UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

LES COMMUNAUTÉS MYCORHIZIENNES ET LEUR EFFET SUR LA CROISSANCE DES ARBRES SUR UN SITE POST-MINIER

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FOREWORD

The dissertation is divided into three chapters. Chapter I includes the general introduction which puts into context the research problem, the literature review and the objectives. Chapter II is presented in the form of a scientific article with authors: "Supun Madhumadhawa Pawuluwage, Philippe Marchand, Nicole J. Fenton, Melanie Roy and Benoit Lafleur". The article will be submitted to a selected journal. I had the main responsibility for collecting and analyzing the data, and writing the article. My directors and research committee members helped design the study, assisted in interpretation of the results and critically and constructively revised the content of the article. Chapter III contains the general conclusion and implications.

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LIST OF ABBREVIATIONS

OTU: Operational Taxonomic Unit

CEC: Cation Exchange Capacity

O_2: Oxygen

CO_2: Carbon dioxide

N: Nitrogen

N_2: Dinitrogen

P: Phosphorus

Zn: Zinc

Cu: Copper

Mn: Manganese

Ni: Nickel

Co: Cobalt

Cd: Cadmium

Pb: Lead

Hg: Mercury

ECM: Ectomycorrhizae

ABS: Arbuscular Mycorrhizae

ppm : Parts Per Million

RÉSUMÉ

La symbiose mycorhizienne joue un rôle clé dans les processus écologiques comme la succession des plantes à travers la redistribution des ressources entre les plantes hôtes ayant des besoins différents. La succession primaire de végétation dans les zones post-minières offre de grandes opportunités pour étudier comment la symbiose mycorhizienne influence la revégétalisation dans les environnements dégradés. Dans cette étude, nous visons à déterminer comment la croissance des semis est affectée par la facilitation souterraine via des réseaux mycorhiziens. Plus précisément, nous déterminons 1) les communautés mycorhiziennes existantes, 2) l'effet de variables telles que l'identité de l'hôte et la structure spatiale sur le partage mycorhizien entre les hôtes et 3) comment ces communautés mycorhiziennes, ainsi que d'autres facteurs physiques et biologiques définis, affectent la croissance des arbres dans un environnement hostile. Le site d'étude est un site de résidus miniers de la mine d'or Beattie près du lac Duparquet dans le nord-ouest du Québec et deux parcelles de 15 x 15 m ont été inventoriées sur ce site. *Betula papyrifera*, *Populus balsamifera*, *Picea glauca* et *Thuja occidentalis* ont été utilisées comme espèces focales et l'effet des plantes voisines a été identifié. Les communautés de champignons mycorhiziens ont été séquencées à l'aide de marqueurs moléculaires et analysées pour détecter un éventuel partage d'espèces. Nous avons identifié 474 unités taxonomiques opérationnelles fongiques (OTU) à partir d'échantillons de plantes amplifiées par l'ADN fongique. Parmi les 474 OTU, 52 OTU ectomycorhiziennes et 20 OTU mycorhiziennes arbusculaires ont été identifiées. La spécificité de l'hôte n'a pas été détectée car les espèces ectomycorhiziennes étaient partagées entre toutes les espèces hôtes et l'effet de priorité détermine la structure de la communauté mycorhizienne des individus de la plante hôte plutôt que la spécificité de l'hôte.

La composition mycorhizienne ne variait pas entre les parcelles et ni spatialement à l'intérieur de chaque parcelle. Les concentrations d'azote et de phosphore dans le sol étaient extrêmement faibles et des niveaux élevés d'arsenic ont été détectés sur le terrain. Les indices de compétition de voisinage, l'azote du sol, la biomasse des semis ou la richesse mycorhizienne, l'abondance mycorhizienne et le partage des mycorhizes entre les plantes hôtes n'ont pas affecté la croissance de la plupart des plantes hôtes. Cette étude conclut que les interactions plante-plante (compétition ou facilitation), les réseaux mycorhiziens et les variations locales de concentration des nutriments du sol ne sont pas des facteurs importants pour la détermination de la croissance et de la végétalisation des plantes dans notre site d'étude en raison des fortes limitations des nutriments. Cette étude aide à développer des stratégies appropriées de reboisement des forêts boréales dans les sites miniers.

Mots clés: végétalisation, dégradation minière, communauté mycorhizienne, nutriments du sol.

SUMMARY

Mycorrhizal symbiosis plays a key role in ecological processes like plant succession through the redistribution of resources among host plants with different needs. Primary succession of vegetation in post-mining areas offers great opportunities to study how mycorrhizal symbiosis influences the revegetation of degraded environments. In this study, we aim to determine how seedling growth is affected by below-ground facilitation via mycorrhizal networks. Specifically, we determine 1) existing mycorrhizal communities, 2) the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts and 3) how these mycorrhizal communities, along with other defined physical and biological factors, affect tree growth in a harsh environment. The study site is the mine tailings site of the Beattie Gold Mine near Lake Duparquet in north-western Quebec and two 15 x 15 m plots were inventoried at this site. *Betula papyrifera, Populus balsamifera, Picea glauca* and *Thuja occidentalis* were used as focal species and the effect of neighbouring plants was identified. Communities of mycorrhizal fungi were sequenced using molecular markers and analyzed to detect possible species sharing. We identified 474 fungal operational taxonomic units (OTUs) from the fungal DNA amplified samples. Among the 474 OTUs, 52 ectomycorrhizal OTUs and 20 arbuscular mycorrhizal OTUs were identified. Host specificity was not detected as ectomycorrhizal species were shared among all host species and priority effects drove the mycorrhizal community structure of host plant individuals rather than host specificity.

Mycorrhizal composition did not vary between plots nor spatially within each plot. Soil nitrogen and phosphorus concentration were extremely low and high arsenic levels were detected in the field. Neighborhood competition indices, soil nitrogen, shoot biomass, mycorrhizal richness, mycorrhizal abundance and sharing of mycorrhizae among host plants did not affect the growth of most host plants. This study concludes that plant-plant interactions (competition or facilitation), mycorrhizal networks and local variations in the concentration of soil nutrients are not important factors for determination of plant growth and vegetalization at our study site due to the harsh environmental conditions. This study helps to develop appropriate strategies for reforestation of boreal forests in mining sites.

Keywords: Vegetalization, Mining Degradation, Mycorrhizal Community, Soil Nutrients.

CHAPTER I

GENERAL INTRODUCTION

1.1 Research problem

The boreal forests of Canada are the largest remaining wilderness in North America and home to a quarter of the world's remaining intact forests (Blancher and Wells, 2005). These Canadian boreal forests are under threat due to anthropogenic activities such as logging, mining, hydroelectric development and oil and gas extraction. There has been significant mining development in the boreal forests of Québec, which has required the construction of many tailings facilities over the last several decades (MERN, 2009). Mining practices, as well as oil and gas extraction, create a variety of disturbances in boreal forests, but there are few studies on the effects of these disturbances in boreal forests (Venier et al., 2014). In particular, mining activities in this region impact and change the nature and structure of boreal forests. Abandoned mining sites in the boreal region are recent surface deposits, poor in organic matter and nitrogen (Johnstone et al., 2016), and they enter a regenerating stage after creation, as they are slowly colonized by spore-bearing plants (bryophytes, pteridophytes), herbs, grasses, and eventually trees. Colonization process in post-mining sites is different from burned or logged forests: when not restored, colonization occurs in post-mining sites through the dispersal of seeds and spores over long or short distances from source populations of plant species in neighbouring forests (Audet et al., 2014); in contrast, colonization in burned or logged forests takes place from biological legacies such as seed banks and stumps of species present before the disturbance. Based on this

phenomenon, the recolonization of these post-mining sites is very similar to primary succession, which also occurs at glacier retreat sites, after a volcanic eruption, and in sand dunes (Walker and Del Moral 2003).

Post-mining lands therefore offer the opportunity to study the first steps of colonization of boreal forests on bare soil that is low in organic matter and nitrogen. Mycorrhizae as a symbiotic relationship between plants and fungi influence plant-plant interactions through the diversity of resources that mycorrhizae provides to their host and resource distribution via mycorrhizal networks to their host plants (Montesinos-Navarro et al., 2018). The effect of mycorrhizae on tree growth in a post mining site could be different compared to a natural environment. Studies of primary succession of vegetation in post-mining areas are very rare and provide great opportunities to study the nature of symbiotic relationships between host plants and fungi for facilitation of the process of revegetation in early stages of primary succession.

1.2 Literature review

1.2.1 Boreal forests in the world and their ecological importance

Boreal forests are conifer-dominated forests that are spread over 15 million km^2 across North America and Eurasia (MERN, 2008). Boreal forests are among the largest biomes in the world and provide numerous services to society, including wood and food production, water purification, and ecosystem services such as carbon storage, nutrient cycling, and soil formation (Gauthier et al., 2015; Hassan et al., 2005). Occupying about 8% of the world's land surface, boreal forests are spread over 20 time zones and split into two major parts: the largest in Russia, Siberia and Scandinavia, the

other in Canada and Alaska. Boreal forests are the largest forest type in Quebec, Canada, covering around 70% of Quebec's area and divided into two major sub-zones, the continuous boreal forest and the taiga forest (MERN, 2008).

Boreal forests are characterized by cold-tolerant species of genera such as *Abies*, *Larix*, *Picea*, *Pinus*, *Populus* and *Betula* (Brandt et. al., 2013). Most plant species of the boreal region are generalists that tolerate harsh environmental conditions and periodic natural disturbances such as forest fires and insect outbreaks (MERN, 2008). Southern Canadian boreal regions are dominated by boreal mixed-woods forests, which are characterized by varying canopy dominance of boreal broadleaf and conifer trees (Bergeron et. al., 2014). *Abies balsamea*, *Populus tremuloides*, *Betula papyrifera*, *Picea mariana*, and *Pinus banksiana* are the main tree species found in Quebec's boreal forests.

Due to changes in soil, climate and topography, boreal forests have a wide range of forests at the local level, providing a home for a large number of faunal and floral species (MERN, 2008). They also strongly contribute to the control of global warming by capturing atmospheric carbon via photosynthesis (Bonan and Shugart, 1989), as about 33% of the total carbon stored in global forests is stored in the boreal forests (60% in soils) (Pan et. al., 2011).

1.2.2 Primary succession as an ecological process

Plant succession is an ecological process that explains the vegetation dynamics in ecosystems over time after various disturbances occur in natural ecosystems (Horn 1974, del Moral 2007, Pickett et al., 2009). Succession can be observed at a broad range

of scales from the microscopic to the continental level (McCook, 1992). Traditionally succession can be divided into two types: primary and secondary succession. Primary succession is a chronological changing of species composition and other ecosystem characteristics from a point with little or no biological legacies (Walker and Moral, 2011). Primary succession can follow natural or man-made disturbances such as volcanic eruptions, landslides, coastal sand dune formation and mine wastes. Initiation of primary succession can be caused by input of seeds and spores, animals and organic matter in the soil from outside the ecosystem or by expansion of vegetation from adjacent habitats (Walker and Moral, 2003).

Secondary succession occurs after temporory disturbances such as fire or insect outbreaks (Horn, 1974). Primary and secondary succession also differ in that primary succession is characterized by a slow process of revegetation due to the low level of soil resources, whereas secondary succession occurs in sites with increased biological activity and higher level of soil resources, ultimately accelerating the revegetation (Miles and Walton 1993; Prachet al., 1993; del Moral 2007, Pickettet al., 2009).

1.2.3 Effects of mining activities on the boreal forests

Natural resource developments such as mining affect the terrestrial biodiversity of southern Canadian boreal forests through changes in the landscape structure, stand structure, age distribution and species composition (Venier, 2014). More than 10,000 mining exploration sites (Tremblay and Hogan 2006), and about 1300 abandoned mining sites can be found in the Canadian boreal region (Brandt et al., 2013). There are 2200 mining sites in Quebec and 14,182 ha of mining-disturbed lands, of which around 3307 ha have been restored by 2000 (Tremblay, 2001).

Mining activities can cause soil infertility and soil toxicity through drastic changes in the soil pH, the amount of organic compounds, bulk density and amount of C in the soil (Walker and Moral, 2011; Latifovic, 2004). Soil infertility and soil toxicity caused by long-term mining practices may also have significant effects on the vegetation due to metal deposition, excess nitrogen fertilization, and deposition and uptake of ground-level ozone (Latifovic, 2004). Quideau et al. (2013) have shown that soil biogeochemical processes in novel ecosystems restored following oil sands mining differ from those in natural boreal ecosystems, especially in terms of organic matter content, microbial communities, and nutrient availability. Also, Venier (2014) reviewed the effect of mining and oil and gas excavation on the microbial, amphibian and mammal communities of Canadian boreal forests, showing changes in community structure, landscape structure, stand structure and age class distribution, as well as the loss of habitat for plant and animal species.

Covering 1% of the Earth's land area, mineral extraction sites create suitable substrates for primary succession (Walker and Moral, 2003). These opportunities for primary succession are created by the removal of topsoil or the deposit of wastes during the mining processes (Walker and Moral, 2011).

1.2.4 Soil as a substrate for seedling establishment and growth

Soil plays a key role for the sustainability of life on earth. By providing a substrate for a wide range of living organisms, it is a fundamental component of terrestrial ecosystems (Thies and Grossman, 2006; Voroney, 2007). Qualitatively, soil properties can be described as chemical, physical or biological. The combination of these

physical, chemical and biological properties determines the ability of a soil to carry out ecological functions, especially plant growth (Brady and Weil, 2008).

Chemical and physical properties, and biological components of soil play important roles in the modification of soil into a better substrate for the establishment of plant communities in disturbed sites (Walker and Del Moral 2003; Frouz et al., 2008). Chemical properties of soil such as pH, cation exchange capacity (CEC), and soil salinity influence the management of soil nutrients in ecosystems. Thus, nutrient concentrations and soil chemical properties can be used as "indices of nutrient supply" (Powers et al., 1998; Schoenholtz et al., 2000).

The chemistry of the components in the soil solution (water, O_2, CO_2, organic matter, inorganic constituents) affects the soil biosphere, especially soil microorganisms, and ultimately has a strong effect on plant growth (Voroney 2007). Soil nutrients influence plant community structure by determining species composition in particular ecosystems (Huston, 1980; McLendon and Redente, 1991). Nutrient availability and limitations affect the primary production of forest ecosystems in the early succession period, as different nutrient availability varies between ecosystem types (Vitouseket al., 1993). For example, P is unavailable under extreme pH conditions (Walker and Del Moral 2003).

Since N is the most limiting factor for plant growth, N cycling processes are crucial for releasing N to the plants. In the boreal forests, early successional plant species such as aspen, balsam poplar and paper birch provide litter that is rich with easily decomposable N to the soil, which enhances soil N availability for other plants (Flanagan and Cleve, 1983). Through symbiotic N fixation, some plants (especially alder shrubs in boreal forests) overcome N limitations and establish successfully in

boreal ecosystems, also enhancing the soil N pool for other plants (Uliassi et al., 2000). Herbivory (e.g. by moose and hares) also enhances N availability in developing soil by recycling 30% of the aboveground biomass of plants (Kielland and Bryant 1998).

In addition to these nutrients, some heavy metals accumulate in the soil substrate due to various sources of pollution including mining, burning of fossil fuels, use of fertilizers and disposal of industrial waste. Some of these heavy metals, such as Zn, Cu, Mn, Ni and Co, act as micronutrients to the plants, but others such as Cd, Pb and Hg are contaminants that do not contribute to the biological function of plants (Gaur and Adholeya, 2004). These contaminants act as soil stressing agents and also change the soil-plant-microbial community interactions (Pawlowska et al., 1997; Krumins et al., 2015). Metal contaminants have a direct effect on plant metabolism. Since plant growth is facilitated by the soil microbial community, these toxic metals in soil also have indirect effects on plant growth through microbial population shifts that result in altered soil metabolism (Krumins et al., 2015). In this case, some plants can benefit from both plant and soil microbe resistance to the soil contaminants, ultimately shortening the toxicity period for plants and microbes (Thurman, 1981; Verkleij and Schat, 1990; Das et al., 2014; Krumins et al., 2015).

Physical properties of soil such as texture, structure, porosity, temperature, and color affect plant growth starting from germination, emergence and establishment of seedlings (Gardner et al., 1999). Texture determines which propagules will be established in the substrate following the processes of trapping and germinating of propagules (Walker and Del Moral, 2003). Soil surfaces with smooth textures (absence of aboveground barriers), along with high wind situations slow down colonization (Fort and Richards, 1998), whereas soil surfaces with a biological crust can easily trap the seeds and spores. These components also facilitate important soil functions such as

water retention, accumulation of organic matters and nutrients to promote succession (Walker and Del Moral 2003).

1.2.5 Mycorrhizae – a symbiotic relationship among plants and fungi

The mycorrhizal symbiosis is a 400 million years old symbiotic relationship between land plants and fungi (Smith and Read, 2010). Being present in approximately 90% of terrestrial plant species and soil fungi (Selosse et al., 2006), it facilitates the mutual exchange of resources where fungi supply limited nutrients to plants and plants provide assimilates such as carbon to fungi (Selosse et al., 2006; Smith and Read, 2010). This symbiotic relationship has important implications for the diversity and productivity of plant communities, since it facilitates plant-plant interactions via connecting plants by their roots (Montesinos-Navarro et al., 2018).

Basically, five categories of mycorrhizae can be found : arbuscular mycorrhizae, ectomycorrhizae, ericoid, orchid and mycoheterotrophic mycorrhizae (Smith and Read, 2010). The cool climate of the boreal biome reduces the availability of plant nutrients, particularly N, through slow rates of mineralization and decomposition. As a result, coniferous boreal forests are the primary niche for ectomycorrhizal (ECM) communites because of the low availability of N, slow mineralization and acidic soils in these ecosystems (Read et al., 2004; Kranabetter et al., 2009). In addition to the above mentioned factors, the composition and distribution of host plants plays a key role in the colonization and distribution of ECM fungi in northern forest ecosystems, because physiological properties of host plants and mycobionts are strongly linked (Twieg et al., 2007). More than 6000 ECM-forming fungal species have been

identified, most of them are basidiomycetes followed by some ascomycete fungi (Smith and Read, 2010).

Almost all ectomycorrhizal plants are woody perennials (Smith and Read, 2010). ECM-associated tree species in plant families Pinaceae, Fagaceae, Betulaceae and Salicaceae are the main constituents of the boreo-temperate biomes of the world (Smith and Read, 2010). ECM fungi found in boreal forests mainly belong to Ascomycota and Basidiomycota, including Russulaceae, Thelephoraceae, Cortinariaceae and Atheliaceae as dominant families (Kernaghan et. el., 2003). Using next generation sequencing, Nagati et. al. (2018) described soil fungal species in mixed boreal forest stands dominated by *Populus tremuloides* and *Picea mariana* in the Abitibi-Témiscamingue and Nord-du-Québec regions of Canada. They found that 15% of fungi were ectomycorrhizal and around 20% were saprophitic. High throughput metabarcoding is a very powerful tool to study the diversity of complex fungal communities including mycorrhizae. Nevertheless, mycorrhizal diversity in boreal forests remains little known even though these fungi are very diverse in that region (Blaalid et. al., 2013; Schmidt et. al., 2013).

1.2.6 Mycorrhizae as a determinant of primary succession

The effects of biotic and abiotic factors on primary succession can be described using different types of approaches. Several decades ago, mycorrhizal symbiosis did not receive much attention from scientists even though it has considerable effects on plant regeneration in early successional stages (Nara et al., 2003). In nutrient-poor environments, mycorrhizae play a key role in facilitating the survival and growth of host plants through the uptake and distribution of plant nutrients (Nara et al., 2003;

Smith and Read 2010). Early plant successional processes influenced by mycorrhizal symbiosis can be identified as a process of directional change in the composition, relative abundance, and spatial pattern of species comprising communities (Frankland 1998; Dighton and White, 2005).

Primary succession in disturbed sites begins with the establishment of non-mycorrhizal and/or facultative mycorrhizal plant species (Allen and Allen, 2012). Then, colonization starts in these sites with AM associated plants followed by ECM associated plants and ultimately ericoid mycorrhizal plant species (Read 1989; Nara et al., 2003; Kikvidze et al., 2010). However, this order can vary based on the types of ecosystems and the dominant plants of those ecosystems. For example, even though AM symbiosis occurs first during primary succession in most ecosystems, since boreal forests are dominated by ECM associated plants, mycorrhizal colonization in those primary successional sites starts with ECM dependent plants (Smith and Read 2010).

Mycorrhizal fungi accelerate the uptake of N, P, Ca, K, Mg and water in plants (Smith and Read, 1997) and facilitate the seedling establishment of most land plants (Horton and Van der Heijden, 2008). In ECM-dependant ecosystems, mycelia arising from ectomycorrhizae act as a nutrient absorption system in seedlings of host plants, such as *Pinus* species in boreal forests, as absorption of nutrients, especially N and P, by mycelia are three times higher than in roots (Leake et al., 2004). According to Dehlin et al. (2004), light and humus fertility have direct impacts on the colonization of ECM in seedlings of the main boreal forest plants, and along with this ECM colonization, interspecific competition from neighbours have important effects on seedling recruitment and subsequently determine the plant community assemblage in successional forest ecosystems.

In many plant species, mycelia of mycorrhizal fungi produce common mycorrhizal networks (CMNs) by colonizing roots of neighbouring trees and seedlings, and these CMNs facilitate the uptake and transportation of nutrients and other resources among plants (Selosse et. al., 2006). Some plants do not share mycorrhizal fungi in their respective root systems as they do not have CMNs and both CMNs and non-shared mycorrhizal fungi can be found in some plant species (Montesinos-Navarro et al., 2018). The network complexity of a CMN can range from two plant species connected by one fungal species to a number of plants of different species with various fungal species, with different frequencies of connections in the network, and even interactions with other soil organisms (Simard and Durall, 2004).

The resource distribution process varies among CMNs, based on the taxa forming the network, since traits of these CMN-forming fungi define the types of resources provided to their respective hosts. Through this asymmetry, CMNs ultimately affect the coexistence of plant species in particular ecosystems by enhancing or suppressing their growth and survivorship (Bever et al., 2010; Montesinos-Navarro et al., 2018). Interactions among plants species can be mutualistic, neutral or antagonistic.

Seedling growth and establishment is a key process in the dynamics of forest ecosystems. Due to the absence of canopy trees in disturbed sites, light is not a major constraint for seedling survival and establishment, but inadequacy of soil nutrients is the main negative effect present during primary succession in boreal forests. Thus, CMNs play a key role to mitigate that negative effect and help seedling establishment through net facilitation by older plants (Booth and Hoeksema, 2010). Heijden and Horton (2009) carried out a literature synthesis to determine the effect of mycorrhizal networks on seedling establishment in natural ecosystems and found that mycorrhizal networks promoted seedling growth in 50% of cases, while in 25% of cases they had negative effects on seedlings associating with ectomycorrhizal fungi and in 25% of

cases had a neutral effect. They concluded that mycorrhizal networks have the ability to socialize the ecosystem as seedlings draw benefits from older plants in the community via these networks.

In the boreal forest, interspecies CMNs were found in seedlings of spruce, aspen, and birch in postfire boreal ecosystems of Alaska. The similarity of ECM root tip-associated fungal communities and type of fungal group present in CMNs had no relationship with the distance between samples (Bent et al., 2011). Selosse et al. (2006) recognized that CMNs associated with ECM dependent seedlings such as *Quercus rubra* highly benefit from neighbouring congeneric *Quercus* species, compared to seedlings with neighbours of AM dependent *Acer rubrum*. In that case, CMNs benefitted the growth, survival, mineral nutrition and symbionts presence in seedlings. They also highlighted that facilitation through CMNs can vary according to soil fertility, types of species involved in plant-fungi symbiosis and age of adult plants in the ecosystem.

Most mycorrhizal fungi are not host-specific, since one fungal species can connect with several plant species (Simard and Durall, 2004). However, host specificity plays a key role for seedling establishment through resource redistribution via mycorrhizae in boreal forests (Hobbie, 2005). Molina et al. (1982) identified three levels of host potential: as fungi with wide mycorrhizal host potential and low specificity, fungi with intermediate mycorrhizal host potential with intermediate specificity, and fungi with narrow mycorrhizal host potential and restricted to form ectomycorrhizae with a specific host species or species within the same genus. Bruns et al. (2002) described the advantages and importance of host specificity to complex ecosystems. They hypothesized that ECM host specificity facilitates the transfer of more resources to the host plant than are available to generalist species that are associated with the same host

plant. Hoeksema (1999) discussed that ECM host specificity facilitates the dominance of their hosts in the forest.

1.2.7 How mycorrhizae affect seedling establishment in post-mining sites

Generally, all mycorrhizae play a key role in regeneration of post-mining sites, and ECM are especially important for forest production and reforestation programs (Smith and Read, 2010). As soil acidity is found to be high in some mining areas, rooting of plants during seedling establishment can be hindered to a certain point. However, in young mining soils, roots and mycorrhizae can utilize that substrate heterogeneity (Hüttl, 2001). In mine tailings, some plant species cannot establish and survive without mycorrhization (Shetty et al.,1994). Frouz et al. (2015) conducted a study to compare the seedling establishment of English oak (*Quercus robur*) and European beech (*Fagus sylvatica*) at reclaimed alder plantations and unreclaimed post mining sites. Based on the results of this study, *Quercus robur* seedlings showed higher mycorrhizal colonization in reclaimed post mining sites than in other habitats.

ECM-associated plants have a greater ability to access N and P in degraded sites, especially in mining sites, which could give a competitive benefit for these plants as nutrients are limited in those disturbed sites (Bauman et al., 2017). ECM colonization also supports plant establishment by increasing soil water use and mitigating the effects of metal toxicity in mining soils (Iordache 2009; Marx 1977 and Nara 2006). In addition, ECM symbiosis helps to protect some plants from pathogen attacks when they are in disturbed soils (Marx 1972). Studying of the role and characteristics of ECM communites already associated in turbulent environments help to identify the most

suitable native ECM species that can facilitate the establishment of plant species in post mining areas (Bauman et al., 2012).

1.3 Objective of the study

The general goal of this study is to determine how seedling growth is affected by below-ground facilitation via mycorrhizal networks on a post-mining site.

Specifically, we were interested in identifying 1) existing mycorrhizal communities, 2) the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts and 3) how these mycorrhizal communities, along with other defined physical and biological factors, affect tree growth in a harsh environment.

CHAPTER II

MYCORRHIZAL COMMUNITIES AND THEIR EFFECT ON TREE GROWTH AT A POST-MINING SITE

2.1 Abstract

Primary succession of vegetation in post-mining areas offers an opportunity to study how mycorrhizal symbiosis influences the revegetation of degraded environments. Mycorrhizae are shared among host plants and influence the uptake and transportation of nutrients among plants. In this study, we characterize the taxonomical and spatial structure of the mycorrhizal community and determine how mycorrhizal species affect seedling growth at a former mining site. The study site is the mine tailings site of the Beattie Gold Mine near the town of Duparquet in north-western Quebec. *Betula papyrifera*, *Populus balsamifera*, *Picea glauca* and *Thuja occidentalis* were used as focal tree species and the influence of neighbourhood competition indices, mycorrhizal communities and soil nutrients on seedling growth were characterized. Mycorrhizal fungi collected from the mine tailings site were sequenced using molecular markers and analyzed to determine richness and abundance, and to detect possible species sharing. With fungal DNA amplified from 40% of plant samples, 474 fungal operational taxonomic units (OTUs) were identified with 52 ectomycorrhizal OTUs and 20 arbuscular mycorrhizal OTUs. Ectomycorrhizal species were shared among all host species with no significant host specificity but were segregated at the scale of individual plants. Priority effects drive the mycorrhizal community structure of host plant individuals rather than host specialization. Soil nitrogen and phosphorus concentration were extremely low and high arsenic levels were detected in the field.

Growth of most host plants was not affected by neighborhood competition indices, variation in soil nitrogen concentration, shoot biomass or mycorrhizal richness, mycorrhizal abundance and sharing of mycorrhizae among host plants. This study concludes that plant-plant interactions (competition or facilitation), mycorrhizal networks and soil nutrients are not important factors for determination of plant growth and vegetalization at our study site due to the strong nutrient limitations and high As contamination.

Keywords: Vegetalization , Mining Degradation, Mycorrhizal Fungi, Soil Nutrients

2.2 Introduction

Boreal forests, the world's largest terrestrial biome, provide a wide range of ecosystem services to society, such as timber and food production, water purification, carbon storage, nutrient cycling, and soil production (Gauthier et al., 2015; Hassan et al., 2005). Boreal forests are the largest forest type in Quebec, Canada, covering about 70% of Quebec's territory (56 million hectares). Quebec's boreal region is divided into two main sub-regions, the continuous boreal forest and the taiga forest (MERN, 2008). Boreal forests are characterized by cold-tolerant species of genera such as *Abies, Larix, Picea, Pinus, Populus* and *Betula* (Brandt et al., 2013). Boreal mixed-woods forests, which are characterized by varying canopy dominance of boreal broadleaf and conifer trees are found in the southern Canadian boreal region (Bergeron et al., 2014). Covering 1% of the Earth's land, areas of surface mineral extractions, such as mining, affects the terrestrial biodiversity of the Southern Canadian boreal forest through changes in the landscape structure, stand structure, age, distribution, and species composition (Venier, 2014). In particular, mining activities can cause soil infertility

and soil toxicity through drastic changes in soil pH, organic compounds, bulk density, and amount of C in the soil, and subsequently create the opportunity for these post-mining sites to undergo a primary succession process (Johnstone et al., 2016; Walker and Moral, 2011). The soil biogeochemical processes in novel ecosystems rehabilitated after oil and sand mining are different from those in natural boreal ecosystems, in terms of organic matter content, microbial community composition, and nutrient availability (Hahn and Quideau, 2013).

Mycorrhizae are a ubiquitous symbiosis between plants and fungi, which can take many different forms, giving different benefits to host plants. Mycorrhizae make symbiotic associations with around 90% of terrestrial plants (Selosse et al., 2006; Brundrett, 2009) and facilitate the mutual exchange of resources and enhance nutrient acquisition in plants (Smith and Read, 2010). The effect of mycorrhizal symbiosis on early plant succession processes can be identified as a process of change in the composition, relative abundance, and spatial patterns of plant species (Frankland 1998; Dighton & White, 2005). Among the five groups of mycorrhizae: arbuscular mycorrhizae (ABS), ectomycorrhizae (ECM), ericoid, orchid and mycoheterotrophic mycorrhizae (Smith and Read, 2010), ABS and ECM are the two most common. The main difference between ECM and ABS symbiosis is that ECM hyphae do not penetrate inside plant cells but make a net around cell walls, while ABS hyphae penetrate inside cells and form arbuscules. Climatic conditions in the boreal region, such as low temperature, reduce the availability of plant nutrients, particularly N, through slow rates of decomposition. The low availability of N and slow rates of mineralization due to a cooler climate make boreal forests a primary niche for ECM communities (Read et al., 2004; Kranabetter et al., 2009). In the black spruce-feather moss forests of western Quebec, ECM and saprotrophs (decomposers) are the most abundant fungal communities and most of the ECM species belong to Ascomycota and Basidiomycota while ABS species belong to Glomeromycota (Nagati et al., 2018).

Mycelia of mycorrhizal fungi produce common mycorrhizal networks (CMNs) when they colonize and link roots of neighboring trees and seedlings. These CMNs facilitate the uptake and transportation of nutrients and other resources among plants and also change the plant's competitive abilities ultimately affecting the physiology and ecology of plants (Selosse et. al., 2006). These differences in mycorrhizal facilitation in terms of resource partitioning and distribution to host plants are created by the presence of CMNs, non-shared or both, based on the taxa forming the network (multiplicity of fungal species and plant species), guild type (ECM, ABS) and ways of trading resources (directly from soil or from neighboring plants) (Bever et al., 2010; Montesinos-Navarro et al., 2018;). Most mycorrhizal fungi are not host specific as is implied by the creation of CMNs where fungi are shared among plant individuals and species (Horton and Van Der Heijden, 2009; Simard and Durall, 2004), however plants with host specific ECM are more efficient in obtaining nutrients compared to plants with non-specific ECM (Hobbie, 2005).

Mycorrhizal symbiosis may affect plant-plant interactions, for instance it alters inter- and intra-specific competition and facilitation between plants (Simard and Durall, 2004). Considering the mycorrhizal sharing among boreal tree species, phylogenetically closely related host plant species (e.g., balsam fir and black spruce) have a higher probability of sharing similar ECM communities compared to less closely related host species (Nagati et al., 2019; Bahram et al., 2014).

Plant-plant interactions in a stressed environment like in a mine tailings site are shaped by the plant's ability to be colonized with shared (CMNs) or non-shared fungi (host specific). In disturbed environments such as mining sites, ECM-dependent plants are more likely to have access to N and P, and these plants can compete successfully with non-ECM-associated plants when nutrients are limited (Bauman et al., 2017). Soil characteristics alter plant-plant interactions by affecting the ability of plants to maintain

microbial communities in the rhizosphere and acquisition of nutrients. Among the heavy metals that accumulate in the soil substrate due to various sources of pollution including mining, some such as Zn, Cu, Mn, Ni and Co act as micronutrients to the plants, but others such as Cd, Pb and Hg act as soil stressing agents and change the soil-plant-microbial community interactions (Pawlowska et al., 1997; Hagmann et al., 2015). Thus, the cumulative effect of mycorrhizal symbiosis, plant-plant interactions, soil properties and interactions including below-ground facilitation and competition between them ultimately determine plant growth in stressed environments.

The general goal of this study is to determine the effect of below-ground facilitation and competition associated with mycorrhizal networks on plant growth. Specifically, we were interested in identifying 1) existing mycorrhizal communities, 2) the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts and 3) how these mycorrhizal communities, along with other defined physical, chemical and biological factors, affect tree growth in a harsh environment. To do that, we conducted our research in the mine tailings site of the abandoned Beattie gold mine, in north-western Quebec, by identifying existing mycorrhizal communities through next-generation sequencing (NGS) and determining the effect of mycorrhizal communities, soil nutrients, plant-plant interactions, and host plant variables on tree growth under these harsh environmental conditions. Giving special emphasis on mycorrhizal communities and plant-plant interactions of plant regeneration in successional environments, we asked the following questions: 1. What is the fungal richness and abundance at the site, overall and by fungi type? 2. Does fungal richness change with the identity and size of the host? 3. How does the mycorrhizal fungi community composition vary among host species? 4. Does the fungal composition vary spatially? 5. How does competition or facilitation from neighbouring plants, mycorrhizal diversity, and host variables influence the growth of the main tree species?

2.3 Materials and methodology

2.3.1 Study site

The study site was located on the mine tailings site of the Beattie Gold Mine (in operation from 1933 to 1957) near the town of Duparquet in north-western Quebec (48° 30' 13" North, 79° 15' 11" West) (Figure 2.1). The town of Duparquet and the Beattie mine site are on the Clay Belt of Quebec and Ontario and located in the Canadian southeastern boreal forest within the Missinaibi–Cabonga forest section (Bergeron, 2000; Girardin et al., 2001; Rowe, 1972). According to the average climatic statistics for La Sarre, the closest meteorological station to the study site, annual average daily mean temperature is 0.7 °C. Average precipitation, average rainfall and snowfall are 889.8 mm, 643.6 mm and 246.5 cm respectively (Canadian climate normals 1971-2000 station data).

Around thirty years after the closure of the mine, between 1981 and 1984, the botanist Jean Gagnon conducted a vegetation survey and a study of the natural process of colonization by vegetation in this tailing site and the surrounding forests (Martineau, 2014). During this survey, more than 190 taxa were inventoried, and several plant species found in this site were never recorded in other sites in this area. Surrounding forested areas of Lake Duparquet are dominated by balsam fir followed by black spruce, white spruce and paper birch (Bergeron, 2000).

2.3.2 Preparation of inventory and mapping of trees and shrubs

In the summer of 2018, two 15 x 15 m plots were chosen to represent the tree diversity present on the site. All woody plants 10 cm or taller were tagged with a permanent aluminum tag, they were identified to species, their position was recorded to the centimeter with a total station theodolite, and their height and diameter at breast height (DBH, only for plants > 2 m tall) were noted.

Figure 2.1 A) Location of Beattie gold mine in Quebec, Canada.

Figure 2.1 continued

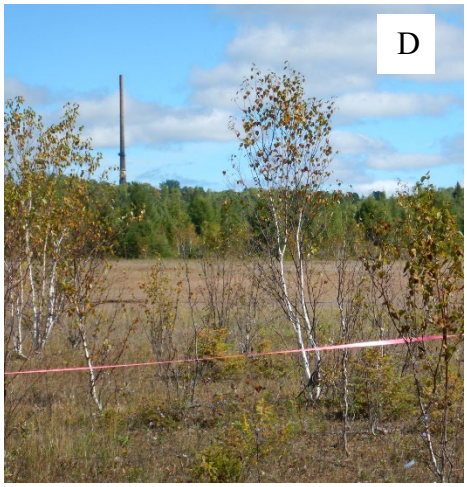


Figure 2.1 B) Location of west and east sampling plots on the Beattie gold mine tailings site, C) and D) Sampling site on the tailings of the former Beattie gold mine.

2.3.3 Sampling of plants

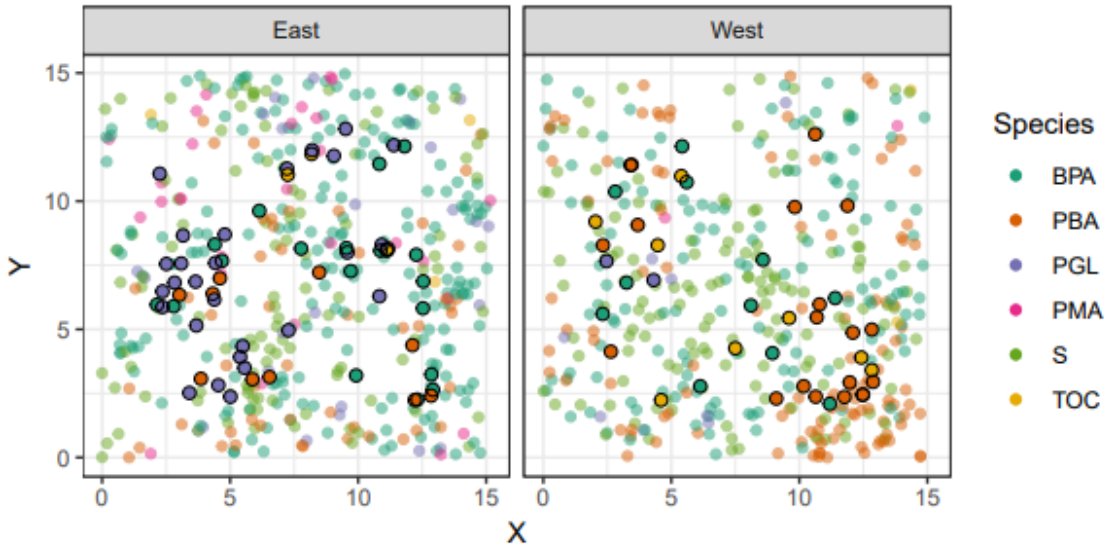


Figure 2.1 Location of focal and neighbour plants in two plots (focal plants are circled with a black outline).

In August 2019, 101 focal plants were selected among surviving tagged plants of the following four species: *Betula papyrifera* (paper birch, BPA), *Populus balsamifera* (balsam poplar, PBA), *Picea glauca* (white spruce, PGL) and *Thuja occidentalis* (eastern white cedar, TOC) (Figure 2.2 and Table 2.1). Focal plants were selected to encompass different height classes and different neighbourhood compositions for each species. All neighbouring plants (316) within a 1-m radius of the selected focal plants were identified, including the above mentioned four species as well as *Picea mariana* (PMA), *Salix* spp. (S), *Betula nana* (BN), *Cornus* spp. (C) and *Larix laricina* (LLA) (Table 2.1). After selecting the focal plants and neighbouring plants, sampling of roots,

soil and leaves was carried out as described below. The difference in height between the two years was measured for all the focal species and the mean annual apical growth of focal plants was measured based on the position of up to three annual bud scars only for BPA, PBA and PGL since bud scars are not apparent for TOC. The root collar diameter of all focal and neighbour plants was recorded for the calculation of competition indices. Fine roots were collected from all the focal and neighbouring plants by excavating them for identification of existing mycorrhizal species. The wet shoot biomass was recorded for all the sampled focal plants.

Table 2.1 Number of focal (F) and neighbour (N) plants sampled in the two plots of the study site, with total counts and expected mycorrhizal types (ECM- ectomycorrhizal, ABS- arbuscular) for each species based on the literature (Kernaghan et. el., 2003; Miller, 1982; Natel and Neumann, 1992; Samson and Fortin, 1986; Treseder et. al., 2004).

Plot	Total	F	N	Species								
				PBA	BPA	PGL	TOC	S	BN	PMA	LLA	C
West	168	39	129	53	51	4	8	51	1	0	0	0
East	249	62	187	30	108	35	4	53	0	13	2	1
Expected mycorrhizal symbiosis				AB-ECM	ECM	ECM	AB	AB and ECM	ECM	ECM	ECM	AB

Populus balsamifera (PBA), *Betula papyrifera* (BPA), *Picea glauca* (PGL), *Thuja occidentalis* (TOC), *Picea mariana* (PMA), *Salix* spp. (S), *Betula nana* (BN), *Cornus* spp. (C) and *Larix laricina* (LLA).

2.3.4 Soil sampling and analysis

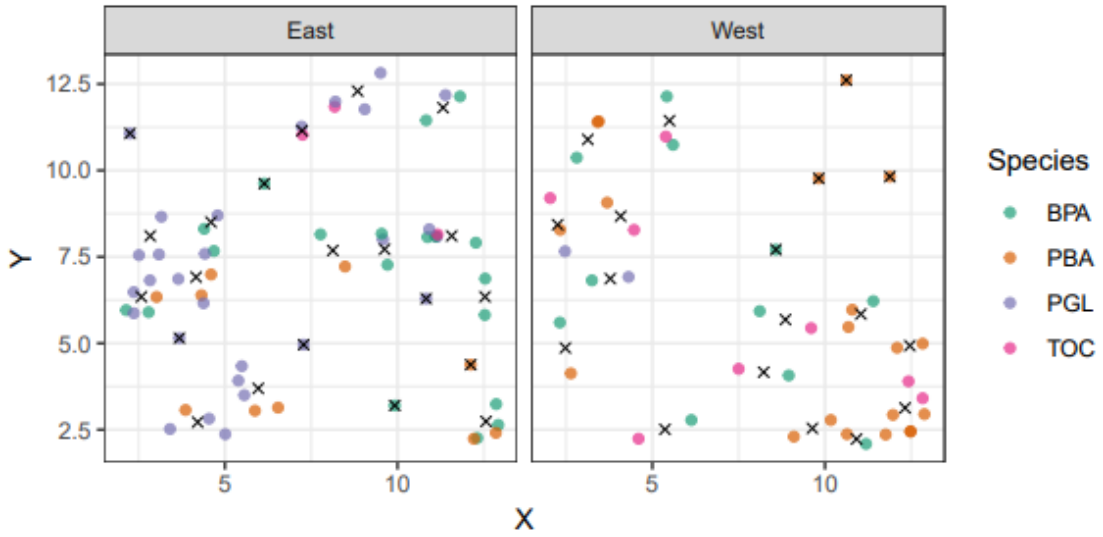


Figure 2.2 Location of soil samples near focal plants (soil sampling sites were indicated with a cross mark).

Forty-two soil samples (around 500 g per sample) were collected near focal plants, after removing the organic crust, for chemical analysis and to identify the below-ground soil fungal community (Figure 2.3). This number was chosen to minimize analysis costs by taking one soil sample for closely clustered focal plants, while ensuring all focal plants were at most 1 m away from the nearest soil sampling point. Root and woody debris were removed from each sample, then each sample was homogenized. Soil samples were air-dried for a week. For each soil sample, C and N concentrations were determined with the Dumas method and read on a thermal conductivity detector, while concentrations of P, K, Ca and Mg were determined from a Mehlich 3 extraction followed by ICP optical emission spectrometry at the Abitibi-

Témiscamingue Agri-Food Research Station in Notre-Dame du Nord, Quebec. The concentration of As, Hg and Cd was measured on two pooled samples (one per plot) in the H2Lab, Rouyn-Noranda (https://h2lab.ca/a-propos/) using the MA.200-Mét.1.2 standard protocol of the Québec government (MELCC, 2020).

2.3.5 Identification of fungal species using molecular analysis

Root system samples were collected from 401 plants. These samples were washed under tap water and observed in clean white plates. Fine roots including ectomycorrhizal root tips and possible arbuscular mycorrhizae were collected with sterile tweezers (sterilized in 2% bleach). All fine roots were dried for 3 days at 50 °C. From these root samples, DNA was extracted from 150 to 250 mg of fine roots using PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA). DNA extraction was stopped before the elution step, to allow transporting samples to the sequencing site (see below) without defreezing the DNA. Finally, DNA was eluted in 50 µl TE Buffer before the PCR process, and then stored at -20 °C.

The nuclear ribosomal Internal Transcribed Spacer (ITS) region is a widely used DNA metabarcoding marker for fungal taxonomy due to its high degree of interspecific variability, conserved primer sites and multicopy nature in the genome (Blaalid et. al., 2013). ITS1 is suitable for analyzing soil fungal communities (Schmidt et. al., 2013). Thus, the ITS1 region was amplified from all fine root DNA extracts using the forward ITS5 primer (primer sequence: GGAAGTAAAAGTCGTAACAAGG) (White et al. 1990) and the reverse primer 5.8S_Fungi (primer sequence: CAAGAGATCCGTTGTTGAAAGTK) (Epp et al., 2012). These primers contained eight different nucleotides with three degenerated nucleotides (=" tag"). PCR was

carried out with a unique combination of tagged primers for each sample. Two replicates were performed for each sample. The 401 samples were send with 49 controls for the PCR amplification. The PCR was initialized with 10 minutes at 95 °C, followed by 35 cycles of: denaturation for 30 seconds at 95 °C, annealing for 30 seconds at 55 °C and elongation for 1 min at 72 °C, and a final step of 7 min at 72 °C. PCR products were stored at 10 °C. Agarose gel electrophoresis was performed on randomly selected samples to confirm successful amplification. Libraries were constructed from PCR-amplified samples and sent to Illumina sequencing.

Illumina metabarcoding was chosen for DNA sequencing since it produces shorter reads but accomplishes sequencing at a greater depth for a given cost compared to the 454 metabarcoding approach (Schmidt et. al., 2013). For this step, samples were sent to the GENOTOUL-GeT-PlaGe sequencing platform (http://www.genotoul.fr/en/, Toulouse, France) using Illumina Miseq next generation sequencer with the TruSeq Nano PCR-free kit using the paired-end sequencing technology (2x250 pb) with the chemistry V3.

2.3.6 Bioinformatics analysis

The OBITools Python package (Boyer et al., 2016) and Unix commands were used to analyze the DNA metabarcoding data obtained from Illumina sequencing. Metabarcoding of different samples sequenced with Illumina technology was done to obtain fungal taxa occurring in each sample. First, as the sequencing is handled on both senses, we then detected paired forward and reverse sequenced based on 50 nucleotides overlap. This step discards amplicons with a missing end and short one-string sequences. From the detection of forward and reverse tag in each sequence, amplicons

were then assigned to PCR reactions, and therefore to samples. We then discarded sequences that were not assigned to any sample, those that were shorter than 50 nucleotides, those with a low phred score (below 50), and reads containing other characters than A, C, G or T (IUPAC code). Following this filtering step, identical sequences were grouped together, assigned a count and singletons were removed. Sequence clustering was done based on distances between sequences using OBITool Sumaclust with a 97% identity since it is the optimal identity for genetic analysis of fungal DNA with ITS1 marker. In order to do taxonomic identification on our dataset, we used OBITool Ecotag function (Boyer et al., 2016) with a GenBank extracted Database as the reference database (Clark et al., 2016). The most similar sequences were stated for Operational Taxonomic Units (OTUs) and OTUs were assinged to a taxonomic rank. Finally, OTUs were assigned to trophic status as symbiotroph (ectomycorrhizal or arbuscular mycorrhizal), saprotroph and plant pathogen using the FunGuild software (Nguyen et al, 2016).

2.3.7 Statistical analysis

All statistical analyses were performed with R version 4.0.2, (R core team, 2020). Community analyses were performed for all fungi, then separately for ectomycorrhizae (ECM) and arbuscular mycorrhizae (ABS). These community analyses were performed for the four focal host species as well as the willow *Salix sp*. For fungal diversity analysis, we calculated the mean richness and abundance (number of sequencing reads) of each guild (ECM, ABS, saprotroph, and plant pathogen) in each host species. The distribution of ABS and ECM mycorrhizal richness and abundance among individuals of each host species were also calculated. To visualize the variation in fungal community composition, we performed a principal component analysis (PCA) using

the Hellinger transformation of raw read counts (abundance of each OTU for each host plant) (Legendre and Legendre, 2012). Here, the Hellinger transformation compares samples based on the proportional abundance of different OTUs (raw number of reads divided by total reads for the sample). Thus, differences in total reads between samples, which could be due to some samples being more amplified than others, do not affect the fungal community analysis. We determined the most important OTUs (weight of magnitude > 0.1 in one of first 3 components in PCA) for all fungi and ECM and ABS separately. To characterize the variation in mycorrhizal community composition between hosts and to see if communities were differentiated based on the host species, we calculated the confidence ellipses for the mean position of each host species on the PCA using the factoextra package (Alboukadel and Fabian, 2020).

Differences in mycorrhizal community composition (for ECM and ABS separately) by plot (East and West) and host species were also tested with PERMANOVA (adonis function of the vegan R package, Oksanen et al., 2019) using the Hellinger distance. If any effect were significant in the PERMANOVA, we performed two post hoc tests. First, the betadisper function in vegan (Oksanen et al., 2019) was used to check if there are significant differences in the spread of samples (i.e the variability in composition) between host species or plots. Then, if the PERMANOVA detected a global difference between host species, we would use the package pairwiseAdonis (Pedro, 2017) to test for differences in composition between all pairs of host species while performing a correction for multiple comparisons.

To verify if the mycorrhizal community compositions were spatially structured within each plot, we performed a Mantel test of the correlations between dissimilarity of fungal communities (measured as the Hellinger distance) and physical distance between host plants. Finally, to check for segregation of species between samples, independent of host species or spatial location, we calculated the checkerboard score

(C-score) the EcoSimR package (Gotelli et al., 2015). This is a global test for the co-occurrence of all pairs of species: if fungal species are more segregated between sites than expected at random, this could indicate competitive exclusion or priority effects (Ulrich et. al., 2017). As recommended by Gotelli (2000), both the sim9 and sim2 null models of EcoSimR were tested for co-occurrence analysis. The sim9 null model permutes the community matrix while maintaining the same number of species by site and the same number of sites by species, whereas the sim2 null model keeps the same number of sites by species (so common species stay common and rare species stay rare), but not the number of species by site.

To account for intraspecific and interspecific competition in our models of tree growth (as described below), Hegyi competition indices were calculated based on the root collar diameter (not with DBH since most of our plants are less than 1 m in height) and physical distance between neighbouring plants as follows:

$$CI_i = \frac{1}{D_i} \sum_j w_{ij} \frac{D_j}{r_{ij}} \quad (1)$$

where D_i and D_j are the root collar diameters of the focal and neighbour plants, respectively, r_{ij} is the distance between them and the sum is over all neighbours j (Ledermann et al., 2011). The weights w_{ij} can be used to define different indices: to measure intraspecific competition, $w_{ij} = 1$ if the focal and neighbour plants are of the same species and 0 otherwise; for interspecific competition, $w_{ij} = 1$ if they are of different species and 0 otherwise.

The Jaccard similarity index can be used to assess mycorrhizal community differences and similarites among neighbouring plants (Andrea et al., 2011; Verbruggen et al., 2014). To represent the proportion of mycorrhizal species shared between focal and neighbour

trees (irrespective of abundance), we calculated the Jaccard index of similarity as follows:

$$\text{Jaccard Index} = c/(n_1 + n_2) \quad (2)$$

where c = number of species common to both plants, $n_1 + n_2$ = total number of species in both plants (Jaccard, 1912). To measure the degree of mycorrhizal sharing with neighbors, we defined a neighborhood mycorrhizal index, a modified version of Hegyi's index where the contribution of each neighbour is weighted (w_{ij} in Eq. 1 above) by the Jaccard index of similarity. If facilitation between neighbouring plants is mediated by common mycorrhizal networks, we expect this index to have a positive effect on growth.

We fit linear models to relate the apical growth of focal plants to the measured plant's wet shoot biomass, intraspecific and interspecific Hegyi competition indices, mycorrhizal richness and abundance (for ECM and ABS separately), neighborhood mycorrhizal index and soil nitrogen concentration, as we expect soil N to be a limiting factor for plant growth). These variables were log-transformed except for mycorrhizal richness and abundance. We used a log(1+x) transformation for the intraspecific and interspecific Hegyi competition indices and neighborhood mycorrhizal index due to the presence of zeros. Separate models were fit for each of the four focal species (PBA, BPA, PGL and TOC). The apical growth was taken as the mean of up to three years of growth (2016-2019) based on bud scars for balsam poplar, paper birch and white spruce, and as the difference in height between the two sample years (2018 and 2019) for white cedar.

2.4 Results

2.4.1 Amplification status of host plants

Of the 401 fine root samples tested from five host species, 157 (39%) were amplified with fungal DNA. The percentage of samples with amplification varied for each species and between plots (Table 2.2). The distribution of root collar diameter was similar for plants with and without amplified sequences (Figure 2.4).

Table 2.2 Number of samples sequenced (n), number and proportion of samples with amplified fungal DNA by host species and plot.

Host_sp	plot	n	With amplification	Proportion amplified
Paper birch	East	111	40	0.36
Paper birch	West	51	24	0.47
Balsam poplar	East	30	8	0.27
Balsam poplar	West	54	20	0.37
Willow	East	53	13	0.25
Willow	West	51	22	0.43
White spruce	East	35	20	0.57
White spruce	West	4	2	0.50
Cedar	East	4	2	0.50
Cedar	West	8	6	0.75

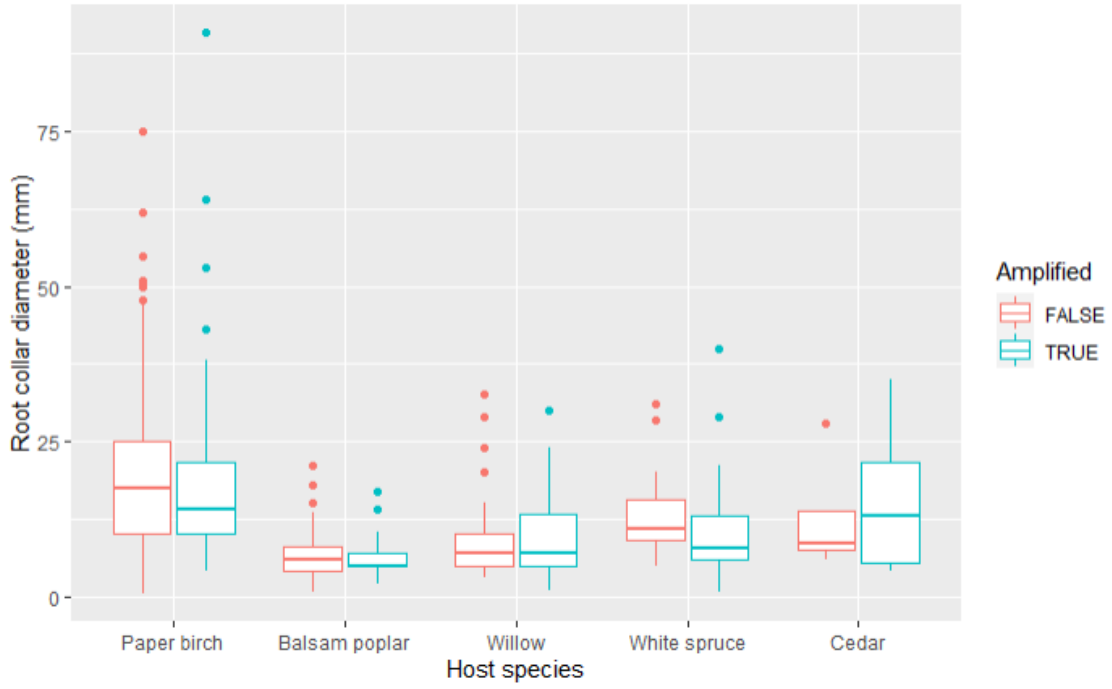


Figure 2.3 Root collar diameter for plants with and without amplified sequences by host species.

2.4.2 Fungal taxonomic diversity

A total of 233,132 sequences were obtained from fine root samples after removal of samples that were blanks. After quality filtering and removal of singletons, filtered sequences were clustered into 474 OTUs (98,935 sequences) with a 97% identity threshold. Using the ecotag function in OBITools, among the 474 OTUs, 260 (54.8%) were assigned to the species level while 48, 54 and 25 OTUs were identified to the genus, family, and order levels respectively. Overall, 387 (81.6%) OTUs were assigned to at least the order level. These 474 OTUs belong to 5 phyla, 59 orders, 115 families and 151 genera. The dominant phylum was Ascomycota (52% of OTUs, 51.2% of

sequences) followed by Basidiomycota (35% of OTUs, 44% of sequences). Mucoromycota, Chytridiomycota and Blastocladiomycota were the other phyla present.

Based on the sequences assigned to a family, genera, or species, Chaetomiaceae was the most abundant fungal family at the site, followed by Cortinariaceae, Psathyrellaceae, Hypocreaceae and Ceratobasidiaceae. Among the fungal families known to form ECM mycorrhizae, Cortinariaceae, Pyronemataceae, Thelephoraceae, Inocybaceae and Suillaceae were the five most abundant families, whereas the most abundant ABS species found at the site belonged to the Glomeraceae, Pervetustaceae, Claroideoglomeraceae and Acaulosporaceae families. Among the 151 distinct fungi genera identified, *Podospora*, *Trichoderma*, *Rhizoctonia*, *Otidea* and *Cadophora* made up a large proportion (~75%) of fungal sequences. We found 18 genera of mycorrhizal fungi (12%), of which twelve were ECM and six were ABS. The most abundant ECM genera were *Otidea* (79% of reads) and *Inocybe* (14% of reads), whereas *Pervetustus* (79% of reads) and *Glomus* (15% of reads) were the most abundant genera for ABS (Figure 2.5).

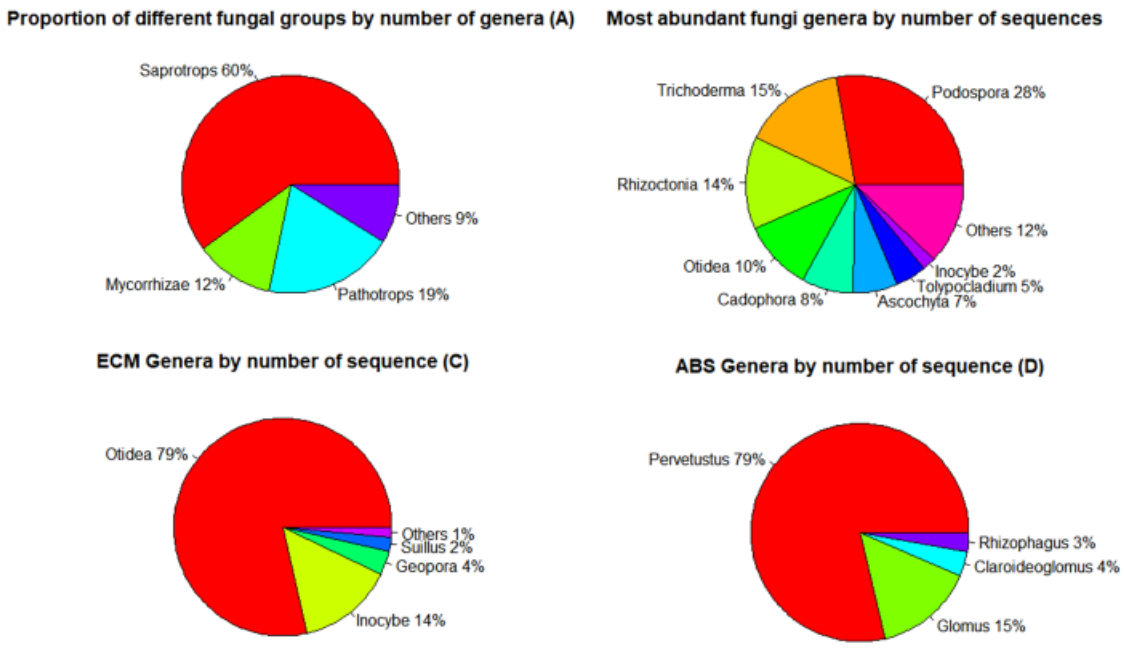


Figure 2.4 Proportion of different fungal groups by number of genera (A), abundance of fungal (B), ectomycorrhizal (ECM) (C) and arbuscular (ABS) (D) genera by number of sequences.

Fungal OTUs were grouped into ECM , ABS, saprotrophs and pathotrophs based on the FUNGuild annotation tool. Saprotrophs were the most common with 41,242 sequences followed by mycorrhizae with 29,623 sequences (23,513 for ECM, 259 for ABS) and pathogens with 25,656 sequences. From 474 fungal OTUs, 184 saprotrophs OTUs, 74 pathotroph OTUs, 54 ECM OTUs and 22 ABS OTUs were recorded. The remaining 140 OTUs (around 30%) were not assigned to a trophic status with FUNGuild. *Podospora intestinacea*, a dung saprotroph (12% reads) and Cortinariaceae sp. I, a mycorrhizal species (11% reads), had the highest number of reads (abundance) among the 474 fungal OTUs whereas, Cortinariaceae sp. I, *Otidea* sp. I, uncultured

Tomentella, uncultured *Agaricales* and uncultured *Inocybe* were the five most abundant ECM OTUs.

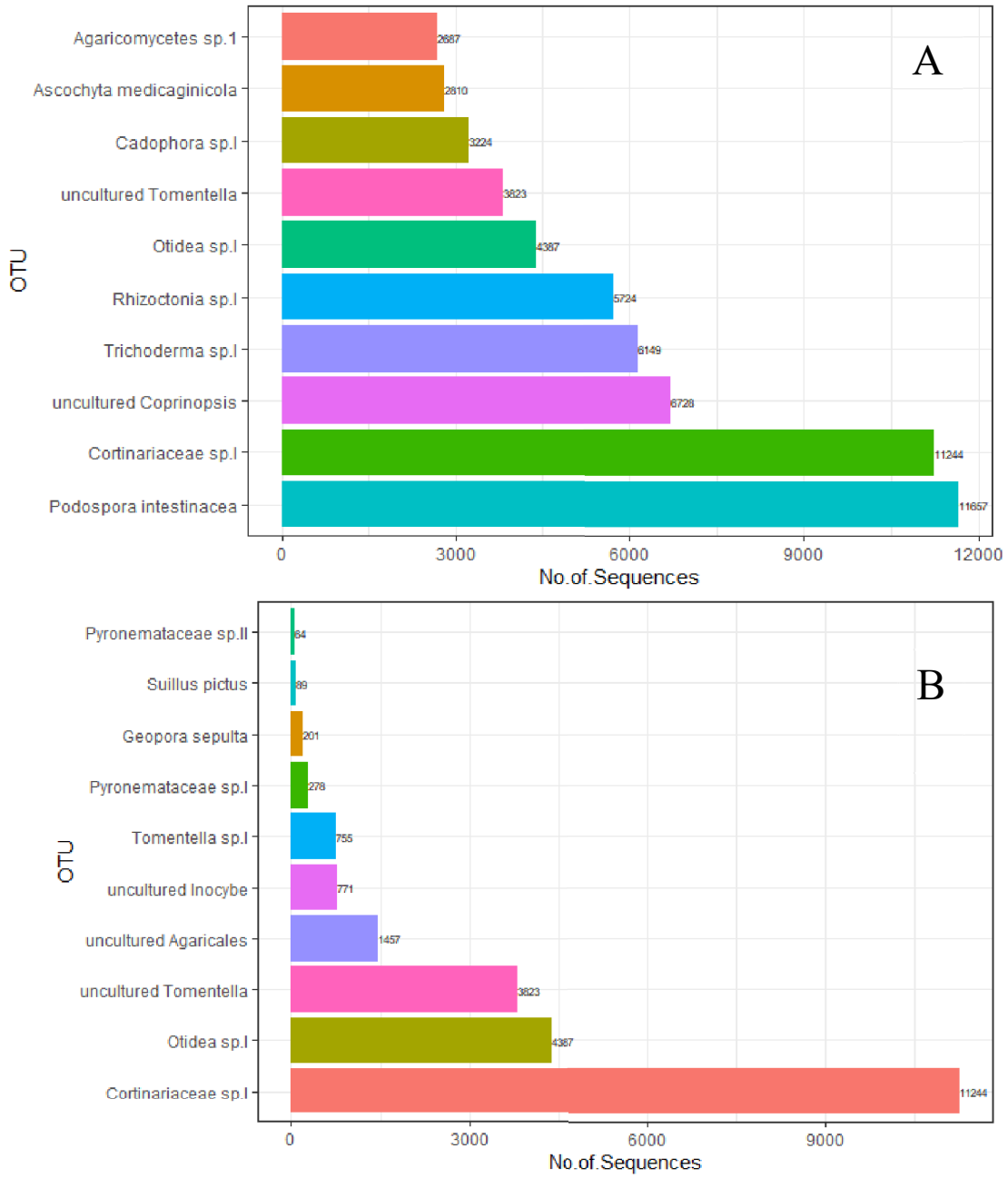
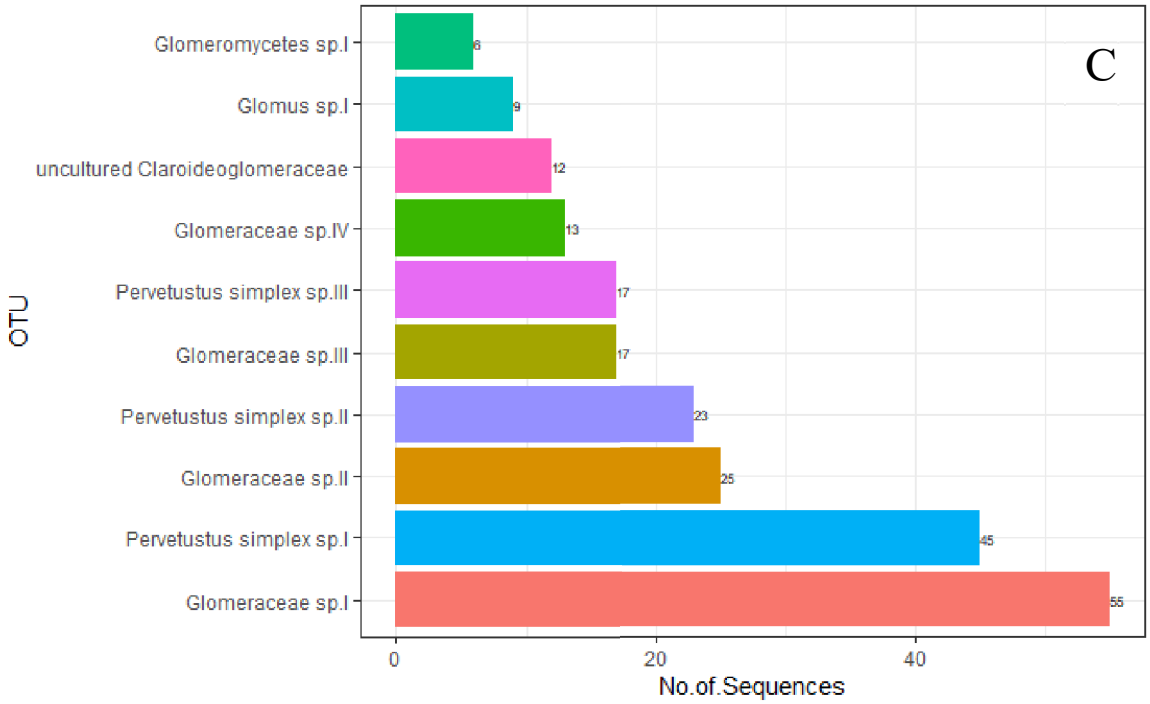


Figure 2.5 Most abundant OTUs among (A) all fungi, (B) ectomycorrhizal fungi and (C) arbuscular mycorrhizal fungi by number of sequences.

Figure 2.6 continued



Among 22 ABS OTUs, Glomeraceae sp.I, *Pervetustus* simplex sp.I, Glomeraceae sp.II, *Pervetustus* simplex sp. II and Glomeraceae sp. III were the most abundant (Figure 2.6).

2.4.3 Effect of host species on fungal richness and abundance

Overall, fungal species richness and abundance were highest for saprotrophs and plant pathogenic fungi, followed by ECM and ABS in all host species (Table 2.3). Fungal richness of each guild type varied slightly among host species whereas substantial variations of fungal abundance of each guild type could be observed (Table 2.3, Figure

2.7, Figure 2.8). The number of fungal sequences amplified by sample ranged from 9 to 7332 while the number of distinct fungal OTUs by sample ranged from 1 to 46 with a mean of 11.8.

Table 2.3 Mean species richness (S) and mean number of reads (N, i.e., abundance) by sample for each host species and for each fungal guild (ECM: ectomycorrhizal, ABS: arbuscular mycorrhizal, Sapro: saprotrophic, Patho: pathogenic).

Host species	Richness				Abundance			
	ECM	ABS	Sapro	Patho	ECM	ABS	Sapro	Patho
Paper birch	1.5	0.3	4.2	3	107.6	1.9	154	179
Balsam poplar	1.4	0.5	4.5	3.9	70	2.4	171.5	152.7
Willow	1.7	0.2	4.6	3.2	238.2	0.3	408.7	181.8
White spruce	1.3	0.4	5	3.4	250	0.8	511	119.9
Cedar	1.4	0.8	3.1	1.2	88.1	1.8	23.2	7.9

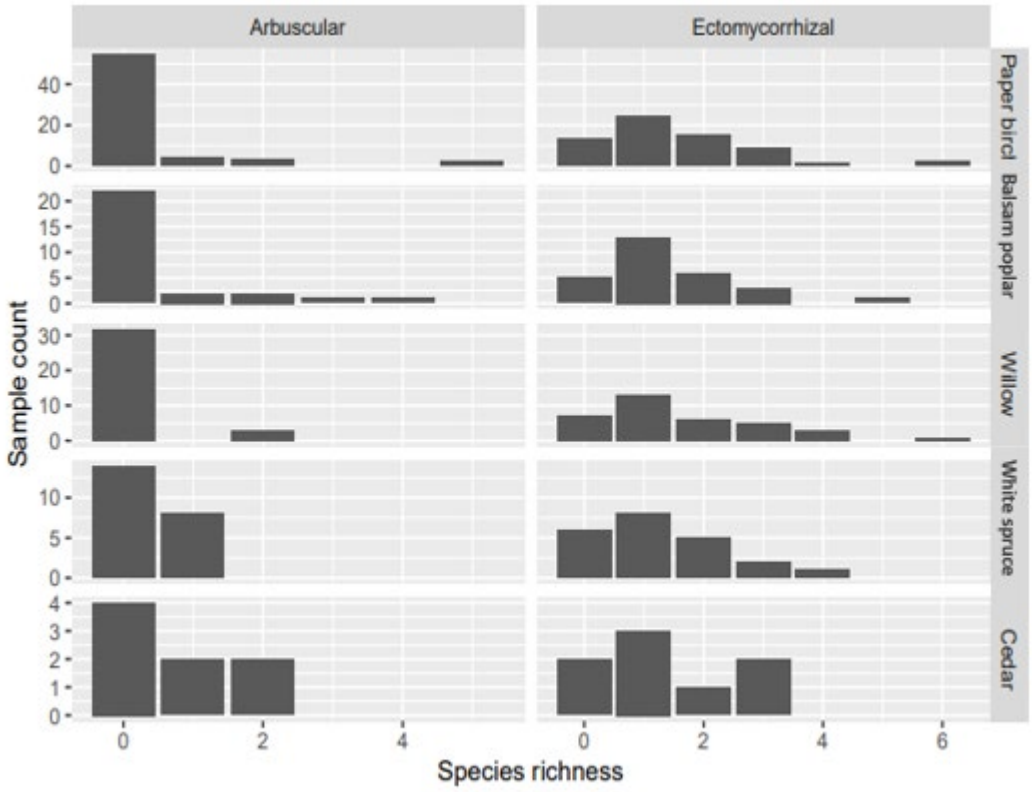


Figure 2.6.Mycorrhizal richness distribution among samples of each host species (note the different y-axis scale in each row).

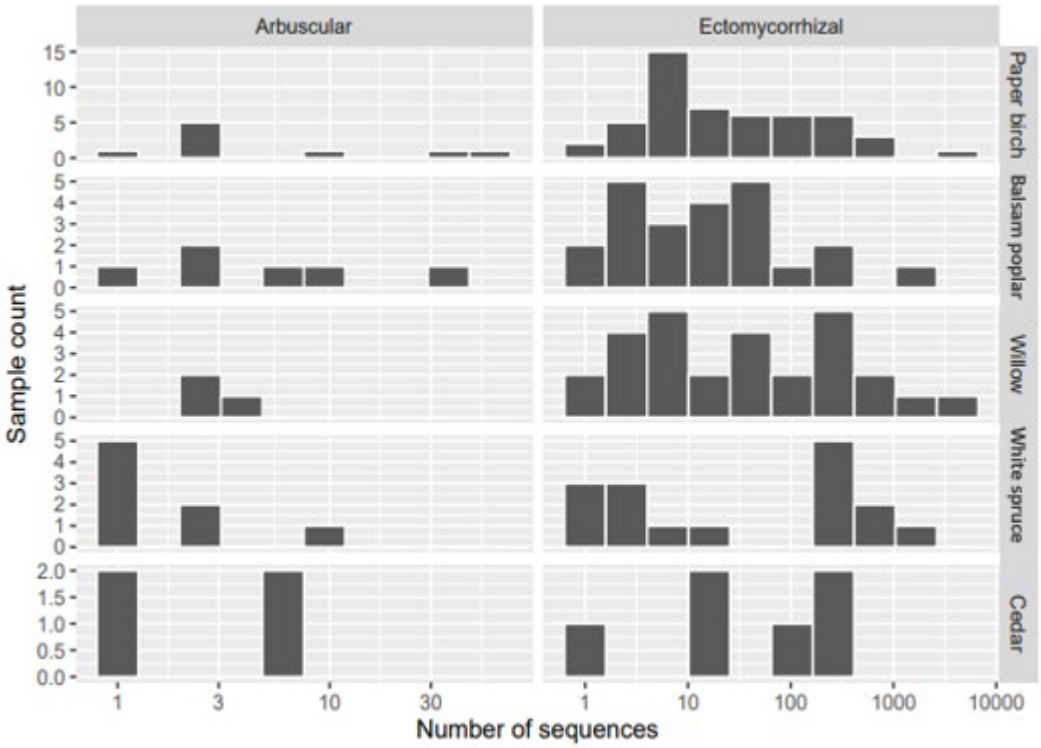


Figure 2.7 Mycorrhizal abundance distribution among samples of each host species (note the different y-axis scale in each row).

In total, 474 OTUs were distributed among the five selected host species. Among the 157 samples with any fungal OTU, 124 samples contained ECM (52 OTUs) and 30 samples had ABS (20 OTUs). Mean ECM richness and mean ABS richness across all samples were 1.5 and 0.3, respectively. The distribution of ECM and ABS richness is similar between host species, with most samples having 0 to 4 ECM OTUs (with a maximum of 6) and 0 to 2 ABS OTUs (with a maximum of 5) (Figure 2.7).

2.4.4 Mycorrhizal community composition by host species.

The PCA graph of Hellinger-transformed data obtained from 124 trees, which hosted 52 ECM OTUs, shows no distinct clusters by host species, as indicated by overlapping 95% confidence ellipses (Figure 2.9). The PERMANOVA results also show no significant effect of host species on mycorrhizal community composition and a low fraction of ECM community variation was explained by host species ($R^2 = 0.03$, $P = 0.3891$) (Table 2.4). On the other hand, for the 30 trees hosting 20 ABS OTUs, the PERMANOVA showed a significant effect of host species ($R^2 = 0.19$, $P = 0.006$) (Table 2.4) and the PCA graph reveals one distinct cluster for cedar (Figure 2.10).

Considering the ECM community distribution and sharing among host species, the highest richness was recorded for willow (34 OTUs), followed by paper birch (31 OTUs), balsam poplar (17 OTUs), white spruce (15 OTUs), and cedar (nine OTUs). The highest richness of ABS OTUs was found in balsam poplar (13 OTUs). There were 15 out of 20 OTUs that were shared by more than one host species.

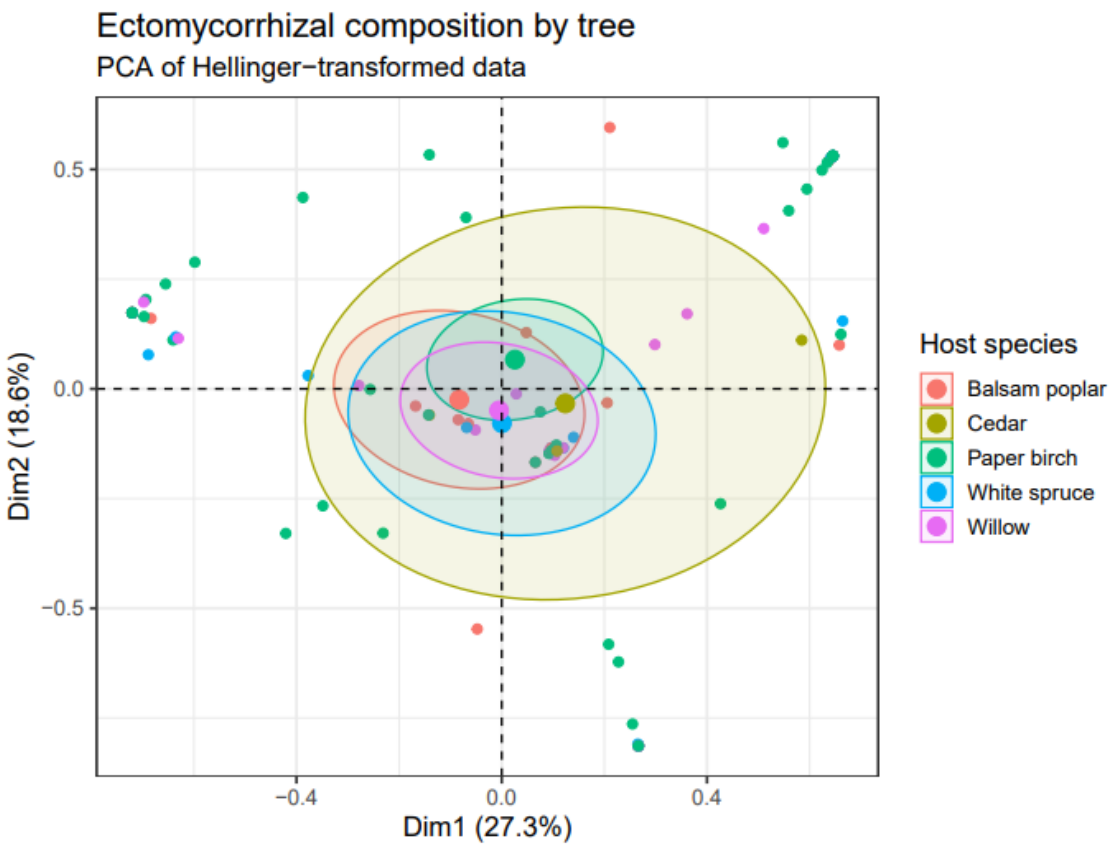


Figure 2.8 PCA graph of ECM fungi abundance data (Hellinger-transformed) by host species (using factoextra).

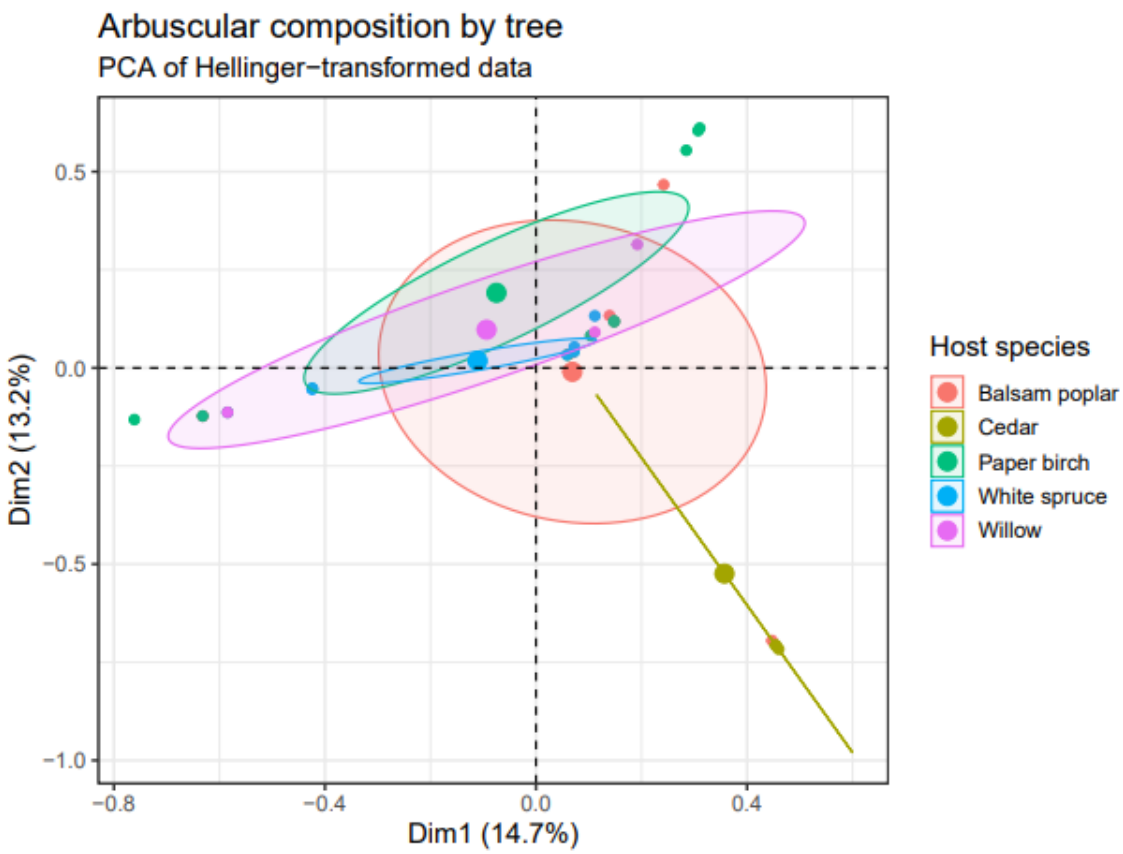


Figure 2.9 PCA graph of ABS fungi abundance data (Hellinger-transformed) by host species (using factoextra).

Table 2.4 PERMANOVA results (based on Hellinger Distance) for ECM and ABS community composition as a function of plot, host species, and root collar diameter.

Fungal dataset	ECM			ABS		
Variable	F.Model	R^2	Pr(>F)	F.Model	R^2	Pr(>F)
Plot	1.310	0.011	0.156	0.974	0.031	0.492
Species	1.000	0.032	0.389	1.492	0.193	0.006 **
Root collar diameter	0.788	0.006	0.753	1.090	0.035	0.365

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

The PERMANOVA test identified a significant effect of host species on ABS community composition (Table 2.4). This significant variation of composition between host species could mean either that the average composition for each host species is different, or the variation in composition (difference among samples) is greater for one host species than the other. Performing a follow-up test using the betadisper function in vegan, we obtained average distances to the median of 0.6148 (Paper birch), 0.6453 (balsam poplar), 0.5979 (white spruce), 0.5770 (willow) and 0.4375 (cedar). These distances were not significantly different according to the ANOVA results for betadisper. (Table 2.5). Therefore, no host species shows significantly more or less inter-individual variation in the ABS community composition.

Table 2.5 Results of ANOVA for betadisper test results for five host species (considering distances as a response).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	4	0.118	0.029	0.661	0.625
Residuals	25	1.114	0.045		

According to PERMANOVA results for ABS community composition, there are differences among host species. Nevertheless, it does not say among which species those significant differences occur, although we found a separate cluster for ABS associated with cedar (Figure 2.10). To identify pairwise differences in composition between host plants, we conducted another follow-up test using the package pairwiseAdonis for the ABS dataset; however, the results revealed no significant differences (Appendix A).

2.4.5 Variation of mycorrhizal composition between plots and spatially within each plot

Among plants with amplified fungal communities, 74 were in the west plot and 83 in the east plot (Table 2.2). Among them, 61 plants in west and 63 plants in east had amplified ECM sequences whereas 13 plants in west and 17 plants in east had ABS sequences. Conducting a PERMANOVA test for mycorrhizal datasets considering plots (west and east plot) as a factor, we found no significant differences in mycorrhizal composition for ECM and ABS (Table 2.4). The Mantel test explains the correlation

between dissimilarity of mycorrhizal communities (computed as the Hellinger distance) and the physical distance between host plants. We could not find significant spatial correlations within each plot for ECM and ABS (Table 2.6).

Table 2.6 Mantel test with Hellinger distance for each plot (west and east) considering fungal data sets (ECM and ABS).

Plot	Mycorrhizal data set	Z.stat	P value
West	ECM	13870.7	0.187
East		14831.7	0.541
West	ABS	664.368	0.493
East		475.709	0.441

2.4.6 Determination of co-occurrence of species

Calculating C-scores by performing co-occurrence analysis using EcoSimR function, we determined whether mycorrhizal species segregated or aggregated across host species for different fungal datasets (Table 2.7). As both null models gave coherent results, the results of sim9 was only presented. Since the observed C-score for both ABS and ECM data was significantly higher than the mean C-score from random permutations, communities of both ABS and ECM have less co-occurrence (thus were more segregated) than expected between individual plants.

Table 2.7 Results of C-score matrices calculated by sim9 algorithm for fungal datasets.

Fungal data set	Observed Index	Mean of Simulated Index	Variance of Simulated Index
ECM	12.969	12.702	0.013
ABS	6.163	5.941	0.003

2.4.7 The influence of neighborhood indices, mycorrhizal richness and abundance and host variables on the growth of focal plants.

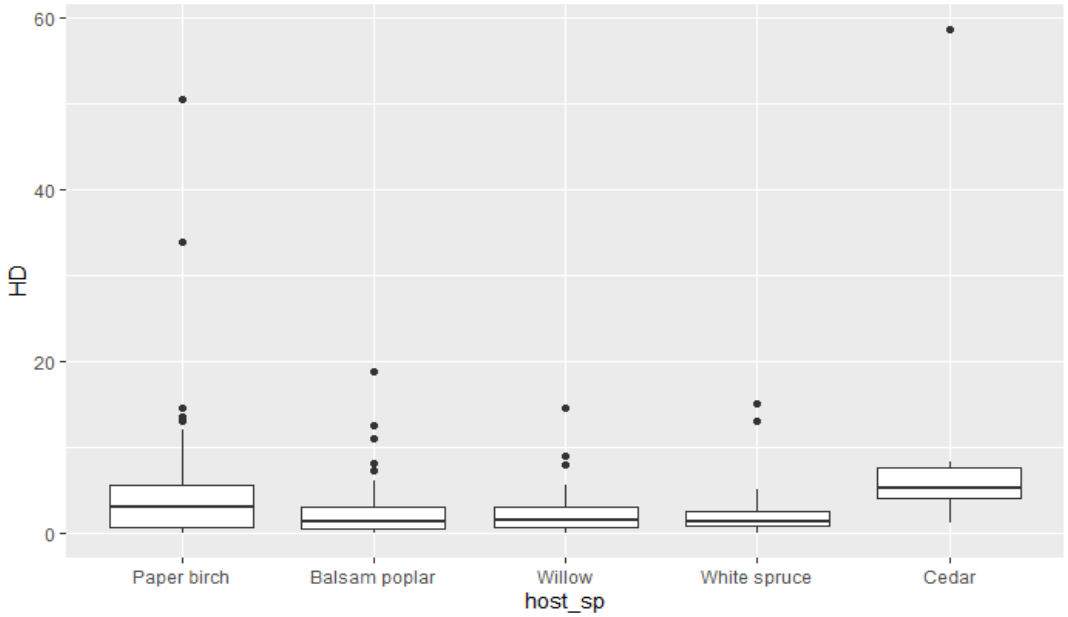


Figure 2.10 Height increment (HD- difference of height between 2019 and 2018 in mm) of host plants (host_sp).

The height increment (difference of height between 2019 and 2018) of plants shows slow growth for all five species (including all focal and neighbour plants) (Figure 2.11). Considering each focal species as a separate dataset, we fitted linear models to explain the influence of neighbouring effects as competition indices (intra-specific competition and inter-specific competition) and neighborhood mycorrhizal index. The shoot biomass of focal plants was also included as a covariate in the models. None of the neighborhood indices were significantly correlated with the apical growth of plants for balsam poplar, paper birch and cedar plants. Also, soil nitrogen concentration (based on the soil sample nearest to the plant) and mycorrhizal richness and abundance were not related to the growth of focal plants for all four plant species. However, the results obtained for the white spruce dataset reveals that intraspecific competition was negatively correlated and shoot biomass was positively correlated with apical growth of plants (Appendix B).

2.5 Discussion

In this study, we aimed to find out how mycorrhizal communities are structured and how they affect plant growth in a mine tailings site. To the best of our knowledge, most studies on mycorrhizae are focused on determining the effects of the factors associated with host species on the mycorrhizal community diversity, not the effect of mycorrhizal comminities on the host plants in a harsh environemnt. In contrast, we were interested in identifying 1) existing mycorrhizal communities, 2) the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts and 3) how these mycorrhizal communities, along with other defined physical and biological factors, affect tree growth in a harsh environment. Results of this study indicate that although mycorrhizae are believed to enhance host plant growth, this did not apply to the plants

at the mine tailings site for shoot apical growth we studied probably due to the harsh growing conditions. Mycorrhizal richness and abundance was low in the field and this could be a reason for non-significant effects of mycorrhizal communities on the plant growth at the site. Also, we did not detect structured mycorrhizal networks and compositional differences based on host species and spatial proximity.

2.5.1 Why was there a low abundance of mycorrhizae in our site?

The fungal community found in this study was species poor (474 OTUs) and fungal isolation and amplification was low in fine roots with only 39% success rate. In contrast, Nagati et al., (2018) recorded higher fungal diversity (1119 OTUs, 89% success rate in DNA amplification) from a mixed boreal forest area in the same region following the same protocol for molecular identification of fungi. The reason for this difference could be that fungal abundance is higher in undisturbed forest soils compared to in a mine tailings site and fungal abundance is also higher under plant canopies and in soil with higher amount of organic matter (Mummey et al., 2002). Our study area, the Beattie gold mine tailings site, is lacking trees forming a canopy layer and has less organic matter in the soil compared to surrounding natural boreal forested areas. These factors could explain the lower detection of soil fungi at this site.

Microbial abundance and community structure can differ with the type of ecosystem and age of the site. As an example, early successional reclaimed sites may have a lower abundance of fungi and a higher abundance of bacteria compared to natural forested areas (Dimitriu et al., 2010). Furthermore, ECM may be even more reduced in early successional sites compared to other fungi as, ECM abundance was low in recently reclaimed sites compared to other fungal groups such as endophytic fungi (Dimitriu et

al., 2010). Our study reveals similar results, as we detected a lower abundance and richness of ECM compared to other fungal groups (Table 2.3).

Interestingly, we detected ABS from white spruce root samples with molecular identifcation. We did not verify the ABS morphological structure by taking plant roots. As whole root and its rhizosphere were extracted, ABS mycelium could be amplified even in the absence of mycorrhizal symbiosis with that plant we sampled. Nonetheless, the presence of ABS in spruce is possible, for example Wagg et al. (2008) found evidence for the co-occurrence of ABS with ECM in some species of the Pinaceae family.

2.5.2 Which factors influence mycorrhizal community composition among host plants?

It is believed that host species identity is the major factor shaping the ECM community composition. Considering plants associated microbial groups (including mycorrhizae), Gagnon et al. (2020) has discussed that plant species is a main factor along with soil pH and acidogenic mine deposits which affects the community structure of plants associated microbial groups. In contrast to this, in disturbed soil, phylogenetically distinct plant groups shared similar ECM communities as they are sharing same soil conditions (Bierza et al., 2020; Cline et al., 2005; Trocha et al., 2012). However, most ECM fungi are not host specific; therefore, they can connect with many host plant species forming mycorrhizal networks (Simard and Durall 2004) though host specific ECM transport nutrients to plants more efficiently than generalist ECM (Bruns et al., 2002; Hobbie 2005). Our study did not find significant evidence of host specificity in ECM communities (Figure 2.9). Also the PERMANOVA results of our study only shows a significant effect of host species on ABS community composition, but our

sample size was insufficient to identify between which pairs of species these significant differences occurred in a post-hoc test. While mature forested ecosystems have both host specific and generalist ECM communities (Natel and Neumann, 1992).The fungi species can be selected more by the conditions at the site than by the host species due to factors such as nutrient scarcity and lack of older well-established plants in a disturbed ecosystem (such as mine tailings).

Mycorrhizal communities (both ECM and ABS) were not significantly different between plants in the west and east plots. Also, the mycorrhizal communities were not spatially structured within each plot. Even though we could not identify significant differentiation of mycorrhizal species (ECM) among host species or via spatial structure (ECM and ABS), our co-occurrence analysis results revealed that both ECM and ABS communities co-occur less often and are more segregated than would be expected by chance. Therefore, this could indicate competitive exclusion or priority effects among the fungi (Kennedy et al., 2009). This means that once a species colonizes a host, there is less chance for other species to establish there as early comers competitively exclude the latecomers via obtaining a shared resource beforehand (Kennedy 2010).

Early colonizing pioneer species are often well adapted fungi to the mining site, maybe they better facilitate the plant growth and survival. Although these pioneer fungi are well adapted to a mining site, the time and the order of fungal arrival is determined by factors such as chance, field abundance, or characteristics that make the fungus a good "pioneer" species (Bauman et. al., 2011). Considering ECM species abundance in our mining site, the exceptionally abundant fungi species may antagonistically inhibit the arrival of ECM species (Stark and Kytöviita, 2005), which can be well adapted under the harsh environmental conditions. Since we did not determine the age of the plants, it is not possible to identify which plants were the surrounding neighbours when a

seedling was first established. If we knew the age of the plants, then we would know which neighbours were established before the focal seedling and which are the dominant fungi in those neighbours. This may explain the lack of (static) spatial structure, as seedlings near each other might have been colonized at different times. Also, this competitive exclusion would explain the lower richness of mycorrhizae per sample in our mining site (Table 2.2).

2.5.3 How do neighborhood indices, mycorrhizal diversity, soil nutrients and host variables influence the growth of focal plants?

Plant growth was very slow in the site. We detected very low N, P and K concentrations and a high concentration of arsenic in the field (Appendices C and D). Lafleur et al. (2012) discussed that N and K are often the limiting nutrients for tree growth on unfertilized plots for poplar plants, and K is a limiting factor for the growth of white spruce (Truong and Gagnon, 1975). With very low concentrations of N, P and K across the site, these are a globally unfavorable conditions for growth of our selected focal species. Interestingly, Ca concentration was higher in the west plot compared to the east plot, and it also contained a higher number of cedar plants. Cedar is considered as a calciphile species since it can grow under high level of Ca (Blanchet, 1982; Imper and Zobel,1983).

According to the linear regression models we tested for the growth of focal plants, intra and inter specific competition did not seem to be important, since harsh environmental conditions at the site seemed to be the limiting factor. Also, mycorrhizal richness and abundance did not predict growth in our study. Thus, plant growth was not influenced by plant-plant competition nor by mycorrhizal facilitation under these harsh environmental conditions. Considering the factors which drive ECM formation and

forest regeneration under a harsh environmental condition, Amaranthus (1998) has discussed that factors like type and severity of disturbances, ectomycorrhizal diversity, climatic conditions, biological conditions, and the impact of non-host plants over time affect to reduction ofECM formation and forest regeneration after disturbances.

Toxicity indices (both total (TITotal) and available (TIBio) concentrations of heavy metals were the most significant factor shaping the ECM communities in heavy metal contaminated areas (Bierza et al., 2020; Stefanowicz et al., 2008). Under these harsh conditions, heavy metal tolerant ECM species have the advantage of survival and subsequently help forest regeneration (Bierza et al., 2020). Our mine tailing site bears naturally regenerating seedlings. The results reveal a low diversity of ECM communities in terms of ECM richness as we detected only 54 (11.4%) ECM OTUs among 474 fungal OTUs. Lack of suitable, well adapted ECM species to tolerate and mitigate effect of extremly high level of As (Appendix D) in the mine tailing site could be one reason for why we did not detect an effect of ECM richness on seedling growth. Species in fungal genera *Russula*, *Scleroderma* and *Rhizopogon* have the ability to make ECM connects with host plants under harsh environments since they have the ability to accumulate and tolerate higher concentrations of heavy metal than other species because of the specific biochemical pathways they possess (Bierza et al. 2020). Thus, planting of inoculated seedlings with heavy metal tolerant ECM species and with large root systems could be a good remedy to restore disturbed sites when older grown plants are absent in the field. However, the prior inoculation of introduced fungal species would not be effective for all the mine tailings sites since indigenous fungi can be found in some sites and negative priority effects can occur (Bauman, 2011).

2.6 Conclusion

This study reveals that our mine tailings site contains both ECM and ABS communities. However overall mycorrhizal richness and abundance was low with higher ECM richness and abundance than ABS. Mycorrhizal composition did not vary between plots nor spatially within each plot. Neighborhood indices, mycorrhizal communities and soil nutrients did not explain plant growth. Considering the community composition and distribution of mycorrhizae in our study, species identity or spatial arrangement did not determine the mycorrhizal diversity and distribution under harsh environmental conditions, but there was significant segregation of mycorrhizal species among individual plants. These results are consistent with important priority effects, where the timing of arrival of fungi determines the mycorrhizal community present in a seedling, and early arriving species exclude latecomers even though the latter may be best adapted to enhance seedling growth.

CHAPTER III

GENERAL CONCLUSION

In this study, our general goal was to determine how seedling growth and survival is affected by below-ground facilitation via mycorrhizal networks. Specifically, we were interested in identifying 1) existing mycorrhizal communities, 2) the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts and 3) how these mycorrhizal communities, along with other defined physical and biological factors, affect tree growth in a harsh environment. Considering these objectives, we asked the following questions: 1. What is the fungal richness and abundance at the site, overall and by fungi type? 2. Does fungal richness change with the identity and size of the host? 3. How does the mycorrhizal fungi community composition vary among host species? 4. Does the fungal composition vary spatially? 5. How does competition or facilitation from neighbouring plants, mycorrhizal diversity, and host variables influence the growth of focal plants?

We detected both ECM and ABS mycorrhizal species in all the five host tree species. Also, the distribution of both ECM and ABS richness was similar among species. The cool climate in boreal regions slows the rate of mineralization and reduces the availability of N, making boreal forests the primary niche for ECM fungi as they are particularly associated with N limited environments. A recent study conducted in a mixed boreal forest Nagati et al. (2018) found that a majority of the taxa were either ECM or saprotrophs. However, in our site, which was in the same region, richness and abundance of ECM was lower, and ABS was much lower than the richness and abundance of saprotrophs. Fungal richness of each guild type varied slightly among

host species, whereas substantial variations in the fungal abundance of each guild type was observed among host species. If we compare the fungal diversity and fungal amplification of our samples from our mine tailings site with natural boreal forest areas, it was low in our site (474 OTUs and 39% of amplification success), while Nagati et al. (2018) recorded higher fungal diversity (1112 OTUs and 89% of amplification success). Our results indicate that fungal abundance and richness was lower in disturbed anthropogenic soils than in the natural soils (Mummey et al., 2002).

In our second objective, determining the effect of variables such as host identity and spatial structure on mycorrhizal sharing among hosts, we found that neither species identity nor spatial arrangement explained the mycorrhizal community structure and sharing of them among hosts. The co-occurrence analysis explains this as, fungal species were more segregated among sites than expected at random, indicating that competitive exclusion or priority effects, where early comers establish in the host taking resources beforehand suppressing the arrival of later comers, were structuring the community. Therefore, the timing, and sequence of arrival of mycorrhizal species determine the structure and community composition of mycorrhizal communities. In contrast to this, a recent study conducted in mine tailings of northwestern Québec shows that host identity influences the community composition of plant-associated microbial groups (Gagnon et al., 2020).

Most mycorrhizal fungi are not host-specific, since they have wide host ranges as one fungal species can connect with many host plant species through mycorrhizal networks. Mature forested ecosystems house both host-specific and generalist ECM communities. Our study revealed no host specific ECM since species were shared among them. Interestingly, we identified a separate cluster of ABS for cedar plants.

In the third objective, results of the linear growth models revealed that none of the neighborhood indices were significantly correlated with the apical growth of plants for balsam poplar, paper birch and cedar plants. Also, soil nitrogen concentration and mycorrhizal variables were not related to the growth of focal plants for all four plant species. Thus, we can conclude that plant apical growth was not influenced by plant-plant competition nor by mycorrhizal facilitation in this harsh environment, as it was globally unfavorable for plant growth.

Generally, mycorrhizal inoculation practices have been proposed in most restoration programs. However, mycorrhizae do not explain the differences of growth of plants in these kind of degraded sites under harsh environmental conditions. Therefore, mycorrhizal inoculations with seedlings might not help for these kinds of environments if the inoculating mycorrhizal species are not well adapted to the harsh environment. Thus, planting of inoculated seedlings with harsh environmental tolerable, heavy metal resistant ECM species might be a good future direction for restoration of degraded sites.

APPENDICES

Appendix **A** PairwiseAdonis results for ABS to test for pairwise differences between host species (Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted	sig
Cedar vs Balsam poplar	1	0.578	1.313	0.141	0.184	1.000	
Cedar vs Paper birch	1	1.003	2.478	0.184	0.011	0.110	
Cedar vs Willow	1	0.701	1.725	0.257	0.148	1.000	
Cedar vs White spruce	1	0.980	2.429	0.195	0.020	0.200	
Balsam poplar vs Paper birch	1	0.363	0.797	0.058	0.626	1.000	
Balsam poplar vs Willow	1	0.272	0.544	0.072	1.000	1.000	
Balsam poplar vs White spruce	1	0.615	1.341	0.101	0.161	1.000	
Paper birch vs Willow	1	0.332	0.751	0.070	0.654	1.000	
Paper birch vs White spruce	1	0.881	2.057	0.121	0.024	0.240	
Willow vs White spruce	1	0.556	1.250	0.122	0.280	1.000	

Appendix **B** The results of linear regression models explaining the influence of neighboring effects as neighborhood indices (intra-specific competition-cci, inter-specific competition-hci and mycorrhizal index-myco_ci), ECM richness-S_ECM, ECM abundance-N_ECM, ABS richness-S_ABS, ABS abundance-N_ABS and shoot biomass of focal plants for growth.

Focal dataset	Predictors	Estimated Coefficient	Standard Error	t value	P-value
Balsam poplar	cci	-0.189	0.198	-0.952	0.353
	hci	0.069	0.206	0.338	0.739
	myco_ci	-0.445	0.509	-0.874	0.395
	S_ECM	-0.028	0.368	-0.075	0.942
	N_ECM	-0.0001	0.001	-0.055	0.957
	S_ABS	0.667	1.041	0.641	0.536
	N_ABS	NA	NA	NA	NA
	shoot mass	0.221	0.267	0.828	0.418
Paper birch	cci	0.079	0.104	0.758	0.456
	hci	-0.159	0.11	-1.438	0.164
	myco_ci	0.172	0.25	0.689	0.498
	S_ECM	0.069	0.092	0.756	0.461
	N_ ECM	5.47E-06	8.65E-04	0.006	0.995
	S_ABS	0.231	0.679	0.341	0.738
	N_ABS	-0.029	0.156	-0.186	0.855
	shoot mass	0.158	0.11	1.43	0.166

Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

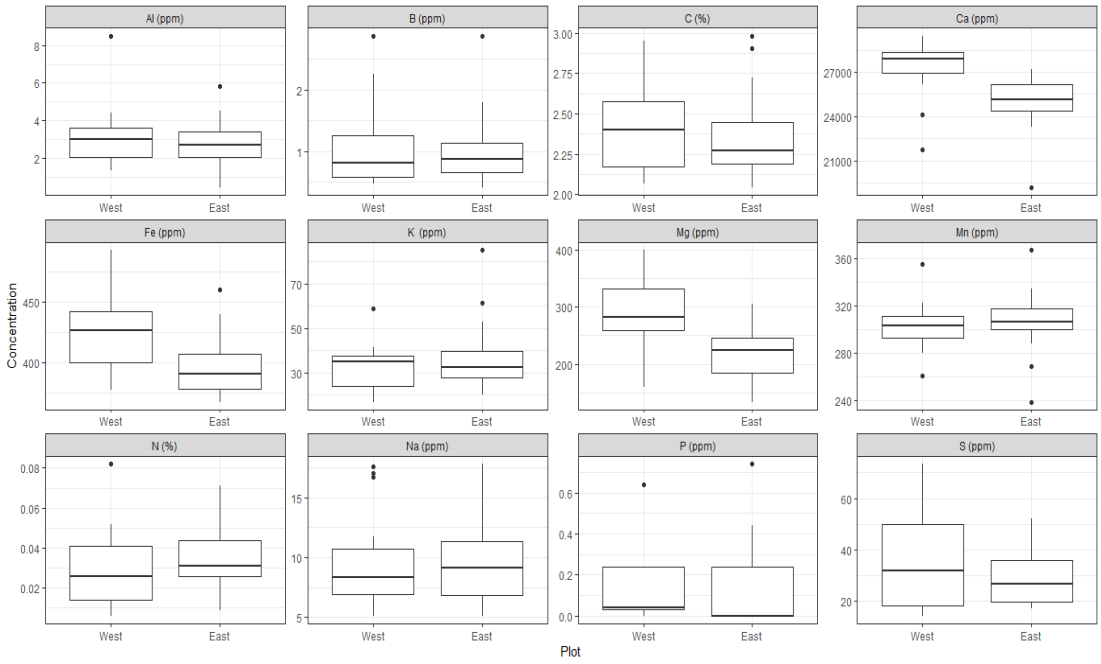
Continuation of Appendix **B**

((intra-specific competition-cci, inter-specific competition-hci and mycorrhizal index-myco_ci), ECM richness-S_ECM, ECM abundance-N_ECM, ABS richness-S_ABS, ABS abundance-N_ABS).

Focal dataset	Predictors	Estimated Coefficient	Standard Error	t value	P-value
White spruce	cci	-0.213	0.103	-2.08	0.048 *
	hci	-0.068	0.092	-0.737	0.468
	myco_ci	0.061	0.44	0.139	0.891
	S_ ECM	-0.035	0.174	-0.204	0.841
	N_ ECM	-0.0004	0.0003	-1.408	0.182
	S_ABS	-0.429	0.883	-0.485	0.636
	N_ABS	0.428	0.605	0.708	0.491
	shoot mass	0.174	0.081	2.157	0.041 *
Cedar	cci	-0.192	1.694	-0.113	0.914
	hci	0.751	0.368	2.039	0.097 .
	myco_ci	0.495	2.992	0.165	0.879
	S_ ECM	0.077	0.154	0.497	0.654
	N_ ECM	-0.0007	0.0015	-0.48	0.664
	S_ABS	-0.586	0.566	-1.035	0.377
	N_ABS	0.207	0.237	0.875	0.446
	shoot mass	0.708	0.417	1.7	0.15

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix **C** Boxplots of concentrations of plant nutrients detected from West plot and East plot (In the P results, 13 out of 17 samples for the west plot and 18 out of 25 samples for the east plot were below the detection limit).



Appendix **D** Concentrations of heavy metals detected from West plot and East plot (there are 3 thresholds A-B-C, C being the highest / most contaminated according to Quebec government standards for contamination).

Plot	Heavy metal	Concentration in PPM	Quebec government standards for contamination
West	Hg	1.41	A-B
	As	560	> C
	Cd	0.14	< A
East	Hg	1.42	A-B
	As	590	> C
	Cd	0.49	< A

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