiazek 2011). Water potentials can also reflect possible differences in osmotic properties due to osmotic adjustment in response to drought (Dar et al. 2018). Reduced water availability under drought conditions leads to reductions in plant water content and cell turgor, which triggers stomatal closure and inhibition of gas exchange and growth processes. The decreases in plant water potential are a combination of reduced tissue water content and decreased osmotic potential as the concentration of solutes increases due to lower water content and accumulation of solutes (Jaleel et al. 2009, Ahmad et al. 2018, Dar et al. 2018).

Mycorrhizal symbiosis improves plant water relations by increasing water uptake and transport through fungal hyphae to the host plants (Hernández-Sebastià et al. 1999, Augé et al. 2007, Zhao et al. 2014), enhanced root hydraulic conductivity (Bárzana et al. 2014) and increased water potential by greater osmotic adjustment, higher stomatal conductance, indirect effect of improved phosphate and other nutrient uptake (Duan et al. 1996, Zarik et al. 2016).

In the present study, drought stress caused a decrease in leaf chlorophyll concentrations in both populations of inoculated and non-inoculated plants. The leaf chlorophyll concentrations in seedlings inoculated with *Pezicula ericae* and *Meliniomyces variabilis* ERM fungi were significantly higher compared with the non-inoculated plants under both drought-stress conditions and in well-watered plants. ERM fungi can facilitate the uptake of nutrients that are required for chlorophyll synthesis (Finlay et al. 1992, Marschner and Dell 1994). Both ecto- (Scattolin et al. 2013) and vesicular-arbuscular (Pinior et al. 2005, Asrar and Elhindi 2011) mycorrhizal associations have been often reported to increase leaf chlorophyll concentrations under various abiotic stress conditions. This effect was attributed largely to an increase in nitrogen and magnesium uptake (Panwar 1992, Panwar 1993, Dixon et al. 1994, Mathur and Vyas 1995).

In my study, plants exposed to drought treatment suffered from significant decreases in net photosynthesis rate (Pn). Interestingly, Pn was significantly higher in inoculated velvetleaf blueberry seedlings compared with the non-inoculated plants both in the drought stress treatment and in well-watered control. The higher Pn may be partly due to higher leaf chlorophyll concentrations and water potentials in both drought-stressed and well-watered treatments. Mycorrhizal symbiosis has been found to enhance Pn of the host plant by increasing root hydraulic conductance and plant water potential, facilitating nutritent uptake and increasing leaf chlorophyll concentrations (Allen 2007, Xu et al. 2013, Augé et al. 2016). In both well-watered and drought treatments, Pn of seedlings inoculated with *Pezicula ericae* was higher than in the seedlings inoculated with *Pezoloma ericae*, *Meliniomyces variabilis* or *Oidiodendron maius*. The higher Pn can also help explaining the reasons for a significantly higher biomass in plants inoculated with *Pezicula ericae* compared with the other examined fungi.

Similarly to the net photosynthesis rate, transpiration rate (E) decreased as a result of

drought treatment. The effects of mycorrhizas on E vary between different mycorrhizal and plant species (Augé 2001). Mycorrhizal symbiosis can stimulate E of the host plants by improving hydraulic conductivity to enhance water transport (Thakur and Panwar 1997, Perotto et al. 2018). However, other studies reported decreases in E (Mathur and Vyas 1995, Cordeiro et al. 2019). In my study, E was higher in seedlings inoculated with ERM fungi compared with non-inoculated plants when exposed to drought stress. This was likely the consequence of greater water delivery to the leaves, consistent with higher water potential in inoculated plants under drought stress conditions. The processes contributing to this response in inoculated plants remain to be determined. Both ectomycorrhizal and vesicular-arbuscular mycorrhizal associations can enhance root water uptake and transport by increasing root hydraulic conductivity (Morte et al. 2000). The increase of root water transport was attributed to the aquaporin-mediated transport (Marjanović et al. 2005, Lee et al. 2010, Xu et al. 2015a) and increased root water absorption area (Muhsin and Zwiazek 2002a). It is noteworthy that in well-watered treatment, E varied between the plants inoculated with different ERM fungi suggesting that the effectiveness of ERM colonization in enhancing water transport properties may vary between different ERM fungal species.

4.4.3 Conclusions

In summary, the results of my research indicate that the ERM fungi significantly increased plant dry weights in upland and lowland velvetleaf blueberry seedlings under drought conditions and when adequate soil moisture was present. Additionally, shoot water potentials and leaf net photosynthetic rates increased as a result of fungal inoculations and they were also higher in inoculated compared with non-inoculated plants under drought conditions. It is plausible that the ERM fungi increased water supply to the plants and facilitated nutrient uptake. It can be speculated that the increased water

delivery in mycorrhizal plants was due to the enhancement of aquaporin-mediated root water transport as reported for other mycorrhizal associations. However, the precise mechanisms contributing to the enhancement of plant growth and drought resistance by ERM fungi remain to be determined. The results of the present study indicate that the upland velvetleaf blueberry population is more adapted to drought conditions compared with the lowland population as observed in non-inoculated plants. However, the population differences in drought resistance could be offset by the inoculation of plants with ERM fungi. The study also demonstrated that the effects of *Pezicula ericae* on growth and physiological parameters of velvetleaf blueberry were greater compared with the other examined ERM fungi. Therefore, *Pezicula ericae* should be considered for further studies aimed at improving the establishment and growth of velvetleaf blueberry planted in sites exposed to harsh environmental conditions such as oil sands reclamation areas.

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4.6 Tables

Table 4.1 Putative taxonomic affinities of isolates obtained from Ericaceous roots as inferred from BLAST queries of ITS sequences in GenBank

	BLAST result		
Sample ID	Closest match in GenBank	Order	Phylum
#17	*Pseudogymnoascus*	*Incertae sedis*	*Ascomycota*
#38	*Pezicula ericae*	*Helotiales*	*Ascomycota*
#48	*Phialocephala fortinii*	*Helotiales*	*Ascomycota*
#49	*Phialocephala fortinii*	*Helotiales*	*Ascomycota*
#51	*Phialocephala fortinii*	*Helotiales*	*Ascomycota*
#50	*Pezoloma ericae*	*Helotiales*	*Ascomycota*
#78	*Meliniomyces variabilis*	*Helotiales*	*Ascomycota*
#80	*Meliniomyces variabilis*	*Helotiales*	*Ascomycota*
#81	*Meliniomyces variabilis*	*Helotiales*	*Ascomycota*
#82	*Meliniomyces variabilis*	*Helotiales*	*Ascomycota*
#94	*Pezicula ericae*	*Helotiales*	*Ascomycota*
#95	*Pezoloma ericae*	*Helotiales*	*Ascomycota*
#96	*Oidiodendron maius*	*incertae sedis*	*Ascomycota*

Table 4.2. Taxonomic affinities of four ERM fungal isolates inferred from BLAST queries of ITS sequences in GenBank

	BLAST result				
Sample ID	Closest match in GenBank (accession no.)	ITS-PCR products (bp)	Query coverage	Identity	E value
#38	*Pezicula ericae* (NR_155653)	547	99%	99%	0.0
#50	*Pezoloma ericae* (KY315940)	538	100%	99%	0.0
#81	*Meliniomyces variabilis* (HM190126)	537	100%	99%	0.0
#96	*Oidiodendron maius* (MH860824)	537	99%	99%	0.0

Table 4.3 Mortality rates of velvetleaf blueberry inoculated with different ERM fungi and subjected to drought treatment and in well watered (control)

Population	Mortality Rate				
	control (#0)	*Pezicula ericae*	*Pezoloma ericae*	*Meliniomyces variabilis*	*Oidiodendron maius*
Lowland (LB)	33.33%	5.56%	5.56%	16.67%	11.11%
Upland (UB)	22.22%	11.11%	11.11%	5.56%	11.11%

Table 4.4 Three-way ANOVA analysis results of watering (drought and well-watered), plant provenance (upland and lowland), and ERM fungi (Four species of ERM fungi) effects on the measured parameters

Source	Significant value									
	shoot dry weights	root dry weights	total dry weights	S/R ratios	Tissue water content	leaf areas	water potentials	leaf chlorophyll concentrations	Pn	E
Drought	<0.0001	<0.0001	<0.0001	0.012	<0.0001	<0.0001	<0.0001	0.088	<0.0001	<0.0001
Population	0.143	0.605	0.208	0.003	0.272	0.19	0.072	0.728	0.724	0.393
Fungi	<0.0001[a]	<0.0001[b]	<0.0001[c]	0.114	0.316	<0.0001[d]	<0.0001[e]	0.012[f]	<0.0001[g]	0.057
Drought * Population	0.276	0.275	0.264	0.803	0.104	0.7	<0.0001	0.051	0.065	0.611
Drought * Fungi	0.056	0.026	0.053	0.076	0.162	0.117	<0.0001	0.931	0.15	0.295
Population * Fungi	0.735	0.341	0.683	0.527	0.568	0.227	0.074	0.963	0.225	0.051
Drought * Population * Fungi	0.712	0.636	0.703	0.504	0.015	0.735	0.063	0.259	0.573	0.495

Note: different letters indicate significant differences between different ERM fungi, please see Post Hoc Tests results in the Appendix.

Table 4.5 Root colonization intensity (M %)

Inoculation	Treatment	Population	M% ± se	
Non-inoculated	Drought	Upland	20.7±6.6	a
		Lowland	17.4±3.8	
	Well-watered	Upland	17.5±3.8	
		Lowland	14.7±4.9	
Pezicula ericae	Rrought	Upland	55.8±3.2	c
		Lowland	63.8±5.0	
	Well-watered	Upland	69.3±4.5	
		Lowland	71.1±4.4	
Pezoloma ericae	Rrought	Upland	67.3±4.0	b
		Lowland	51.3±4.2	
	Well-watered	Upland	56.9±3.8	
		Lowland	53.3±7.0	
Meliniomyces variabilis	Drought	Upland	55.2±4.1	bc
		Lowland	51.8±3.4	
	Well-watered	Upland	62.5±6.3	
		Lowland	78.2±4.2	
Oidiodendron maius	Drought	Upland	66.0±4.1	d
		Lowland	78.7±3.8	
	Well-watered	Upland	77.4±2.7	
		Lowland	78.3±3.3	

Data are means (n = 6) ± SE. Different letters indicate statistically significant differences between plants inoculated with different ERM fungi and non-inoculated control determined by the three-way ANOVA (Duncan's test $p \leq 0.05$).

4.7 Figures

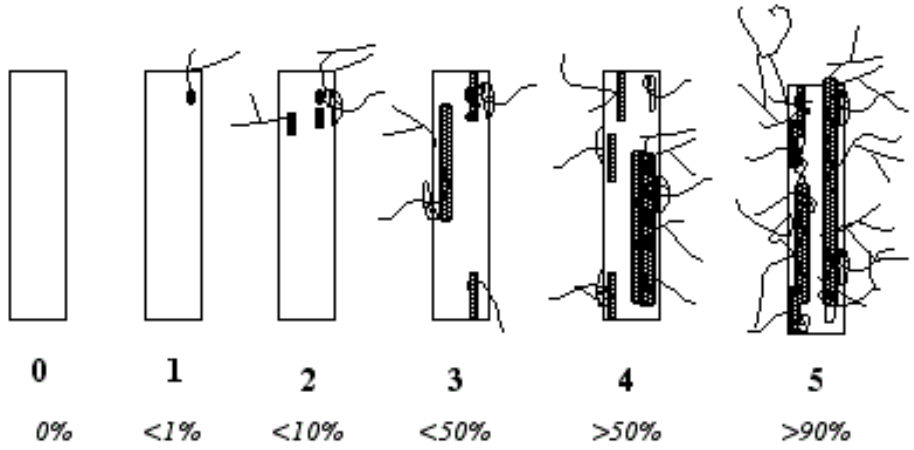

Figure 4.1 Examples of root colonization by ERM fungi for calculations of mycorrhizal intensity according to Trouvelot et al. (1986). Root sample rated 5 (intensity of each fragment > 90%); root sample rated 4 (intensity of each fragment > 50%); root sample rated 3 (intensity of each fragment < 50%); root sample rated 2 (intensity of each fragment < 10%); root sample rated 1 (intensity of each fragment < 1%); root sample rated 0 (intensity of each fragment = 0%).

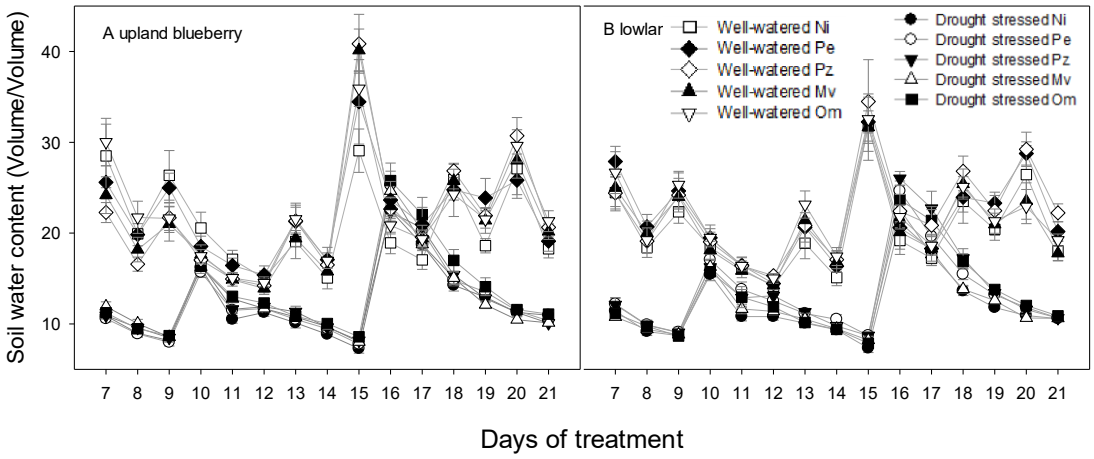

Figure 4.2 Changes of soil water content in pots with upland (A) and lowland (B) velvetleaf blueberry plants inoculated with different ERM fungi (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius*) and Ni - non-inoculated control. Plants were subjected to drought-stress or well-watered. Means (n = 9) ± SE are shown.

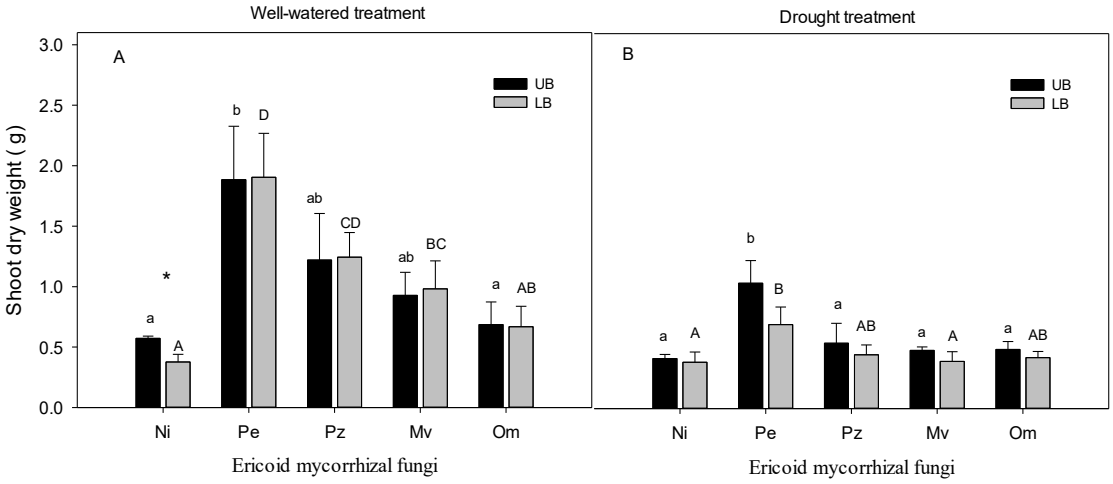

Figure 4.3 Shoot dry weights in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

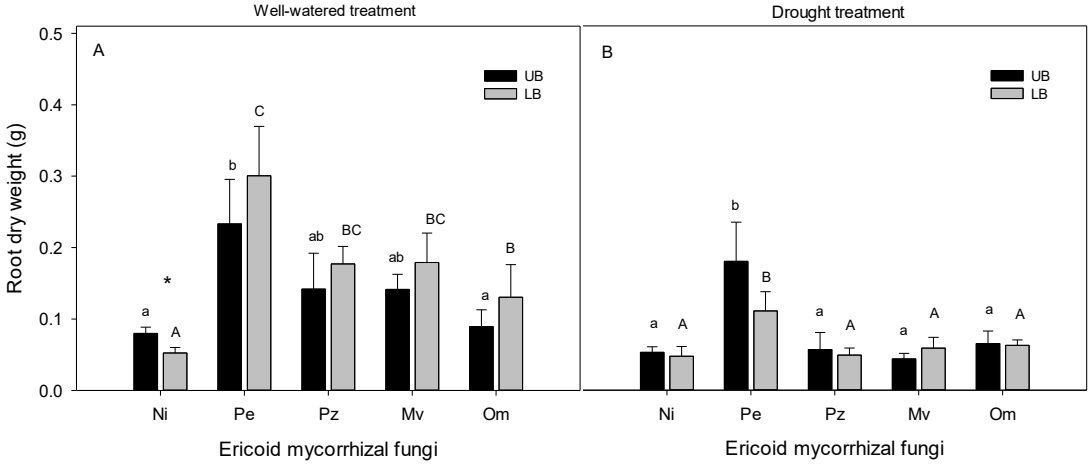

Figure 4.4 Root dry weights in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-

inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

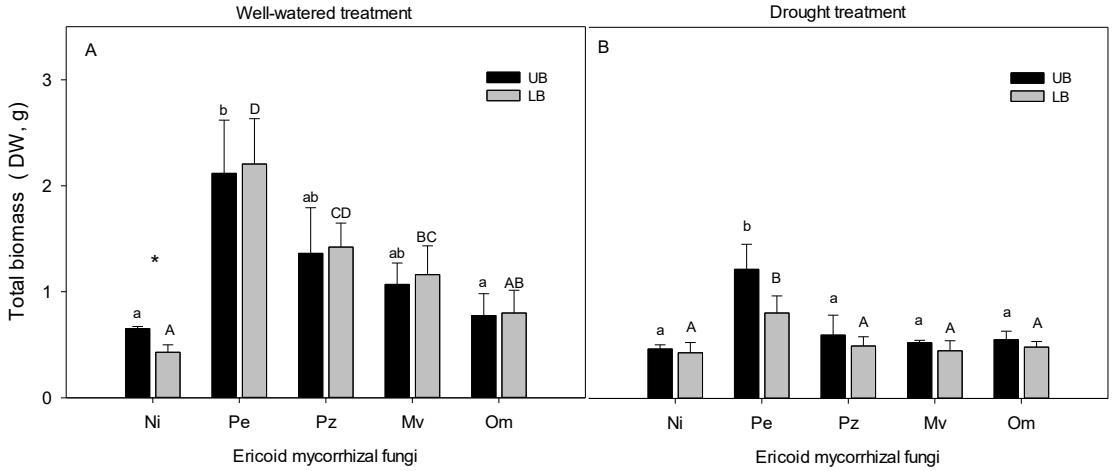

Figure 4.5 Total dry weights in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

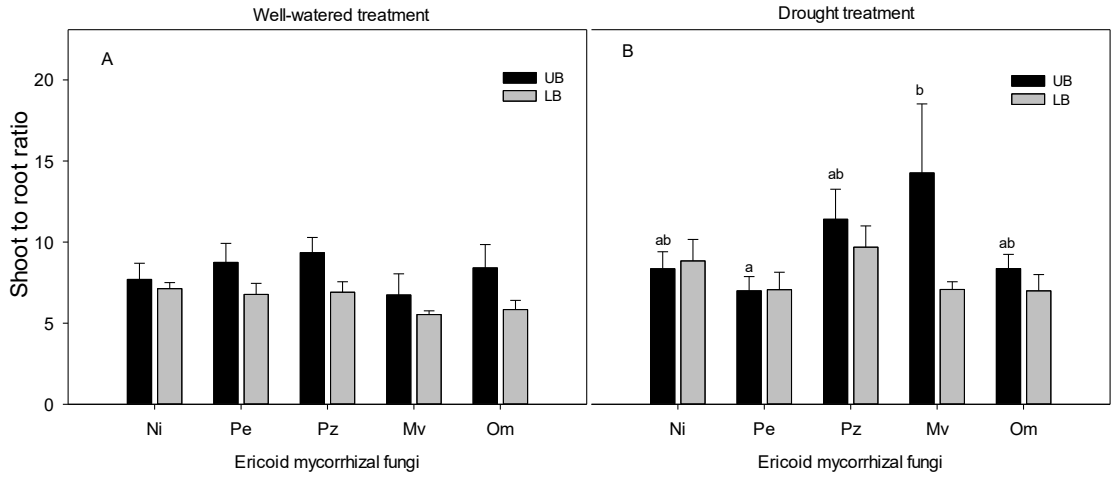

Figure 4.6 Shoot to root ratios in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered.

Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

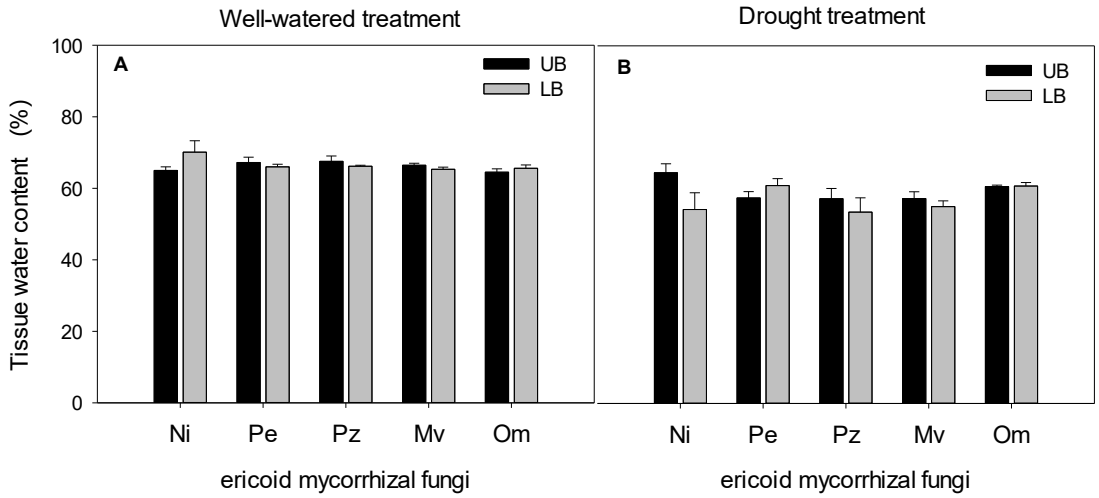

Figure 4.7 Plant tissue water content in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

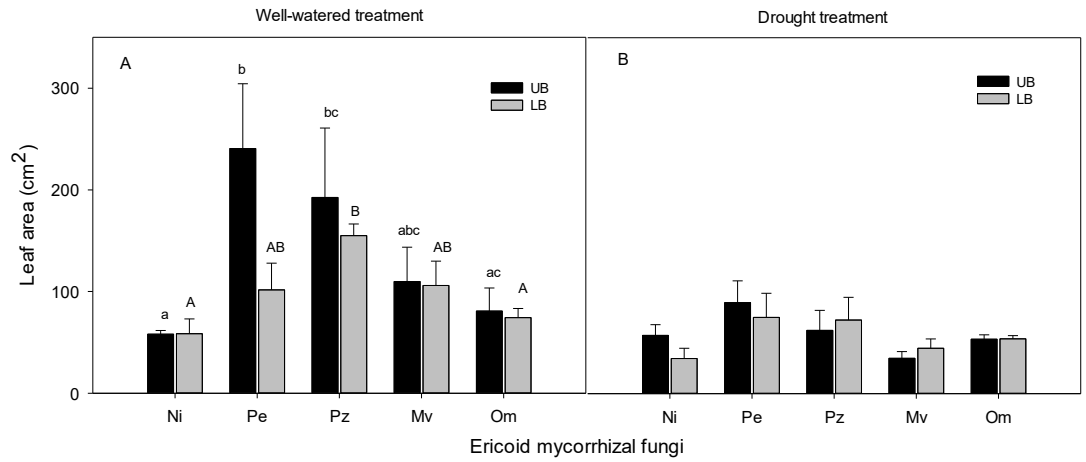

Figure 4.8 Leaf areas in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

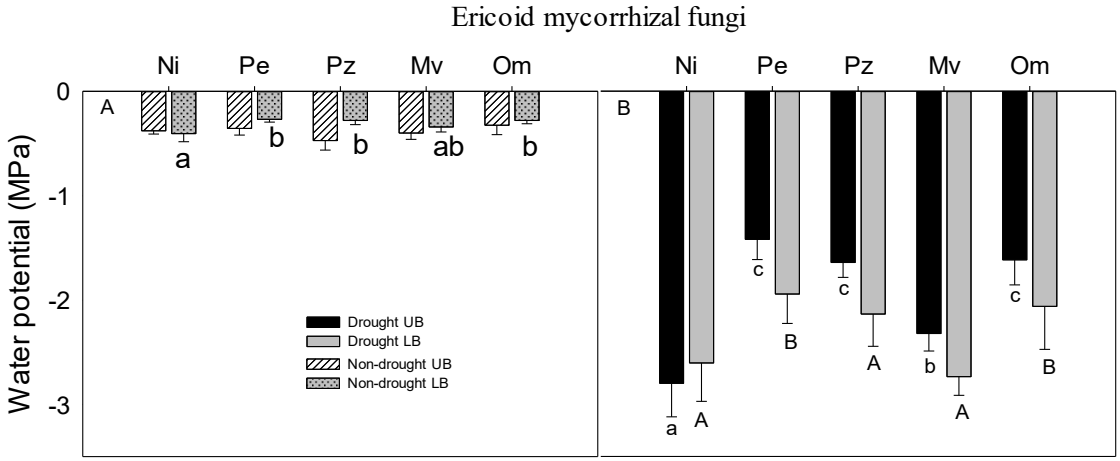

Figure 4.9 shoot water potential in well-watered (A) and drought stressed (B) upland and lowland velvetleaf blueberry plants inoculated with different ERM fungi. Data are means (n = 6) ±SE. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$). Different lowercase letters indicate significant differences in different ERM fungi treatment (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control, either subjected to drought-stress or well-watered.); * indicates significant difference between upland and lowland velvetleaf blueberry populations.

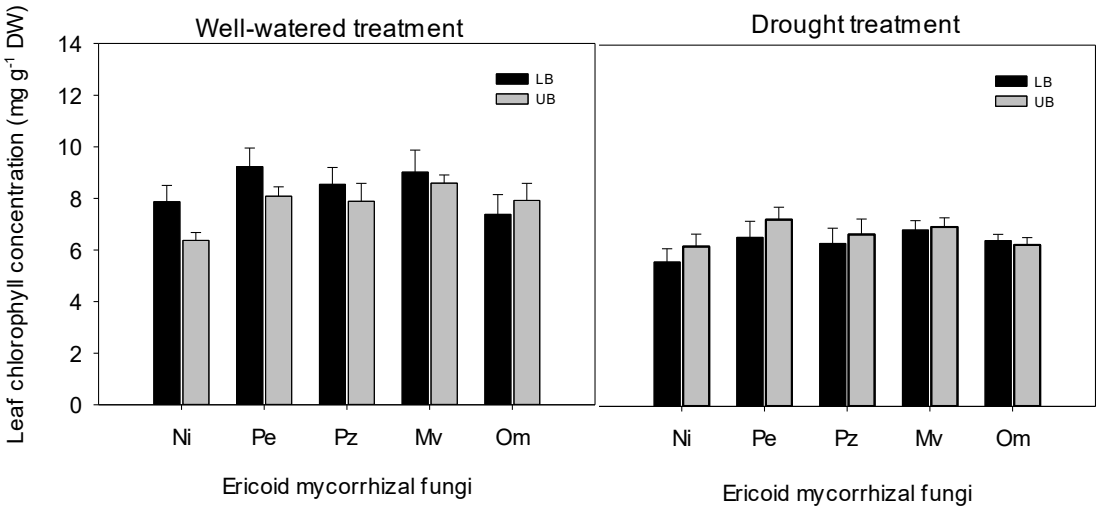

Figure 4.10 Leaf chlorophyll concentration in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

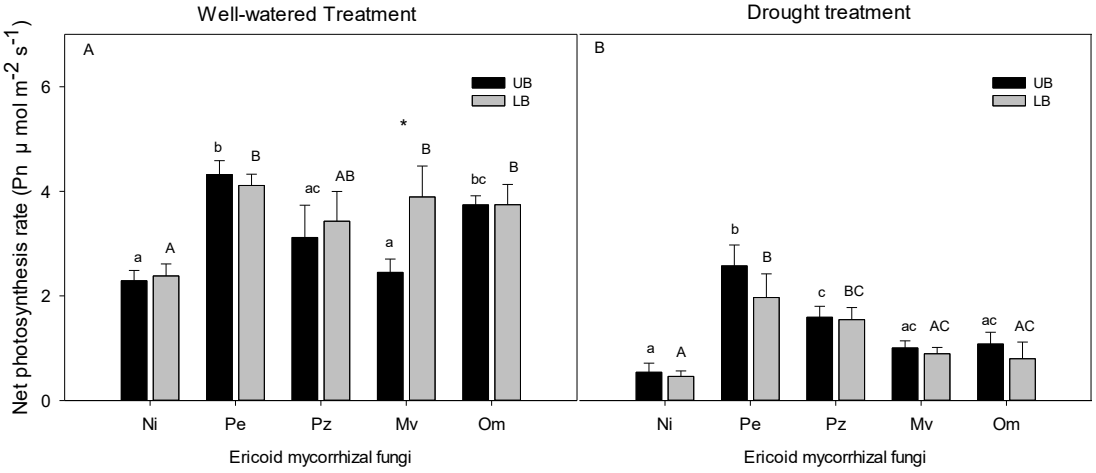

Figure 4.11 Net photosynthesis rate in upland (UB) and lowland (LB) of velvetleaf blueberry seedlings inoculated with ERM fungi and subjected to drought stress or well-watered after 21 days of treatment. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,*

Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

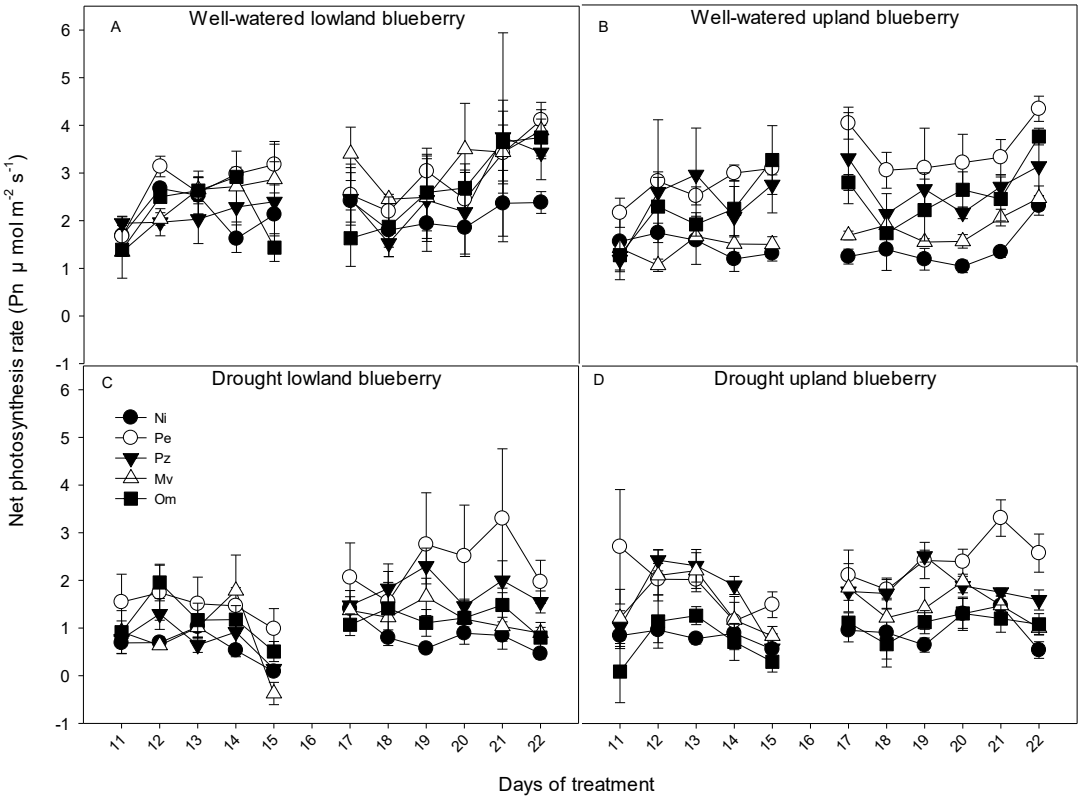

Figure 4.12 Daily change of net photosynthesis rate in upland velvetleaf blueberry and lowland velvetleaf blueberry under drought and well-watered treatment inoculate with different ERM fungi. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

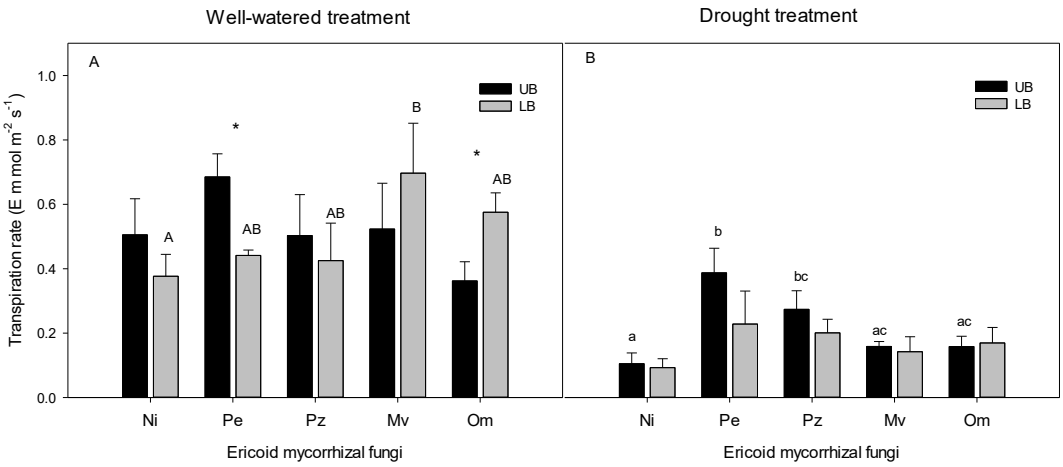

Figure 4.13 Transpiration rate in upland velvetleaf blueberry and lowland velvetleaf blueberry under drought and well-watered treatment inoculate with different ERM fungi after 21 days of treatment. Data are means (n = 6) ±SE. Different letters indicate significant differences between the plants subjected to different ERM inoculation treatments (Pe - *Pezicula ericae*, Pz - *Pezoloma ericae,* Mv - *Meliniomyces variabilis,* Om - *Oidiodendron maius* and Ni - non-inoculated control). The plants were subjected to drought-stress or well-watered. * indicates significant difference between plants from the upland and lowland population. One-way ANOVA was performed followed by Duncan's test ($P \leq 0.05$).

Chapter 5

General discussion and conclusion

5.1 General discussion

Reclamation of oil sands mining areas involves reconstruction of landforms, soil placement and revegetation. Revegetation of some of these sites is challenging due to the presence in some areas of elevated salinity (Kessler et al. 2010, Lazorko and Van Rees 2012), high pH (Allen et al. 2010, Lilles et al. 2012) , low content of beneficial soil microorganisms (Fadaei 2019), and drought conditions (Peng et al. 2011, Lilles et al. 2012). Relatively little is known about the effects of salinity on plant growth and physiology in the presence of other confounding environmental factors (Renault et al. 1999, Kopittke and Menzies 2004). The effects of combined environmental stresss on the development and growth of plants in oil sands reclamation areas need to be better understood to improve revegetation success. In my first research study of this dissertation, I examined the effects of combined drought and salt stresses on the processes of redistribution of water and salt by trembling aspen (*Populus tremuloides*). The process of hydraulic lift (redistribution) is believed to be a widespread phenomenon in various plant species (Zapater et al. 2011), but potential redistribution of salt through this process had received less attention. My study provided evidence for hydraulic redistribution in trembling aspen exposed to drought, salinity and combined drought and

salinity stress. Hydraulic lift contributed to an approximately 13% increase of the volumetric water content in the upper soil layer under drought and under drought combined with salt (NaCl) stress. However, this increase was not accompanied by a significant increase in Na^+ in the upper soil layer when the lower soil layer was immersed in NaCl solution. These results indicate that salt redistribution in the soil through the root system of trembling aspen is not a significant concern on salt-affected sites. Hydraulic lift in plants is believed to help maintain plant water balance in numerous ecosystems such as savannas and temperate and boreal forests (Ludwig et al. 2003, Moreira et al. 2003, Anderegg et al. 2018). Therefore, in oil sand reclamation areas and other sites exposed to soil salinity and drought, trembling aspen could improve soil water dynamics and benefit neighboring plants during the periods of drought. However, soil texture and other soil properties should also be considered in future research as potential factors that may affect the efficiency of hydraulic lift in plants (Wang et al. 2009, Prieto et al. 2010).

In the second research study (Chapter 3), I examined the growth and physiological responses of plants exposed to different pH levels and NaCl when directly affecting only the lower part of the root system. I found that the growth of slender wheatgrass was sharply inhibited when only the lower part of the root system (10% of total root biomass) was exposed to NaCl and/or high pH. It is plausible that high pH and NaCl played a role as stress signals in plant cells (Wilkinson 1999, Kader and Lindberg 2010). Therefore, an

exposure of only a small part of the root system triggered a series of different physiological responses, which contributed to growth inhibition. However, the same treatments had less effect on the growth of yellow sweet clover compared with slender wheatgrass. This demonstrates that yellow sweet clover is relatively less sensitive to NaCl and high pH compared with slender wheatgrass (Ogle 2002, Ogle et al. 2008) and the stress signals were not sufficiently strong to produce general stress responses in plants. In addition, root nodules were obserived in most of the stress treated (NaCl and/or high pH) yellow sweet clover plants. In geneal, N_2-fixing is the main function of the root nodules in leguminous plants due to the presence of N_2-fixing bacteria (Singh 2018). Besides, the nodules could also improve the supply other nutrient elements such as phosphorus (Sharma et al. 2013). They could also be the source of siderophores (Arora et al. 2001) as well as phytohormones such as IAA (Afzal and Bano 2008, Weyens et al. 2009) and cytokinins (Senthilkumar et al. 2009). The root nodules have been found to be effective in alleviating the effects of abiotic stresses including high and low pH, salinity, and drought (Gopalakrishnan et al. 2015). What's more, a study of clover (*Trifolium subterranemn* and *Trifolium fragiferum*) roots showed that higher vacuolar pH was associated with a higher nodulation frequency (Oliveira et al. 1992). Therefore, the nodulation in yellow sweet clover roots may be among the reasons for protecting plants against the effects of high pH and NaCl. However, the effects of root nodules with N_2-

fixing bacteria on growth of yellow sweet clover exposed to high pH and salinity conditions should be further examined through more research.

In my third research study (Chapter 4), I focused on the role of ericoid mycorrhizal associations (ERM) in protecting ericaceous plants against drougt stress. The study was carried out due to low success rate of oil sands revegetation with ericaceous plants and because little is known about the effects of ERM on plant stress resistance. Mycorrhizal associations are present in most of the terrestrial vascular plants (Al-Karaki 2013). Over 90% of the world's vascular plant species are associated with different kinds of mycorrhizal fungi (Brundrett and Tedersoo 2017). I studied the effects of four ericoid mycorrhizas fungi (*Pezicula ericae*, *Pezoloma ericae*, *Meliniomyces variabilis*, and *Oidiodendron maius*) on drought resistance of velvetleaf blueberry (*Vaccinium myrtilloides*) under controlled-environment conditions. The fungi were isolated from the roots of velvetleaf blueberry plants growing in undisturbed forest in the north-eastern Alberta and cultured before plant inoculation. The study demonstrated an enhancement of growth and survival of velvetleaf blueberry plants exposed to several cycles of drought stress. Althought it is difficult to assess drought stress effects on ericoid mycorrhizal because of the paucity of studies on this type of mycorrhizas (Gehring et al. 2017), most of the studies (Augé 2001, Tedersoo et al. 2010, Jayne and Quigley 2014) point out that the inoculation with AM and ECM fungi can improve the growth of host plants under

drought stress. Both AM fungi and ECM fungi could influence plant water relations, however, the drought tolerance mechanisms may vary. Usually, the ECM and AM fungi could help the host plants increase the stomatal conductance, alter hydraulic conductance, and facilitate the tissue osmotic adjustments as well as nutrient uptake (Lehto and Zwiazek 2011). I found that in velvetleaf blueberry, the improved drought resistance of ERM plants was largely due to increased water supply to the plants and through facilitated nutrient uptake. The effects of mycorrhizal fungi on drought tolerance of the host plants may vary depending on the mycorrhizal fungal species (Gehring et al. 2017). Differences in the effectiveness of drought protection by the different ERM fungi were also demonstrated in my study for the velvetleaf blueberry plants. Inoculation of velvetleaf blueberry with *Pezicula ericae* was more effective in increasing growth and improving physiological parameters compared with the other examined ERM fungi in both drought and non-drought treatments. A study that examined the responses of ERM velvetleaf blueberry plants to NaCl, also found that the degree of plant stress protection varied between the ERM fungi (Fadaei 2019, Fadaei et al. 2020) demonstrating the importance of proper ERM fungal selection for the maximum protection against environmental stresses. However, it may also be necessary to match the ERM fungi with the ericaceous plant species as well as the site conditions before recommending plant inoculation with a specific ERM fungus for planting in oil sands reclamation sites.

5.2 Suggestions for oil sands reclamation

I carried out three studies on the effects of salt, high pH and drought stress in plants that are used for the reclamation of oil sands areas. The results of my first study showed that trembling aspen seedlings can hydraulically redistribute water, but not NaCl, through the root system from the deeper soil layers to the surface soil layers. The process of water redistribution has important potential consequences to the survival and growth of neighboring plants and my study showed that there shoud be no concern that significant amounts of NaCl will be redistributed with water in plants growing in salinity-affected soil. Therefore, in oil sands reclamation sites, trembling aspen could improve soil water dynamics and provide water to neighbouring plants during the periods of drought. Results of the second study demonstrated that yellow sweet clover was better adapted to the higher root zone pH and salinity compared with slender wheatgrass. Combined with the nitrogen-fixing properties of yellow seet clover roots, these features make the plants highly suitable during the early stages of oil sands revegetation. . For the third study, I determined that ERM colonization of velvetleaf blueberry is highly beneficial to growth of plants, especially under the drough stress conditions. I also determined that *Pezicula ericae* was the most effective of the examined ERM fungi in improving growth and physiological parameters. Due to the loss of ERM fungal diversity in stockpiled soil that is used for oil sands reclamation (Fadaei 2019), the ERM colonization rate of velvetleaf

blueberry may be very low root in oil sand reclamation site. to the results of the study point to the importance of velvetleaf blueberry inoculation with *Pezicula ericae* prior to planting in oil sands reclamation areas.

5.3 Suggestions for future research

One of the outcomes of all research studies is better understanding of what else needs to be done to fill some of the important knowledge gaps and new questions generated by the research. Although I have already mentioned in previous chapters some of the additional studies that could help address these questions, I listed below the highest priority areas that require more research to understand plant response to salinity, high pH, drought and root hydraulic redistribution in reclamation areas:

1) Examine the horizontal water redistribution by the root system of trembling aspen and determine through field studies the contribution of root hydraulic redistribution in oil sands reclamation area by trembling aspen and other plant species.

2) Investigate the uptake and utilization of mineral nutrients in yellow sweet clover and slender wheatgrass under the heterogeneous soil pH and salt gradients.

3) Examine the effects of ERM associations on the growth of ericaceous plants under the challenging soil conditions characterized by a combination of elevated pH, salinity and drought.

4) Investigate the significance of hydraulic redistribution by plants in oil sands

reclamation areas and its benefit to the survival of mycorrhizal associations under diverse soil conditions.

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Appendix 1

(Supplementary results of Chapter 3)

Table a1.1 Two-way ANOVA tests of treatment effects on wheatgrass shoot dry weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig. (P)
Corrected Model	2599.176[a]	5	519.835	16.590	.000
Intercept	17930.473	1	17930.473	572.231	.000
salt	2077.830	2	1038.915	33.156	.000
pH	422.591	1	422.591	13.487	.001
salt * pH	98.755	2	49.377	1.576	.228
Error	752.024	24	31.334		
Total	21281.674	30			
Corrected Total	3351.201	29			

a. R Squared = .776 (Adjusted R Squared = .729).

Table a1.2 Two-way ANOVA tests of treatment effects on clover shoot dry weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37.899[a]	5	7.580	2.606	.051
Intercept	2904.513	1	2904.513	998.527	.000
salt	11.180	2	5.590	1.922	.168
pH	20.814	1	20.814	7.156	.013
salt * pH	5.905	2	2.952	1.015	.377
Error	69.811	24	2.909		
Total	3012.223	30			
Corrected Total	107.710	29			

a. R Squared = .352 (Adjusted R Squared = .217).

Table a1.3 Two-way ANOVA tests of treatment effects on wheatgrass root dry weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	104.554[a]	5	20.911	9.096	.000
Intercept	1457.182	1	1457.182	633.840	.000
salt	83.010	2	41.505	18.054	.000
pH	13.824	1	13.824	6.013	.022
salt * pH	7.721	2	3.860	1.679	.208
Error	55.175	24	2.299		
Total	1616.911	30			
Corrected Total	159.729	29			

a. R Squared = .655 (Adjusted R Squared = .583).

Table a1.4 Two-way ANOVA tests of treatment effects on clover root dry weight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	363.190[a]	5	72.638	2.189	.089
Intercept	6854.662	1	6854.662	206.597	.000
salt	72.548	2	36.274	1.093	.351
pH	240.581	1	240.581	7.251	.013
salt * pH	50.061	2	25.030	.754	.481
Error	796.292	24	33.179		
Total	8014.144	30			
Corrected Total	1159.482	29			

a. R Squared = .313 (Adjusted R Squared = .170).

Table a1.5 Two-way ANOVA tests of treatment effects on wheatgrass biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3666.427[a]	5	733.285	17.236	.000
Intercept	29610.757	1	29610.757	695.999	.000
salt	2917.706	2	1458.853	34.290	.000
pH	589.278	1	589.278	13.851	.001
salt * pH	159.443	2	79.721	1.874	.175
Error	1021.062	24	42.544		
Total	34298.246	30			
Corrected Total	4687.489	29			

a. R Squared = .782 (Adjusted R Squared = .737).

Table a1.6 Two-way ANOVA tests of treatment effects on clover biomass

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	556.068[a]	5	111.214	3.960	.009
Intercept	18683.177	1	18683.177	665.334	.000
salt	126.979	2	63.489	2.261	.126
pH	402.922	1	402.922	14.349	.001
salt * pH	26.167	2	13.084	.466	.633
Error	673.941	24	28.081		
Total	19913.186	30			

Corrected Total	1230.009	29

a. R Squared = .452 (Adjusted R Squared = .338).

Table a1.7 Two-way ANOVA tests of treatment effects on wheatgrass leaf water potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	344.768[a]	5	68.954	11.113	.000
Intercept	12730.636	1	12730.636	2051.683	.000
salt	299.904	2	149.952	24.166	**.000**
pH	44.602	1	44.602	7.188	**.010**
salt * pH	.262	2	.131	.021	**.979**
Error	260.609	42	6.205		
Total	13336.013	48			
Corrected Total	605.377	47			

a. R Squared = .570 (Adjusted R Squared = .518).

Table a1.8 Two-way ANOVA tests of treatment effects on yellow sweet clover leaf water potential

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	147.358[a]	5	29.472	12.827	.000

Intercept	2919.601	1	2919.601	1270.662	.000
salt	134.665	2	67.333	29.304	.000
pH	7.308	1	7.308	3.181	.085
salt * pH	5.385	2	2.692	1.172	.324
Error	68.931	30	2.298		
Total	3135.890	36			
Corrected Total	216.289	35			

a. R Squared = .681 (Adjusted R Squared = .628).

Table a1.9 Two-way ANOVA tests of treatment effects on wheatgrass Pn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	100.464[a]	5	20.093	24.369	.000
Intercept	1145.888	1	1145.888	1389.780	.000
Salt	93.122	2	46.561	56.471	.000
pH	6.693	1	6.693	8.118	.008
Salt * pH	.649	2	.324	.394	.678
Error	24.735	30	.825		
Total	1271.087	36			
Corrected Total	125.199	35			

a. R Squared = .802 (Adjusted R Squared = .770).

Table a1.10 Two-way ANOVA tests of treatment effects on clover Pn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	91.128[a]	5	18.226	27.917	.000
Intercept	856.290	1	856.290	1311.624	.000
salt	71.658	2	35.829	54.881	.000
ph	18.514	1	18.514	28.359	.000
salt * ph	.956	2	.478	.732	.489
Error	19.585	30	.653		
Total	967.004	36			
Corrected Total	110.713	35			

a. R Squared = .823 (Adjusted R Squared = .794).

Table a1.11 Two-way ANOVA tests of treatment effects on wheatgrass E

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.461[a]	5	.292	25.315	.000
Intercept	.784	1	.784	67.912	.000
Salt	1.343	2	.672	58.177	.000
pH	.046	1	.046	3.985	.055
Salt * pH	.072	2	.036	3.118	.059
Error	.346	30	.012		
Total	2.591	36			
Corrected Total	1.807	35			

a. R Squared = .808 (Adjusted R Squared = .776).

Table a1.12 Two-way ANOVA tests of treatment effects on clover E

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.844[a]	5	.169	11.224	.000
Intercept	.427	1	.427	28.370	.000
salt	.524	2	.262	17.410	.000
ph	.247	1	.247	16.403	.000
salt * ph	.074	2	.037	2.449	.104
Error	.451	30	.015		
Total	1.722	36			
Corrected Total	1.295	35			

a. R Squared = .652 (Adjusted R Squared = .594).

Table a1.13 Two-way ANOVA tests of treatment effects on wheatgrass Leaf area

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.188[a]	5	.238	40.483	.000
Intercept	330.224	1	330.224	56287.275	.000
salt	1.182	2	.591	100.765	.000
pH	.005	1	.005	.843	.368
salt * pH	.000	2	.000	.022	.978
Error	.141	24	.006		

Total	331.553	30
Corrected Total	1.328	29

a. R Squared = .894 (Adjusted R Squared = .872).

Table a1.14 Two-way ANOVA tests of treatment effects on clover Leaf area

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	380657.062[a]	5	76131.412	1.480	.233
Intercept	32314207.608	1	32314207.608	628.253	.000
salt	80548.350	2	40274.175	.783	.468
pH	199618.819	1	199618.819	3.881	.060
salt * pH	100489.893	2	50244.946	.977	.391
Error	1234439.915	24	51434.996		
Total	33929304.586	30			
Corrected Total	1615096.977	29			

a. R Squared = .236 (Adjusted R Squared = .076).

Appendix 2

(Supplementary results of Chapter 4)

Table a2.1 Three -way ANOVA analysis of drought, population and ERM fungi effect on shoot dry weights

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	5.386[a]	19	.283	6.237	.000
Intercept	5.177	1	5.177	113.906	.000
Drought	1.936	1	1.936	42.600	.000
Population	.099	1	.099	2.185	.143
Fungi	2.673	4	.668	14.703	.000[b]
Drought * Population	.054	1	.054	1.199	.276
Drought * Fungi	.435	4	.109	2.393	.056
Population * Fungi	.091	4	.023	.501	.735
Drought * Population * Fungi	.097	4	.024	.532	.712
Error	4.545	100	.045		
Total	15.109	120			
Corrected Total	9.931	119			

a. R Squared = .542 (Adjusted R Squared = .455) b. Post Hoc Test see Table a2.2.

Table a2.2 Post Hoc Tests of drought, population and ERM fungi effect on shoot dry weights

Fungi	N	Subset		
		1	2	3
#0	24	-.3902		
Oidiodendron maius	24	-.2986	-.2986	
Meliniomyces variabilis	24		-.2267	
Pezoloma ericae	24		-.1771	
Pezicula ericae	24			.0540
Sig.		.140	.064	1.000

Based on observed means (Log transformed).

Table a2.3 Three -way ANOVA analysis of drought and ERM fungi effect on root dry weights

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	7.385[a]	19	.389	6.420	.000
Intercept	141.265	1	141.265	2333.042	.000
Drought	3.124	1	3.124	51.593	.000
Population	.016	1	.016	.270	.605
Fungi	3.043	4	.761	12.564	.000[b]
Drought * Population	.073	1	.073	1.206	.275
Drought * Fungi	.698	4	.174	2.881	.026
Population * Fungi	.277	4	.069	1.143	.341
Drought * Population * Fungi	.155	4	.039	.639	.636
Error	6.055	100	.061		
Total	154.705	120			
Corrected Total	13.440	119			

a. R Squared = .549 (Adjusted R Squared = .464) b. Post Hoc Test see Table a2.4.

Table a2.4 Post Hoc Tests of drought and ERM fungi effect on root dry weights

Fungi	N	Subset		
		1	2	3
#0	24	-1.2769		
Oidiodendron maius	24	-1.1418	-1.1418	
Meliniomyces variabilis	24		-1.1220	
Pezoloma ericae	24		-1.0914	

Pezicula ericae	24			-.7929
Sig.		.060	.509	1.000

Based on observed means (Log transformed).

Table a2.5 Three -way ANOVA analysis of drought and ERM fungi effect on total dry weights

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.521[a]	19	.291	6.429	.000
Intercept	2.727	1	2.727	60.335	.000
Drought	2.045	1	2.045	45.242	.000
Population	.073	1	.073	1.606	.208
Fungi	2.706	4	.677	14.968	.000[b]
Drought * Population	.057	1	.057	1.261	.264
Drought * Fungi	.439	4	.110	2.427	.053
Population * Fungi	.104	4	.026	.573	.683
Drought * Population * Fungi	.098	4	.025	.545	.703
Error	4.520	100	.045		
Total	12.769	120			
Corrected Total	10.041	119			

a. R Squared = .550 (Adjusted R Squared = .464) b. Post Hoc Test see Table a2.6.

Table a2.6 Post Hoc Tests of drought and ERM fungi effect on total dry weights

Fungi	N	Subset		
		1	2	3
#0	24	-.3356		
Oidiodendron maius	24	-.2373	-.2373	

Meliniomyces variabilis	24			-.1671	
Pezoloma ericae	24			-.1281	
Pezicula ericae	24				.1144
Sig.		.112		.096	1.000

Based on observed means (Log transformed).

Table a2.7 Three -way ANOVA analysis of drought and ERM fungi effect on S/R ratios

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.836[a]	19	.044	2.030	.013
Intercept	92.355	1	92.355	4259.046	.000
Drought	.141	1	.141	6.519	.012
Population	.196	1	.196	9.046	.003
Fungi	.166	4	.041	1.911	.114
Drought * Population	.001	1	.001	.062	.803
Drought * Fungi	.189	4	.047	2.185	.076
Population * Fungi	.069	4	.017	.801	.527
Drought * Population * Fungi	.073	4	.018	.838	.504
Error	2.168	100	.022		
Total	95.359	120			
Corrected Total	3.005	119			

a. R Squared = .278 (Adjusted R Squared = .141).

Table a2.8 Three -way ANOVA analysis of drought and ERM fungi effect on Tissue water content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.283[a]	19	.015	5.843	.000

Intercept	46.582	1	46.582	18258.391	.000
Drought	.203	1	.203	79.673	.000
Population	.003	1	.003	1.218	.272
Fungi	.012	4	.003	1.199	.316
Drought * Population	.007	1	.007	2.692	.104
Drought * Fungi	.017	4	.004	1.675	.162
Population * Fungi	.008	4	.002	.739	.568
Drought * Population * Fungi	.033	4	.008	3.247	.015
Error	.255	100	.003		
Total	47.120	120			
Corrected Total	.538	119			

a. R Squared = .526 (Adjusted R Squared = .436).

Table a2.9 Three -way ANOVA analysis of drought and ERM fungi effect on DW/FW ratios

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.091[a]	19	.005	5.446	.000
Intercept	5.559	1	5.559	6308.760	.000
Drought	.065	1	.065	73.346	.000
Population	.000	1	.000	.511	.476
Fungi	.005	4	.001	1.475	.215
Drought * Population	.002	1	.002	2.394	.125
Drought * Fungi	.004	4	.001	1.258	.292
Population * Fungi	.002	4	.000	.450	.773
Drought * Population * Fungi	.013	4	.003	3.622	.008
Error	.088	100	.001		
Total	5.738	120			
Corrected Total	.179	119			

a. R Squared = .509 (Adjusted R Squared = .415).

Table a2.10 Three -way ANOVA analysis of drought and ERM fungi effect on leaf areas

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.657[a]	19	.192	4.032	.000
Intercept	271.797	1	271.797	5693.791	.000
Drought	1.568	1	1.568	32.842	.000
Population	.084	1	.084	1.754	.190
Fungi	1.255	4	.314	6.575	.000 [b]
Drought * Population	.007	1	.007	.150	.700
Drought * Fungi	.369	4	.092	1.933	.117
Population * Fungi	.278	4	.069	1.456	.227
Drought * Population * Fungi	.096	4	.024	.501	.735
Error	2.864	60	.048		
Total	278.317	80			
Corrected Total	6.521	79			

a. R Squared = .561 (Adjusted R Squared = .422) b. Post Hoc Test see Table a2.11.

Table a2.11 Post Hoc Tests of drought and ERM fungi effect on leaf areas

Fungi	N	Subset	
		1	2
0	16	1.6714	
81	16	1.7791	
96	16	1.7906	
50	16		1.9736
38	16		2.0015
Sig.		.150	.719

Based on observed means (Log transformed). The error term is Mean Square (Error) = .048. The error term is Mean Square (Error) = .044. Duncan: Uses Harmonic Mean Sample Size = 16.000. Alpha = .05.

Table a2.12 Three-way ANOVA analysis of drought and ERM fungi effect on water potentials

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	254.884[a]	19	13.415	128.929	.000
Intercept	1187.652	1	1187.652	11414.359	.000
Drought	228.790	1	228.790	2198.870	.000
Population	.345	1	.345	3.317	.072
Fungi	13.861	4	3.465	33.305	.000 [b]
Drought * Population	2.633	1	2.633	25.301	.000
Drought * Fungi	7.378	4	1.845	17.728	.000
Population * Fungi	.916	4	.229	2.201	.074
Drought * Population * Fungi	.961	4	.240	2.308	.063
Error	10.405	100	.104		
Total	1452.941	120			
Corrected Total	265.289	119			

a. R Squared = .961 (Adjusted R Squared = .953), b. Post Hoc Test see Table a2.13.

Table a2.13 Post Hoc Tests of drought and ERM fungi effect on water potentials

Fungi	N	Subset			
		1	2	3	4
96	24	2.7969			
38	24	2.8590	2.8590		
50	24		2.9981		
81	24			3.3891	
0	24				3.6867
Sig.		.506	.139	1.000	1.000

Based on observed means (square root transformed). The error term is Mean Square(Error) = .104. Duncan: Uses Harmonic Mean Sample Size = 24.000. Alpha = .05.

Table a2.14 Three-way ANOVA analysis of drought and ERM fungi effect on leaf chlorophyll concentrations

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	31.078[a]	19	1.636	1.437	.127
Intercept	5343.214	1	5343.214	4693.375	.000
Drought	3.378	1	3.378	2.967	.088
Population	.139	1	.139	.122	.728
Fungi	15.356	4	3.839	3.372	.012 [b]
Drought * Population	4.436	1	4.436	3.897	.051
Drought * Fungi	.968	4	.242	.213	.931
Population * Fungi	.674	4	.169	.148	.963
Drought * Population * Fungi	6.126	4	1.531	1.345	.259
Error	113.846	100	1.138		
Total	5488.138	120			
Corrected Total	144.924	119			

a. R Squared = .214 (Adjusted R Squared = .065), b. Post Hoc Test see Table a2.15.

Table a2.15 Post Hoc Tests of drought and ERM fungi effect on leaf chlorophyll concentrations

Fungi	N	Subset	
		1	2
0	24	6.192625	
96	24	6.359394	
50	24	6.677949	6.677949

81	24		7.061524
38	24		7.072703
Sig.		.140	.231

Based on observed means. The error term is Mean Square (Error) = 1.138. Duncan: Uses Harmonic Mean Sample Size = 24.000. Alpha = .05.

Table a2.16 Three-way ANOVA analysis of drought and ERM fungi effect on leaf Pn

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	187.016[a]	19	9.843	14.947	.000
Intercept	633.090	1	633.090	961.383	.000
Drought	132.511	1	132.511	201.225	.000
Population	.082	1	.082	.125	.724
Fungi	41.848	4	10.462	15.887	.000 [b]
Drought * Population	2.289	1	2.289	3.476	.065
Drought * Fungi	4.554	4	1.139	1.729	.150
Population * Fungi	3.807	4	.952	1.445	.225
Drought * Population * Fungi	1.925	4	.481	.731	.573
Error	65.852	100	.659		
Total	885.958	120			
Corrected Total	252.868	119			

a. R Squared = .740 (Adjusted R Squared = .690), b. Post Hoc Test see Table a2.17.

Table a2.17 Post Hoc Tests of drought and ERM fungi effect on leaf Pn

Fungi	N	Subset		
		1	2	3

0	24	1.417860		
81	24		2.059776	
96	24		2.341420	
50	24		2.421301	
38	24			3.244142
Sig.		1.000	.149	1.000

Based on observed means. The error term is Mean Square(Error) = .659. Duncan: Uses Harmonic Mean Sample Size = 24.000. Alpha = .05.

Table a2.18 Three-way ANOVA analysis of drought and ERM fungi effect on leaf E

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.175[a]	19	.220	5.519	.000
Intercept	14.746	1	14.746	370.358	.000
Drought	3.031	1	3.031	76.124	.000
Population	.029	1	.029	.736	.393
Fungi	.379	4	.095	2.377	.057
Drought * Population	.010	1	.010	.260	.611
Drought * Fungi	.199	4	.050	1.249	.295
Population * Fungi	.391	4	.098	2.455	.051
Drought * Population * Fungi	.136	4	.034	.853	.495
Error	3.982	100	.040		
Total	22.903	120			
Corrected Total	8.157	119			

a. R Squared = .512 (Adjusted R Squared = .419).