Role of host identity, stand composition, soil type and disturbance severity in structuring ectomycorrhizal communities in the boreal forest

by

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Abstract

The symbiosis between trees and ectomycorrhizal fungi (EMF) is essential for tree establishment and survival in the boreal forest because it is a disturbance prone ecosystem characterized by long harsh winters and low nutrient mobility. Ectomycorrhizal fungal community composition can be influenced by factors such as host tree identity and subsequently stand composition, soil type and disturbance severity. However, a less explored concept is the fact that these factors can interact and influence one another. Because of the essential role EMF play in tree productivity in the boreal forest it is important to understand the driving factors and interactions between them in structuring the fungal community. To that end, I investigated the EMF community on roots of planted seedlings in soils from (1) an oil sands reclamation site constructed with different salvaged surface soils and (2) a site that experienced a gradient of harvest disturbances to address the influence of disturbance severity, soil type and host identity on EMF community composition. Additionally, I utilized the oil sands reclamation site to test if mixed-species stands have overall additive or synergistic effects on EMF richness and composition. The EMF community was significantly influenced by host identity, disturbed soil the interaction between them ; the different species of planted seedlings hosted different fungal communities depending on which disturbed soil they were planted in. Factoring in stand composition on the forest reclamation site, the interaction between host identity and soil type was a stronger influence in structuring ectomycorrhizal fungal communities. Additionally, the strong host identity preference for EM fungal communities meant that overall composition and species richness of EMF in mixed-species stands was the additive result of combining different tree species. Taken together, this research suggests that host identity, soil type and level of disturbance can interact to influence ectomycorrhizal community composition and therefore,

both biotic and abiotic factors should be taken into consideration when measuring fungal communities in the boreal forest. Also, at this point in time, stand composition does not have an influence on EM fungal communities of planted seedlings on a forest reclamation site compared to host identity and soil type.

Preface

Data from Chapter 2 has been used as part of a synthesis paper that was submitted to *Ecological Applications* on 31 October 2017. The submitted publication is titled "Disturbance severity drives the recovery of ectomycorrhizal fungi in the boreal forest more than host identity or soil type" and authored by Gregory J. Pec, Natalie Scott, Stefan F. Hupperts, Shanon L. Hankin, Simon M. Landhäusser, and Justine Karst. My contribution to the submitted manuscript were the provision of data and associated field work and the editing of drafts of the manuscript.

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Chapter One: General Introduction

The boreal forest is a circumpolar biome that extends as wide as 1000 km in parts of North America and Eurasia (Larson 1980). These forests are globally important for the benefits they provide such as a provision of forest products, protection of soil resources, and storing a large amount of carbon in the soil, permafrost and wetlands (Bonan 2008). Ectomycorrhizal fungi (EMF) are both abundant in and vital to the boreal forest because of the role they play in tree establishment and survival, and formation of soil, mainly through the weathering of mineral and rock particles as well as mobilizing organic nutrients and contribution of biomass to the organic matter pool through extensive mycelia (Dahlberg et al. 2001, Finlay 2008). However, the boreal forest is prone to both natural and anthropogenic disturbances, which can transform the forest structure and consequentially influence many factors that structure EM fungal communities. The main goal of my thesis is to better understand the driving factors and interactions between them in structuring EM fungal communities, and furthermore, using this information to identify management implications for boreal forest reclamation sites and provide future directions for research. I will first introduce the boreal forest with a focus on the disturbances that influence the ecosystem. I continue with a brief description of EMF and what factors can influence their community composition. Finally, I will provide an overview of my specific research objectives for my thesis.

1.1 The boreal forest: a disturbance prone ecosystem

The boreal forest covers a large portion of northern forests worldwide, but is mainly dominated by four tree genera; *Picea*, *Abies*, *Larix* and *Pinus*, while *Betula* and *Populus* are also abundant in some areas, such as the central and south-central portions of Canada (Larson 1980).

Overall growing conditions are stressful because the window for growth is short due to long harsh winters, while additionally nutrients are tied up in organic compounds, making them unavailable for uptake (Larson 1980). The boreal forest is also unique compared to other forest systems in that it is rarely free from disturbance for more than a few decades (Larson 1980). Many types of disturbances are common in the boreal forest across continents, with some regional variation. North America experiences severe stand-replacing wild fires, while Eurasia experiences a gradient of fire severity from more severe fires in Russia to low severity fires in Europe (Shorohova et al. 2011). Insect pathogens can also be common, such as the spruce budworm in eastern North America and the spruce bark beetle in Siberia (Shorohova et al. 2011). Additionally, the boreal forest has many valuable commercial resources, which leads to anthropogenic disturbances such as forest harvesting (Jones, Durall and Cairney 2003) and surface mining for belowground resources (Macdonald et al. 2015a).

1.2 Ectomycorrhizal fungi in the boreal forest

The term 'myckorrhiza' was first used by Frank 1885 (English translation (Frank 2005)) to describe the symbiosis between plants and fungi and since been updated and described in detail by Smith and Read (2008). Ectomycorrhizal fungi (EMF) represent one group of mycorrhizal fungi that provide their host trees with nutrients and water in exchange for photosynthetically derived carbon (Smith and Read 2008). Ectomycorrhizal fungi are differentiated from other types of mycorrhizal fungi because of (1) the characteristic sheath or mantle of fungal tissue covering the root tips of their tree partners, (2) a Hartig net, which is an ingrowth of fungal hyphae between plant cells and (3) the outward growth of hyphae that interacts with the rhizosphere (Smith and Read 2008). Because of the harsh growing conditions present in the boreal forest, trees rely upon the particular abilities of EMF for nutrient uptake

(Courty et al. 2010a, Hawkins, Jones and Kranabetter 2015). Additionally, EMF play an essential role in ecosystem functions such as weathering and solubilization of minerals, as well as mobilization of organic nutrients and carbon cycling which drives soil respiration (Finlay 2008, Courty et al. 2010a).

Having a diverse EMF community may be essential to maintain ecosystem resilience, particularly in regions that experience disturbance and have lower nutrient availability, such as the boreal forest (Miller et al. 1998, Byrd et al. 2000, Jonsson et al. 2001). Ectomycorrhizal fungi differ in enzymatic abilities to break down nutrients in the soil, such as organic forms of N (Pena and Polle 2014) and P (Conn and Dighton 2000), so having a diverse community is important to overall ecosystem function (Korkama, Pakkanen and Pennanen 2006, Rineau and Garbaye 2009, Courty, Franc and Garbaye 2010b). Therefore, because of the functional diversity EMF express and their importance in both tree establishment and soil processes, it is important to understand what factors can play a role in structuring fungal communities.

1.3 Factors influencing ectomycorrhizal fungal communities

1.3.1 Host identity

Host identity may influence the composition of EM fungal communities. At one extreme, sometimes tree species host a select group of EMF, such as the high specificity of EMF in the genus *Alpova* for *Alnus* (Molina, Massicotte and Trappe 1992). Differences in EMF community composition are sometimes observed between broadleaf deciduous and coniferous host trees (Bills, Holtzman and Miller 1986, DeBellis et al. 2006, Ishida, Nara and Hogetsu 2007). More commonly, trees form relationships with many different EMF and are considered generalists (Smith and Read 2008). Different species of trees may select similar fungal communities. For

examples, similarities have been seen in EM fungal communities between Douglas-fir and paper birch (Jones et al. 1997, Simard et al. 1997a), Douglas-fir and bishop pine (Horton and Bruns 1998) and ponderosa pine and pinyon pine (Hubert and Gehring 2008). However, while EMF may be host-specific in monodominant stands in mixed-species stands they may form relationships with a variety of tree species (Molina and Trappe 1982, Heslin et al. 1992, Kranabetter, Hayden and Wright 1999, Massicotte et al. 1999, Lang, Seven and Polle 2011). In consequence, it is essential to also consider the potential impacts different stand compositions can have on EM fungal communities.

1.3.2 Stand composition

Stand composition can potentially influence both the composition and richness of an EMF community. Mixed-species stands may have additive effects on EMF community composition, in that the fungal community is composed of fungi colonizing single tree species (Jones et al. 1997, Simard et al. 1997a, Hubert and Gehring 2008). The effect is additive, in that no EM fungal species unique to the mixed-species stands are found, but rather communities are comprised of only the combined fungal communities that can be found on each individual species in single-species pure stands. Alternatively, mixed-species stands may have synergistic effects on EMF community composition by harbouring EMF species that are unique to mixed stands and are not found in pure stands (Durall et al. 2006), which in turn would lead to overall higher fungal richness compared to the individual communities found in pures stands when combined. Synergistic effects could be brought about by mixing characteristics of different tree species that in turn create unique properties not found in single-species stands. These characteristics include differences in litter and organic matter content, soil nutrient status, temperature and moisture (Larson 1980, Cavard et al. 2011) that can all in turn influence EM

fungal communities (Bills et al. 1986, Last, Dighton and Mason 1987, Rumberger et al. 2004, Douglas, Parker and Cullings 2005, Walker, Miller and Horton 2005, Matsuoka et al. 2016). However, as also noted by Cavard et al (2011), it is challenging to address the effect mixed-species stands have on diversity measurements because of the difficulty in finding single-species stand comparisons of similar age, site history and characteristics.

1.3.3 Soil type

Soil properties, such as texture (Bois et al. 2005), nutrient status (Huang et al. 2012, Huang et al. 2015, Leduc et al. 2013), pH (Grebenc et al. 2009) and horizon (Rosling et al. 2003, Courty et al. 2008), can all influence EMF community composition. Particularly, higher levels of EM fungal richness are often associated with the litter and organic horizon (Stendell, Horton and Bruns 1999, Jones et al. 2003, Hartmann et al. 2012). Ectomycorrhizal fungi can respond strongly to soil conditions as shown by studies that pre-inoculated tree seedlings with certain EMF species, but those species were replaced by other fungal species once planted (Danielson and Visser 1989, Gagne et al. 2006). This indicates that EMF have preference for and thrive in different types of soils as the inoculated fungal species could not compete with the species adapted to the environment. Aboveground tree composition directly influences nutrient status in the organic horizon by differences in litter input (e.g. needles versus leaves), which can in turn influence EMF composition (Conn and Dighton 2000).

1.3.4 Disturbance severity

Ectomycorrhizal fungal communities can be altered by fire (Baar et al. 1999, Tuininga and Dighton 2004, Kipfer et al. 2011), forest harvesting (Byrd et al. 2000, Grebenc et al. 2009, Ding et al. 2011, Walker and Jones 2013) and mining disturbances (Fay and Mitchell 1999,

Gebhardt et al. 2007, Huang et al. 2012). The legacy material left behind after a disturbance will influence the EMF community composition because fungi can colonize trees and seedlings from surviving roots systems and resident spores and sclerotia in the soil (Horton, Cazares and Bruns 1998, Jones et al. 2003). However, the impact of disturbances on a forest is related to the severity, defined by the amount of overstory and understory vegetation, forest floor, and soil removed (Gilliam 2014). For example, low severity fires (Jonsson et al. 1999) and partial harvesting or thinning (Dahlberg et al. 2001, DeBellis, Widden and Messier 2002, Holden and Treseder 2013) do not have a pronounced effect on EMF community composition.

Therefore, the severity of a disturbance plays a large role in the diversity of EMF left to re-colonize a site after an event. There are several studies focussing on the impact of disturbance severity on EMF community structure (Barker et al. 2013, Jones et al. 2010, Mah et al. 2001, Smith et al. 2005), but are limited to only comparing fire and clearcut disturbances. To better understand the influence of disturbance severity on EMF community composition, more comparisons between different types of disturbances, such as surface mining which represents a high severity disturbance, are needed. Consequently, disturbances directly affect other factors that structure EM fungal communities in that they can influence soils and tree composition and thus the interlinkedness among these factors needs to be addressed.

1.4 Overview of thesis

The primary objective of this thesis is to understand underlying ecological factors and the possible interactions between them in affecting EM fungal communities in the boreal forest. In chapter two, I assay EM fungal communities with different species of tree seedlings on soils from (1) an oil sands reclamation site constructed with different salvaged surface soils and (2) a site that experienced a gradient of harvest disturbances to address the influence of disturbance

severity, soil type and host identity on EMF community composition. In chapter three, I use an oil sands reclamation site to explore the possible interaction between different soil types and stand composition on EM fungal communities and if mixed species stands have overall additive or synergistic effects on EMF richness and composition. Finally, in chapter four, I will provide a synthesis of my research as well as possible management implications for reclamation sites and suggest future research directions.

Chapter 2: Disturbance severity determines ectomycorrhizal fungal community recovery in boreal forests

2.1 Introduction

Ectomycorrhizal fungi (EMF), which form symbiotic relationships with roots of trees, provide nutrient and water uptake for their hosts in exchange for photosynthetically derived sugars (Frank 2005, Smith and Read 2008). They play an essential role in ecosystem functions such as weathering and solubilization of minerals, as well as mobilization of organic nutrients and carbon cycling which drives soil respiration (Finlay 2008, Courty et al. 2010a). The community composition of EMF can affect host tree performance (e.g. growth and nutrient uptake) (Jonsson et al. 2001, Franco et al. 2014, Pena and Polle 2014, Moeller et al. 2016, Hazard et al. 2017), therefore it is important to understand what factors structure the composition of fungal communities.

It has been well documented that the physical, chemical and biological properties of soils (Jones et al. 2003, Douglas et al. 2005, Huang et al. 2014, Walker, Ward and Jones 2016) and identity of host trees (Massicotte et al. 1999, Ishida et al. 2007, Smith and Read 2008, Bent et al. 2011, Ding et al. 2011) influence EMF community composition. Disturbances that frequent the boreal forest , such as fire, insect outbreaks, forest harvesting and other resource extractions can have strong influences on forest soils and composition. For example, in the boreal forest, disturbances such as fire or harvesting can impact soils and vegetation including overstory trees directly or indirectly; while some tree species are adapted to rapid recovery (i.e. *Populus tremuloides*) after these disturbances, others are not (i.e. *Picea glauca*) (West, Shugart and Botkin 1981). These responses to disturbance has a profound effect on the diversity and composition of EM fungal communities, in particular the later successional species (Durall et al.

1999, Horton and Bruns 1998, Kranabetter 1999, Hagerman and Durall 2004, Lazaruk et al. 2005, Teste, Simard and Durall 2009, Kranabetter, De Montigny and Ross 2013). Coupled with the impact of tree death, disturbances can also cause major changes to soils, such as the loss of the organic soil horizon, which contain the majority of EMF (Dahlberg et al. 2001, Tuininga and Dighton 2004, Heinonsalo, Koskiahde and Sen 2007, Hartmann et al. 2012, Barker et al. 2013).

However, disturbances vary greatly in severity defined by the amount of overstory and understory vegetation, forest floor, and soil removed (Gilliam 2014). Therefore, it can be expected that disturbances varying in severity will have different impacts on EMF community composition, but studies investigating this are limited (Mah et al. 2001, Smith et al. 2005, Jones et al. 2010, Barker et al. 2013). Hence, the interlinkedness of disturbance type and severity, host identity and soil type influence on EMF community composition need to be further investigated.

Here, EM fungal communities are compared on a reclaimed open-pit mine site to a range of ecological benchmarks in a natural unmined setting, representing a range of disturbance severities to explore possible interactions among substrate, host identity and disturbance severity on the composition of EM fungal communities. Open-pit mining is an extreme disturbance that involves excavation of surface soils to a depth of 100 meters deep to reach a resource. The overburden material is used to create new landforms that are capped with a range of surface soil materials salvaged from surrounding areas that are slated to be mined.

The ecological benchmarks allow comparisons to these forest reclamation sites to a gradient of disturbance severities that can be found in response to forest harvesting. Within these ecological benchmarks, it was also tested whether EM fungal communities on roots of naturally established trees are similar to those on planted seedlings of the same species. Though many studies have noted distance between mature trees and planted seedlings influences EMF

community composition (Durall et al. 1999, Hagerman et al. 1999, Kranabetter 1999, Cline, Ammirati and Edmonds 2005, Dickie and Reich 2005, Outerbridge and Trofymow 2004, Jones et al. 2008), few directly compare the fungal communities between trees and seedlings. It would be valuable to have direct evidence that established trees are able to host EMF and provide a source of inoculum that can also colonize planted seedlings.

In this study the following questions were asked: (1) Does the EMF community composition differ among soils that have experienced varying extents of disturbance? Reconstructed soils (composed of salvaged surface soil materials, typically used in boreal forest reclamation sites) were compared to different surface soil conditions in unmined forests. These latter soils differ in the severity of soil surface disturbance from an undisturbed control to a forest floor removal and provide context for understanding successional trajectories of EM fungal communities at reclaimed sites. (2) Do EMF show host specificity, and does soil type and conditions affect this response? Based on previous research showing host specificity by fungi present on the reclamation site (Hankin, Karst, and Landhäusser 2015, Hupperts 2016) I predicted that EMF community composition would vary among species of planted seedlings but disturbance severity would have a stronger influence because of the strong possibility of different fungal inoculum and properties of the disturbed soils (Jones et al. 2003, Bois et al. 2005, Rowland et al. 2009). (3) Does EMF community composition vary between roots of naturally established trees versus those planted as seedlings? I predicted that established trees would host later successional species due to age differences between the hosts, but share many widely distributed species with the planted seedlings.

2.2 Methods

2.2.1 General Description of Region

The study area is located in the Dry Boreal Mixedwoods region of Alberta, Canada. This region was covered by the Laurentide ice sheet 10,000 to 12,000 years ago, and left behind glaciofluvial and lacustrine deposits (Johnson and Miyanishi 2008). Due to the deposition of these sediments and disturbances that frequent the landscape, the region is a rich mosaic of uplands, lowlands and lakes. The Boreal Mixedwoods region is characterized by long harsh winters and short warm summers (Beckingham and Archibald 1996.). Depending on soil type, upland forests can range from mesic conditions, supporting a forest dominated by a mixture of trembling aspen (*Populus tremuloides* Michx) and white spruce (*Picea glauca* (Moench) Voss) or xeric conditions where coarser soils are dominated by jack pine (*Pinus banksiana* Lamb.) (Johnson and Miyanishi 2008). In this region soils are young and characterized by a thin eluvial A horizon, a distinct Bm (Brunisols) or Bt horizon (Luvisols), and a C horizon. Lowland forests are dominated by organic soils and the tree species common to these forests include tamarack (*Larix laricina* (Du Roi) K. Koch) and black spruce (*Picea mariana* (Mill.) B.S.P.) (Johnson and Miyanishi 2008).

2.2.2 Forest Reclamation Site

Experimental plots were located on a large-scale research study (36 ha, the Aurora Soils Capping Study hereafter 'Reclamation Site') in which different vertical configurations of different surface soils and substrate types were tested for their use in forest reclamation of capping lean oil-sands overburden. The site was constructed on the Syncrude Canada Ltd.-Aurora mine lease, approximately 75 km north of Fort McMurray, Alberta, Canada (57°19'20"N, 111°30'24"W). The different surface soil materials tested in this study had been

salvaged from areas slated for mining and directly placed (without stock-piling) in the winter of 2011/12 on the experimental site. Direct placement of the salvaged soil material is preferred as propagules contained in the material can lose their viability when stock-piled (Koch et al. 1996, Rokich et al. 2000, Macdonald et al. 2015b). Surface soil materials were salvaged from upland jack pine froests or from black spruce dominated lowland forests. The surface soils included (1) a upland forest floor material (organic forest floor horizons including the A and portion of the B horizon salvaged to a depth of 15 cm depth ('FFM'); (2) B horizon material (the soil material found below the salvaged upland FFM salvaged to a depth of 100 cm) ('Subsoil'), and (3) an organic soil ('Peat') that was salvaged to a depth 200 cm from lowland forests. Each of these salvaged soil materials were placed last and were positioned at the soil surface. For more information on the initial soil characteristics see Hankin et al (2015).

Each treatment plot with different surface soils was 1 ha in size and replicated three times. Each treatment plot contained four 25 × 25 m tree plots consisting of three single species plantings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) and a fourth plot with an even mixture of the tree species (Appendix I). Mixed-species tree plot are described in more detail in Chapter 3. The seedlings of all three species were grown at the Smoky Lake Forest Nursery (Smoky Lake, Alberta) from mixed open pollinated seed sources collected from several populations near Fort-McMurray. Specifically, the seedlings were container-grown and 1-year old, aspen and spruce were grown in containers 6 cm in diameter and 15 cm deep while pine was grown in containers 4 cm in diameter and 12 cm deep. The seedlings were planted in May 2012 at 1 meter spacing equivalent to a density of ten thousand stems per hectare.

2.2.3 Reference Site

To extend the gradient of disturbance severity, a range of ecological benchmarks comprising the 'Reference Site' was included. The Reference Site was an area in a mature jack pine forest located approximately 5 km northeast (57°21'49.1"N, 111°25'45.6"W) from the Reclamation Site and could be considered the eventual 'target' forest of the Reclaimed Site. At the Reference Site areas representative of three disturbance severities were selected: 1) intact jack pine forest ('Control'), 2) clearcut harvest with overstory removed with an intact forest floor ('Disturbed') and 3) clearcut harvest with forest floor and overstory removed ('Removed'). The intact forest was a mature jack pine forest growing on an Eutric Brunisol, with an understory dominated by *Arctostaphylos-uva ursi* and *Vaccinium vitis ideae*. The clearcut 'Disturbed' area had been harvested approximately 17 years prior to this study, the remaining forest floor material and associated vegetation closely resembled those of the 'Control'. Both the 'Disturbed' and 'Removed' areas had jack pine trees that had naturally re-established after the harvest disturbance.

Within each disturbance severity areas of the Reference Site, uniform plots in sizes equivalent to the surface soil plots on the Reclamation Site were selected. Plots were replicated three times. Plots were separated by at least 20 m and each contained three smaller 2.5 × 2.5 m planted tree plots that were separated by at least 2 m (Appendix II). Each tree plot was planted with a mixture of six to eight seedlings of trembling aspen, white spruce and jack pine each. This resulted in a total of 18-24 seedlings per tree plot that were planted at a spacing of approximately 70 cm. The same seedling stock used in the planting of the Reclamation Site was used and planted at the same time.

2.2.4 Molecular Identification

Planted seedlings at the Reference Site were harvested in August 2015, after four growing seasons. Whole seedlings were excavated and then the shoot and root systems were separated. Planted seedlings at the Reference Site had previously been sub-sampled in August 2013 (Hupperts 2016). For the current study, all planted seedlings that had remained alive in each tree plot were collected for a total of 223 samples (1-6 planted seedlings × 3 species × 3 tree plots × 3 disturbance severity plots × 3 types of disturbance severity). Note that there was no trembling aspen alive in the 'Control' tree plots and so no data was used for trembling aspen in the 'Control' soil type for further analysis.

To compare EM fungal communities between naturally established trees and planted seedlings on the Reference Site, height and DBH (diameter at breast height) were measured for the mature and established trees with five 50 cm² circular plots per 'Removed', 'Disturbed' and 'Control' areas in August 2015. Within each of the five circular plots, either one established tree were cut down to determine age in the 'Disturbed' and 'Removed' areas or one mature tree in the 'Control' forest were cored to determine age. Jack pine was the dominant tree species across the site. In the 'Control' forest trees were on average 63 years old and 44 cm in DBH, classified as young adults (Carey 1993). In the 'Disturbed' and 'Removed' areas established trees were on average 7 years old and 3.5 cm in DBH, classified as saplings (USDA Forest Service 2016). To compare the fungal communities on these trees and saplings with the planted jack pine seedlings, five established jack pine trees or saplings in each disturbance severity plot were randomly selected and root samples were collected by excavating around the base of the tree, finding lateral roots and following them to carefully collect fine roots. Approximately 300 fine roots

were sampled from each tree or sapling. A total of 45 established jack pine trees and saplings were sampled (5 trees/saplings × 3 disturbance severity plots × 3 types of disturbance severity).

Root samples at the Reclamation Site were taken in a similar fashion to the established jack pine tree and sapling root collection at the Reference Site. For each planted seedling, starting at the base and following lateral roots outward, approximately 300 fine roots were collected. A total of 135 planted seedlings were sampled at the Reclamation Site (5 planted seedlings × 3 species × 3 tree plots × 3 surface soils). All roots collected were placed on damp paper towels, sealed in bags and kept on ice for no more than 48 hours. Roots were then stored at -20°C until further processing.

Root samples were thawed at 2°C and gently washed by hand over a 1.2 mm sieve to remove adhering soil. Cleaned roots were cut into 1 cm fragments, placed in trays filled with water and thoroughly mixed. A subsample of the fragments was placed under a dissection microscope at 100 × magnification to identify the presence of fungi on the root tips, according to absence of root hairs, presence of a hyphae, mantle structure, color and texture (Goodman et al. 1998). To determine the number of root tips to sequence per planted seedling, a total of 20 root tips from three planted seedlings from the 'Control' forest was extracted and Sanger sequenced (see methods below). The 'Control' forest was used as a baseline because it was assumed to have the most diverse fungal community. Results from this initial analysis showed that on average planted seedlings hosted one to three fungal species. Based on these findings for each planted seedling I sampled a total of five ectomycorrhizal root tips for fungal DNA analysis.

Root tips were placed in 96 well plates and DNA template were extracted by adding 10μL of Sigma Extraction Buffer (Sigma Aldrich, St. Louis, Missouri, USA) to each well that each contained one root tip. The buffer and root tips were then incubated at 65°C for 10 minutes,

then 95°C for 10 minutes. After, 30µL of Neutralization Solution B was added. DNA template was then either immediately used for PCR or stored in a -20°C freezer until PCR could be performed.

Extractions were amplified using fungal specific primers ITS1-F (5'-cttggtcatttagaggaagtaa-3') for forward and ITS4 (5'- tcctccgcttattgatatgc -3') for reverse directions (Gardes and Bruns 1993) at 1.0µL of DNA extract, 6.5µL of autoclaved de-ionized water, 12.5µL of EconoTaq PLUS 2X Master Mix (Lucigen, Middleton, Wisconsin, USA), 2.5µL of 10µM ITS1-F and 2.5µL of 10µM ITS4. Specifically the ITS1-F binds on the SSU conservative region and the ITS4 binds on the LSU conservative region, with both primers amplifying the ITS1 and ITS2 region (Gardes and Bruns 1993). Thermal cycling conditions were as follows: initial denaturation of 95°C for 5 minutes followed by 40 cycles of (denaturation at 95°C for 1 minute and 30 seconds, annealing at 55°C for 1 minute and extension at 72°C for one minute and 30 seconds), and a final extension at 72°C for 10 minutes.

All PCR products at 5 µl were visualized on a 1.7% agar run at 100 volts for 30-35 minutes. Only samples that produced clear single bands were used for further analysis. PCR products were cleaned enzymatically by Exo-SAP IT (New England Biolabs, Massachusetts, USA). After purification, bi-directional sequencing was performed with BigDye Terminator v3.1 (Applied Biosystems, Foster City, California, USA) using the ITS1-F/ITS4. Sequence reactions were cleaned using primers EDTA and ethanol and run on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

2.2.5 Bioinformatic Analysis

Sequences were edited with Geneious version 6.1.8 (Kearse et al. 2012). First, ends were trimmed with an error probability limit of 3%. Base pairs with a phred score below 20 were

changed to N. Complementary forward and reverse directions were assembled into contigs using the CAP3 assembler in the BioEdit version 7.2.5 software (Hall 1999). For sequences that did not form contigs, single directions were kept for the final assembly if there were overall less than 2% base pairs labelled as N. The resulting contigs and single direction sequences were then clustered into OTU (operational taxonomic units) using the CAP3 plugin in Geneious with the following settings: ≥ 97% identity, overlap percentage cutoff = 97, maximum overhang percentage length= 60, match score factor =5, clipping range = 6. OTUs were then run through the GenBank database (National Center for Biotechnology Information, Bethesda, Maryland) using BLASTn to identify the best match. Identity was assigned to an OTU if percent identity was ≥ 97 and query coverage was ≥ 80%.

2.2.6 Statistical Analysis

All statistics were run with R version 3.3.1 (R Development Core Team 2016). First, a measure of relative fungal OTU abundance for each planted seedling was taken and then averaged at the surface soil plot or disturbance severity plot level (Appendix III-IV). To determine whether soil type or host identity structured EM fungal communities on planted seedlings, a perMANOVA was run using the *adonis* function in the *vegan* package with 9999 permutations (Oksanen et al. 2016). Soil type included: 'FFM', 'Peat', 'Subsoil', all reconstructed soils sampled from the Reclamation Site and 'Control', 'Disturbed' and 'Removed', all soils sampled from the Reference Site. If no interaction was found between host identity and soil type, a pairwise comparison was made using the *RVAideMemoire* package (Herve 2016). Multivariate homogeneity of dispersion was tested following perMANOVA to check for equal variance within predictor variables (a conservative alpha of 0.01 was used to avoid Type I error (Underwood 1997), soil type p=0.4, host identity p=0.01, interaction p=1.0))

using the *betadisper* function in the *vegan* package (Oksanen et al. 2016). Due to the low p-value found in the *betadisper* test for the host identity, some caution should be taken in interpreting host effect on fungal community composition as some variation could be caused by within group differences. To visualize the structure of the EMF community on the planted seedlings a rank abundance curve was made using the *rank abundance* function in the *BiodiversityR* package (Kindt and Coe 2005). This curve allowed for visualization of the evenness of the EM fungal communities and dominance of OTUs. The curve was visually analyzed for changes in slope, in which fungal OTUs with the highest relative abundance values were considered the most common in colonizing the root tips. This information was then further used to look for differences between the EM fungal communities on the different soil types and host identities. Indicator species analysis was run using the *multipatt* function in the *indicspecies* package (De Caceres and Legendre 2009) to identify strongly responding fungal OTUs found within the different soil types and host identities.

To determine if the EMF community composition on planted jack pine seedlings differed from that on roots of naturally established jack pine trees/saplings, a similar statistical model was used as above. However, in this analysis soil type necessarily was a subset including only 'Control', 'Disturbed' and 'Removed' as these samples were taken from the Reference Site. A measure of relative fungal OTU abundance for each planted seedling or established tree/sapling was taken and then averaged at the disturbance severity plot level (Appendix IV-V). A perMANOVA was run using the *adonis* function in the *vegan* package with 9999 permutations followed by testing multivariate homogeneity of dispersion using the *betadisper* function in the *vegan* package (soil type p=1.0, host type p=0.1) (Oksanen et al. 2016). If no interactions were found between the soil type and host type, a pairwise comparison was made using the

RVAideMemoire package (Herve 2016). To visualize the structure of the EMF community on planted jack pine seedlings compared to roots of established jack pine trees/saplings, a rank abundance curve was made using the *rank abundance* function in the *Biodiversity R* package (Kindt and Coe 2005). Indicator species analysis was run using the *multipatt* function in the *indicspecies* package (De Caceres and Legendre 2009) to identify strongly responding fungal OTUs found within the different soil types and host types.

2.3 Results

2.3.1 Response of ectomycorrhizal fungal communities to disturbance severity, soil type and host identity

Overall the fungal community on the planted seedlings clustered into 66 operational taxonomic units (OTUs) (Table 2-1). Of the 66 OTUs identified on root tips of the planted seedlings, six were most common on the root systems: *Amphinema byssoides*, *Suillus brevipes*, Uncultured fungus 1, Uncultured fungus 2, *Suillus variegatus* and Pezizaceae 1. The other 60 OTUs were found at relatively lower abundances (i.e. OTU abundance ≤ 2) (Figure 2-1).

The EMF community composition differed both among soils that experienced varying extents of disturbance and host identity (Table 2-2). The soil type significantly influenced the host specificity response in that fungal communities differed on each species of seedling depending on what soil type they were planted in. There was a general trend of 'FFM' surface soils to have similar EM fungal communities as the 'Control', 'Disturbed' and 'Removed' soils, and the 'Subsoil' and 'Peat' soils to have similar EM fungal communities (Figure 2-2).

2.3.1.1 Trembling Aspen

Ectomycorrhizal fungal communities were similar on trembling aspen seedlings when growing on 'FFM' surface soil and in the 'Disturbed' and 'Removed' soils. However, those

communities differed from those that developed on the 'Peat' and 'Subsoil' surface soils. Specifically, indicator species analysis revealed that Uncultured fungus 5 was an indicator for EM fungal communities in the 'Disturbed' and 'Removed' soils of the Reference Site, while Uncultured fungus 1 was an indicator for the 'FFM' surface soil at the Reclaimed Site (Table 2-3). Ectomycorrhizal fungal communities on trembling aspen growing in the 'FFM' surface soil and 'Disturbed' soil was colonized by Uncultured fungus 1. However, Uncultured fungus 1 was found at a much higher abundance in the 'FFM' surface soil. The EM fungal communities on trembling aspen in the 'Peat' and 'Subsoil' surface soils differed from the 'FFM' surface soil and the 'Disturbed' soil in that they were colonized by Pezizaceae 1 (Figure 2-3a).

2.3.1.2 Jack Pine

Jack pine seedlings hosted a high abundance of *Suillus brevipes*, *Suillus variegatus* and Pezizaceae 1 and generally communities were similar between the 'Control' and 'Disturbed' soils of the Reference Site, and 'FFM' surface soil differed from the 'Removed' soil of the Reference Site and the 'Peat' and 'Subsoil' surface soils of the Reclamation Site. Specifically, indicator species analysis revealed that Uncultured fungus 2 was an indicator for jack pine growing in 'Disturbed' soil and 'FFM' surface soil, while *Suillus brevipes* was an indicator for soil types 'Removed', 'Peat' and 'Subsoil' (Table 2-3). Also, *Piloderma* 2 was an indicator for jack pine growing in the 'Control' soil and Pezizaceae 1 was an indicator for the 'Subsoil' surface soil. Accordingly, there were similarities between EM fungal communities in the 'Disturbed' soil and 'FFM' surface soil that had a high relative abundance of Uncultured fungus 2, while jack pine in the 'Removed', 'Peat' and 'Subsoil' soil types were similar in the high abundance of *Suillus brevipes*. Also, the jack pine in the 'Subsoil' surface soil had a high abundance of Pezizaceae 1 (Figure 2-3b).

2.3.1.3 White Spruce

White spruce seedlings hosted a high abundance of *Amphinema byssoides* and Pezizaceae 1 and generally communities showed similarities among the 'Control', 'Disturbed' and 'Removed' soils of the Reference Site and the 'FFM' and 'Peat' surface soils of the Reclamation Site and differed from the 'Subsoil' surface soil. Specifically, indicator species analysis results showed Uncultured fungus 5 to be an indicator for 'Control', 'Disturbed' and 'Removed' soils (Table 2-3). White spruce growing in the 'Control', 'Disturbed' and 'Removed' soils and the 'FFM' and 'Peat' surface soils all had a high abundance of *Amphinema byssoides.* In contrast, white spruce growing in the 'Subsoil' surface soil was heavily colonized by Pezizaceae 1 compared to all the other soil types (Figure 2-3c).

2.3.2 Comparison of ectomycorrhizal fungal communities on planted seedlings versus naturally established trees and saplings of jack pine

Overall the fungal communities on roots of planted jack pine seedlings and established jack pine trees and saplings clustered into 35 OTUs (Table 2-1). Of the 35 OTUs identified on the jack pine seedlings and trees/saplings, there were four that were most common and found in the highest abundance: *Suillus variegatus*, *Russula decolorans*, *Suillus brevipes* and *Cenococcum geophilum*. The other 31 OTUs were found at relatively lower abundances (i.e. OTU abundance ≤ 1.5) (Figure 2-4).

The differences in the EMF community composition of jack pine on the Reference Site were mainly driven by soil type and to a lesser extent the host type (seedling versus tree/sapling) (Table 2-4). Jack pine, regardless of host type, had significantly different communities in 'Removed' soils than from 'Control' and 'Disturbed soil types (Table 2-5). Specifically, indicator analysis revealed the four most abundant fungal OTUs were indicators for different soil

types with *Suillus variegatus* and *Russula decolorans* as indicators for 'Control' and 'Disturbed' soil types and *Suillus brevipes* and *Cenococcum geophilum* as indicators for 'Removed' soils (Table 2-6). Also, *Russula decolorans* was also an indicator for established trees and saplings, suggesting a preference for older hosts. Accordingly, the 'Control' and 'Disturbed' soil types had similar EM fungal communities with a high abundance of *Suillus variegatus* and *Russula decolorans*, while jack pine in the 'Removed' soils were heavily colonized by *Suillus brevipes* and *Cenococcum geophilum* (Figure 2-5b). Established trees and saplings hosted a higher abundance of the four more common OTUs compared to the planted seedlings that also hosted many of the less frequent fungi (Figure 2-5a).

2.4 Discussion

Ectomycorrhizal fungal communities were assayed with three species of tree seedlings across soils varying in disturbance severity to determine the importance of soil type and host identity on the composition of EM fungal communities. Some of these soils were reconstructed as part of the forest reclamation process on an open-pit mine site. The other assayed soils differed in the extent of surficial soil and forest canopy disturbance. The EMF community diverged depending on the different disturbances and associated soil types, and host identities.

2.4.1 Response of ectomycorrhizal fungal communities to disturbance severity, soil type and host identity

After four years since planting of the seedlings, the EMF community retained species already identified in previous studies (Hankin et al 2015, Hupperts 2016) (Table 2-1), but increased from 27 EMF in the second growing season to 66 in the fourth growing season. Additional fungal species found in the fourth growing season included *Russula* and *Cortinarius* species that have been described as mid- to late-successional species (Cline et al. 2005, Douglas

et al. 2005, Ashkannejhad and Horton 2006, Leduc et al. 2013, Huusko et al. 2015). The retention of resistant or early-successional species with a slow increase in mid to late successional species is consistent with previous studies on the recovery of EM fungal communities after mining, fire and harvesting disturbance events (Visser 1995, Gebhardt et al. 2007, Twieg, Durall and Simard 2007, Kipfer et al. 2011, Leduc et al. 2013, Huang et al. 2015).

The EM fungal communities on the planted seedlings responded to the soil types varying in disturbance severity in different ways. Similarly, soil from an undisturbed forests versus a forest that had been clearcut significantly affected EMF community composition, but magnitude of the effect varied with host tree species (Ding et al. 2011). The trembling aspen seedlings in the intact forest did not survive for the analysis in the fourth growing season, likely the conditions were too shaded (Landhausser and Lieffers 2001). Ectomycorrhizal fungal communities in the 'Disturbed' and 'Removed' soils were more similar compared to the 'Peat' and 'Subsoil' soils on the Reclamation Site. Comparatively, it was found that several years after tree harvest, trembling aspen roots still hosted many dominant EMF that were present before the disturbance (Visser, Maynard and Danielson 1998). Despite hosting a high abundance of fungi found in the first growing season, the fungal community associated with trembling aspen in the 'FFM' soil more closely resembled the fungal community in the harvest disturbances compared to the other two surface soils on the Reclamation Site. This may imply that the 'FFM' surface soil can provide fungal inoculum that is also found on less severe disturbed site with similar soil properties. Across all soil types, trembling aspen had lower abundances of the overall most common fungi on their root tips compared to jack pine and white spruce seedlings. One possible explanation is that EM fungal communities differ significantly between broadleaf deciduous and coniferous species (Durall et al. 2006, Ding et al. 2011, Huang et al. 2014). Differences between fungal

communities on coniferous and deciduous trees could derive from the amount of carbon their hosts allocate to support symbionts. For example, higher C^{13}concentrations in EMF fruiting bodies was found to be associated with coniferous tree species compared to deciduous (Hogberg et al. 1999, Taylor et al. 2003).

The variation in EM fungal communities associate with planted jack pine seedlings in the 'FFM' soil compared to the 'Peat' and 'Subsoil' soils is not unexpected as several other studies on jack pine and Masson pine have shown that EM fungal communities varied among different substrate types used in mine reclamation (Bois et al. 2005, Huang et al. 2012, Huang et al. 2015). The difference in EMF community composition on jack pine seedlings in the 'Removed' soil compared to the 'Disturbed' and 'Control' soil is must likely due to the combination of both tree (Byrd et al. 2000) and forest floor removal (Stendell et al. 1999, Hartmann et al. 2012). Similar to trembling aspen seedlings, the EM fungal communities on jack pine in the 'FFM' soil were more similar to the 'Disturbed' and 'Control' soils compared to the 'Peat' and 'Subsoil' soils, implying the 'FFM' soil can host fungi similar to less severe disturbed sites with similar soil properties. Two of the EMF species, *Suillus variegatus* and *Suillus brevipes,* were found abundantly on the jack pine seedlings, which corresponds with other studies that have reported these fungal species presences after harvest and fire disturbances (Visser 1995, Jonsson et al. 1999, Hartmann et al. 2012, Leduc et al. 2013)

Compared to both trembling aspen and jack pine, white spruce seedlings hosted a high abundance of *Amphinema byssoides,* a ruderal species (Kernaghan, Sigler and Khasa 2003a), which, in the past, has been found in high abundance on white spruce (Danielson 1991, Lazaruk et al. 2005, Gagne et al. 2006). Interestingly, white spruce seedlings hosted a higher abundance of *Amphinema byssoides* regardless if they were planted on the 'FFM' and 'Peat' soils or the

'Control', 'Disturbed' and 'Removed' soils. It is possible that the white spruce seedlings may be slower at responding to the effect the different disturbances have on the fungal communities in the soils. In comparison, Danielson (1991) found that after four years *Amphinema byssoides* was the dominant EMF species on white spruce grown in oil sands tailings, but eventually decreased in abundance after seven years as other EMF species colonized the roots. In contrast, there is also some evidence suggesting that EM fungal communities change on *Picea* species after various types of harvesting practices compared to undisturbed forests (Lazaruk et al. 2005, Menkis et al. 2010, Walker and Jones 2013, Huusko et al. 2015, Walker et al. 2016). All three species of planted seedlings hosted some amount of Pezizaceae 1 in the 'Subsoil' surface soil suggesting that this species can tolerate highly disturbed mineral soil environments and has been found at high abundances in an earlier study on forest reclamation sites (Bois et al. 2005).

Despite differences in the EMF community being explained by soil type, host identity and their interaction, there was still a large amount of variation left unexplained (43%) (Table 2-2). One unaccounted factor was the characteristics of the different soils (e.g. nutrient status, pH, organic matter content), which has been shown to influence EMF community composition (Douglas et al. 2005, Hartmann et al. 2012, Leduc et al. 2013, Huang et al. 2014, Hawkins et al. 2015, Sun et al. 2015). Another factor that was not measured was potential dispersal limitation. Ectomycorrhizal fungi vary widely in the distance they can travel, meaning changes with community composition over time will depend on nearby sources of other inoculum (Peay et al. 2012). Moreover, the planted seedlings on the Reference Site could potentially differ from those on the Reclaimed Site by access to more sources of EMF inoculum via the nearby intact forest. The maximum distance to undisturbed forest cover at the Reference Site was ~ 200 m and at the Reclamation Site, ~ 5 km.

2.4.2 Comparison of ectomycorrhizal fungal communities on planted seedlings versus naturally established trees and saplings of jack pine

The EMF community composition varied among the different disturbance severities in that the area that had both trees and forest floor removed had different EM fungal communities compared to the area with trees removed and the intact forest. This corresponds with other research showing removal of the organic horizon can cause major changes to the EMF community composition (Hartmann et al. 2012, Barker et al. 2013). Specifically, differences in the EMF community composition among disturbance severities could be explained by the 'Removed' soils having a higher abundance of *Cenococcum geophilum*, which is an ubiquitous species that does well on disturbed sites (Byrd et al. 2000, Douglas et al. 2005, Heinonsalo et al. 2007), while the 'Control' and 'Disturbed' soils had a higher abundance of *Russula decolorans*, a later successional EMF species. The EMF community composition was also influenced by host type. The naturally established trees and saplings hosted a species of *Cortinarius* and *Russula* not found on the planted seedlings in addition to having a higher abundance of *Russula decolorans*. The planted seedlings hosted a larger abundance of the less common EMF species compared to the established trees and saplings. However, despite differences in the EMF community composition between host types, my initial prediction was partially supported in that both the established trees and planted seedlings hosted high abundances of EMF species common to both host types. The similarities in the communities between the seedlings and established trees lends supports to the theory that established trees can act as inoculum sources able to colonize planted seedlings (Kranabetter 1999, Cline et al. 2005, Teste et al. 2009), and in consequence distance to an intact forest can influence the EMF community composition of planted seedlings (Durall et al. 1999, Hagerman et al. 1999, Outerbridge and Trofymow 2004, Dickie and Reich 2005, Luoma et

al. 2006, Jones et al. 2008, Kranabetter et al. 2013). In other words, trees left behind after a disturbance event can potentially act as ‘refuge plants’ and provide a diverse source of fungal inoculum for regenerating or planted seedlings (Jones et al. 2003) and possibly form advantageous common mycorrhizal networks and share resources (van der Heijden and Horton 2009, Courty et al. 2010a).

Tables

Table 2-1. Operational taxonomic units from the best BLAST in the NCBI database and the corresponding UNITE species hypothesis (SH), assembled from quality filtered sequences from amplified fungal rDNA. Two study sites in Alberta, Canada, a Reclamation Site with reconstructed soils and a Reference Site with soils differing in forest disturbance were assayed for ectomycorrhizal fungi with planted seedlings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) grown in 2012-2015. Also, ectomycorrhizal fungal communities were assayed on naturally established *Pinus banksiana* trees and saplings at the Reference Site in August 2016. *OTU identified in Genbank from 2012 survey (Hankin et al 2015) **OTU identified to same accession number as 2013 survey (Hupperts 2016)

Best Match	Blast ID	Query Length	Max Score	Query Cover	% ID	UNITE SH	UNITE Accession	Planted Seedling	Established Tree/Sapling
Uncultured fungus 1*	KJ938039	572	1057	99%	100%	*Cenococcum*	SH199612	X	X
Uncultured fungus 2	KP889629	665	1229	99%	100%	Fungi	SH203891	X	X
Uncultured fungus 3	GU566255	579	1042	99%	99%	Fungi	SH20532	X	
Tomentella	JX630694	654	1175	99%	99%	Thelephoraceae	SH184538	X	
Phellodon	KF879488	618	1005	87%	97%	Bankera	SH007662	X	
Uncultured fungus 4	AB669512	596	1081	100%	99%	Fungi	SH214265	X	
*Suillus variegatus***	JQ711926	728	1293	100%	99%	*Suillus variegatus*	SH176741	X	X
Lactarius 1	FJ769532	690	1264	93%	99%	Russulaceae	SH220161	X	
Uncultured fungus 5	HM164652	871	1589	98%	99%	*Meliniomyces bicolor*	SH181080	X	
Russula 1	GU143030	691	1190	99%	98%	*Russula*	SH219258	X	
Cortinarius 1	KC840652	532	970	100%	99%	Cortinariaceae	SH197813	X	
Thelephora terrestris	HM189958	693	1280	99%	100%	*Thelephora terrestris*	SH184510	X	X
Uncultured fungus 6	LC013889	591	1075	90%	99%	Fungi	SH189869	X	
Wilcoxina 1	GU452514	634	1155	99%	99%	*Wilcoxina rehmii*	SH211927	X	
Uncultured fungus 7	AJ633601	652	1164	90%	98%	*Tricholoma*	SH219347	X	
Tricholoma 1	AF349688	715	1290	99%	99%	*Tricholoma*	SH190415	X	X
Agariomycetes 1	FJ553633	562	1026	96%	99%	Hygrophorus	SH202867	X	
Tricholoma flavovirens	AF458449	721	1327	99%	99%	*Tricholoma flavovirens*	SH220594	X	X
Amphinema byssoides	KP814511	625	1155	98%	100%	*Amphinema*	SH197944	X	

*Russula decolorans***	FJ845432	721	1325	99%	99%	*Russula decolorans*	SH219855	X	X
Cenococcum geophilum	JX630462	989	1748	99%	98%	*Cenococcum geophilum*	SH199612	X	X
Piloderma 1	JQ711935	667	1229	99%	99%	*Piloderma*	SH203892	X	
Cortinarius murinascens	KP165573	604	1048	99%	98%	*Cortinarius murinascens*	SH188592	X	
Thelephoraceae1	KP403045	669	1216	100%	99%	Thelephoraceae	SH2189362	X	
Piloderma 2	JQ711984	572	1022	100%	99%	*Piloderma*	SH212907	X	
Lactarius 2	EF685048	485	881	99%	99%	*Lactarius*	SH220112	X	
Uncultured fungus 8	KP889633	604	994	100%	97%	Fungi	SH188469	X	
Uncultured fungus 9*	KJ938040	690	1271	100%	99%	Tuber	SH188859	X	
Thelephoraceae 2	JN704829	678	1186	99%	98%	Thelephoraceae	SH189381	X	
*Suillus brevipes***	FJ845440	716	1312	100%	99%	*Suillus brevipes*	SH176743	X	X
Russula katarinae	KP966377	719	1290	99%	99%	*Russula*	SH190324	X	
Inocybe jacobi	HQ604812	601	1110	97%	100%	*Inocybe jacobi*	SH211829	X	
Pezizaceae 1	JN704828	592	1068	98%	99%	Pustularia	SH222144	X	
Uncultured fungus 10*	KJ938035	620	1146	99%	100%	Fungi	SH194156	X	
Uncultured fungus 11*	KJ938030	633	1140	99%	99%	Fungi	SH197943	X	
Uncultured fungus 12*	KJ938032	710	1291	99%	99%	*Hebeloma*	SH215994	X	
Wilcoxina 2**	EU668262	635	1168	99%	99%	Pyronemataceae	SH194158	X	
Tylospora	AB456674	639	1107	99%	98%	Athelieaceae	SH193510	X	X
Uncultured fungus 13	KP889652	724	1282	99%	99%	Fungi	SH218421	X	
Helotiales	FJ475771	574	1029	99%	99%	Helotiales	SH2014986	X	
Wilcoxina 3	DQ320129	567	983	99%	98%	*Wilcoxina*	SH194157	X	

Pezizales 1**	JN704819	597	1088	100%	99%	Pezizales	SH212010	X	
Lactarius scrobiculatus	JF908281	742	1284	100%	98%	*Lactarius scrobiculatus*	SH220109	X	
Thelephoraceae 3	U83467	687	1219	97%	99%	Thelephoraceae	SH177833	X	X
Uncultured fungus 14	KM596883	700	1242	98%	98%	Fungi	SH221083	X	
Uncultured fungus 15	HM164660	813	1395	97%	98%	*Cadophora finlandica*	SH214265	X	
Uncultured fungus 16	EF433987	610	1109	98%	99%	*Amphinema*	SH197944	X	
Thelephora 1	KT334743	653	1170	96%	99%	Thelephoraceae	SH184510	X	
Suillus 1	JQ711787	719	1284	98%	99%	*Suillus*	SH76743	X	
Hebeloma	KX355262	712	1240	100%	97%	*Hebeloma*	SH215995	X	
Helotiaceae 1	KF428231	510	942	90%	100%	Helotiaceae	SH214265	X	
Tomentellopsis	KP403093	655	1199	99%	99%	Thelephoraceae	SH184845	X	
Piloderma olivaceum	KP814367	609	1064	98%	98%	*Piloderma olivaceum*	SH203894	X	
Russula 2	FJ554452	675	1230	97%	99%	Russulaceae	SH186202	X	
Uncultured fungus 17	KF617260	585	1053	97%	99%	Athelieaceae	SH197944	X	
*Russula lacata***	HQ604844	696	1254	100%	99%	*Russula lacata*	SH218421	X	
Uncultured fungus 18	HM164669	863	1528	97%	98%	*Meliniomyces bicolor*	SH214265	X	
Thelephora 2	KF498575	666	1216	94%	99%	Thelephoraceae	SH184510	X	
Meliniomyces bicolor	HQ157880	504	902	89%	99%	*Meliniomyces bicolor*	SH214265	X	
Uncultured fungus 19	JQ975978	698	1203	98%	98%	Russulaceae	SH219861	X	
Uncultured fungus 20*	KJ938033	735	1334	99%	99%	*Rhizopogon pseudoroseolus*	SH221091	X	
Suillus 2	FJ554247	663	1162	97%	98%	Suillaceae	SH176743	X	
Cortinarius 2	JQ711887	703	1266	99%	99%	*Cortinarius*	SH222334	X	

Pyronemataceae 1	KR019795	610	1074	98%	98%	Pyronemataceae	SH194157	X		
Uncultured fungus 21	JF300662	642	1171	100%	99%	Fungi	SH196824	X		
Inocybe lacera	HQ604430	697	1238	98%	98%	*Inocybe lacera*	SH201230	X		
Cortinarius 3	EU597028	621	1099	98%	98%	*Cortinarius*	SH188544			X
Russula odorata	JQ711900	655	1142	100%	98%	*Russula odorata*	SH219856			X

Table 2-2. Results of permutational multivariate analysis of variance testing the effects of soil type and host identity and their interaction on ectomycorrhizal community composition (n=3). Soil type includes three reconstructed soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining) and three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)). Both the Reference and Reclamation Site were located in northern Alberta, Canada and soil were assayed for fungi by planted seedlings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) grown in 2012-2015.

	DF	**Sum Sq.**	**Mean Square**	***F***	**R^2**	***P-value***
Soil type	5	4.9626	0.99252	3.8012	0.24131	**0.001**
Host identity	2	3.4820	1.74098	6.6677	0.16931	**0.001**
Soil type × Host identity	9	3.2432	0.36036	1.3801	0.15770	**0.008**
Residuals	34	8.8777	0.26111		0.43168	
Total	50	20.5654			1.00000	

DF: degrees of freedom, Sum Sq: sum of squares, note DF for interaction term is represented as 9 owing to no data for the *Populus tremuloides* in the 'Control' intact forest

Table 2-3. Indicator species analysis (α = 0.05) using operational taxonomic units of ectomycorrhizal fungi from roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) collected in 2015 grown in different soil types. Soil type includes three reconstructed soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining) and three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada.

Host identity	**Soil type**	**OTU**	**Stat**	***P-value***
Trembling aspen	FFM	Uncultured fungus 1	0.923	0.0218
	Disturbed, Removed	Uncultured fungus 5	0.913	0.0209
Jack pine	Control	*Piloderma* 2	1.00	0.0071
	Subsoil	Pezizaceae 1	1.00	0.0077
	Disturbed, FFM	Uncultured fungus 2	0.894	0.0256
	Removed, Peat, Subsoil	*Suillus brevipes*	0.977	<0.0001
White spruce	Control, Disturbed, Removed	Uncultured fungus 5	0.882	0.0226

Table 2-4. Results of permutational multivariate analysis of variance testing the effects of soil type and host type and their interaction on ectomycorrhizal community composition (n=3). Soil type includes three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)) located in northern Alberta, Canada. Host type refers to *Pinus banksiana* seedlings that were planted on the Reference Site and collected in 2015 or naturally established *Pinus banksiana* trees and saplings on the Reference Site collected in 2016.

	DF	**Sum Sq.**	**Mean Square**	***F***	**R^2**	***P-value***
Soil type	2	2.0235	1.01174	4.5606	0.34066	**0.001**
Host type	1	0.5237	0.52366	2.3605	0.08816	**0.025**
Soil type × Host type	2	0.7306	0.36531	1.6467	0.12300	0.073
Residuals	12	2.6621	0.22184		0.44818	
Total	17	5.9399			1.00000	

DF: degrees of freedom, Sum Sq: sum of squares

Table 2-5. Pairwise comparison for the perMANOVA testing differences in the composition of ectomycorrhizal fungal communities found on planted *Pinus banksiana* seedlings collected in 2015 and naturally established *Pinus banksiana* trees and saplings collected in 2016 from a Reference Site that includes three soils which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)) located in northern Alberta, Canada.

	Control	Disturbed
Disturbed	0.7621	
Removed	**0.0062**	**0.0062**

Values in table are p-values (α=0.05)

Table 2-6. Indicator species analysis (α=0.05) using operational taxonomic units of ectomycorrhizal fungi found on the roots of planted *Pinus banksiana* seedlings collected in 2015 and naturally established *Pinus banksiana* trees and saplings collected in 2016 from a Reference Site that includes three soils which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)) located in northern Alberta, Canada.

	OTU	**Stat**	***P-value***
Soil type			
Removed	*Suillus brevipes*	0.846	0.0075
	Cenococcum geophilum	0.816	0.0143
Control, Disturbed	*Suillus variegatus*	0.943	0.0034
	Russula decolorans	0.816	0.0392
Host type			
Established Tree/Sapling	*Russula decolorans*	0.755	0.0194

Figures

Figure 2-1. Rank abundance curve representing all fungal operational taxonomic units (OTU) ranked from highest to lowest abundance on roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) collected in 2015 grown in different soil types. Soil type includes three reconstructed soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining) and three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged at the surface soil plot or disturbance severity plot level. A natural break point for abundance was visually accessed by the slope of the curve; OTU left of the line were considered the most common fungi. *OTU identified in Genbank from 2012 survey (Hankin et al 2015) **OTU identified to same accession number as 2013 survey (Hupperts 2016)

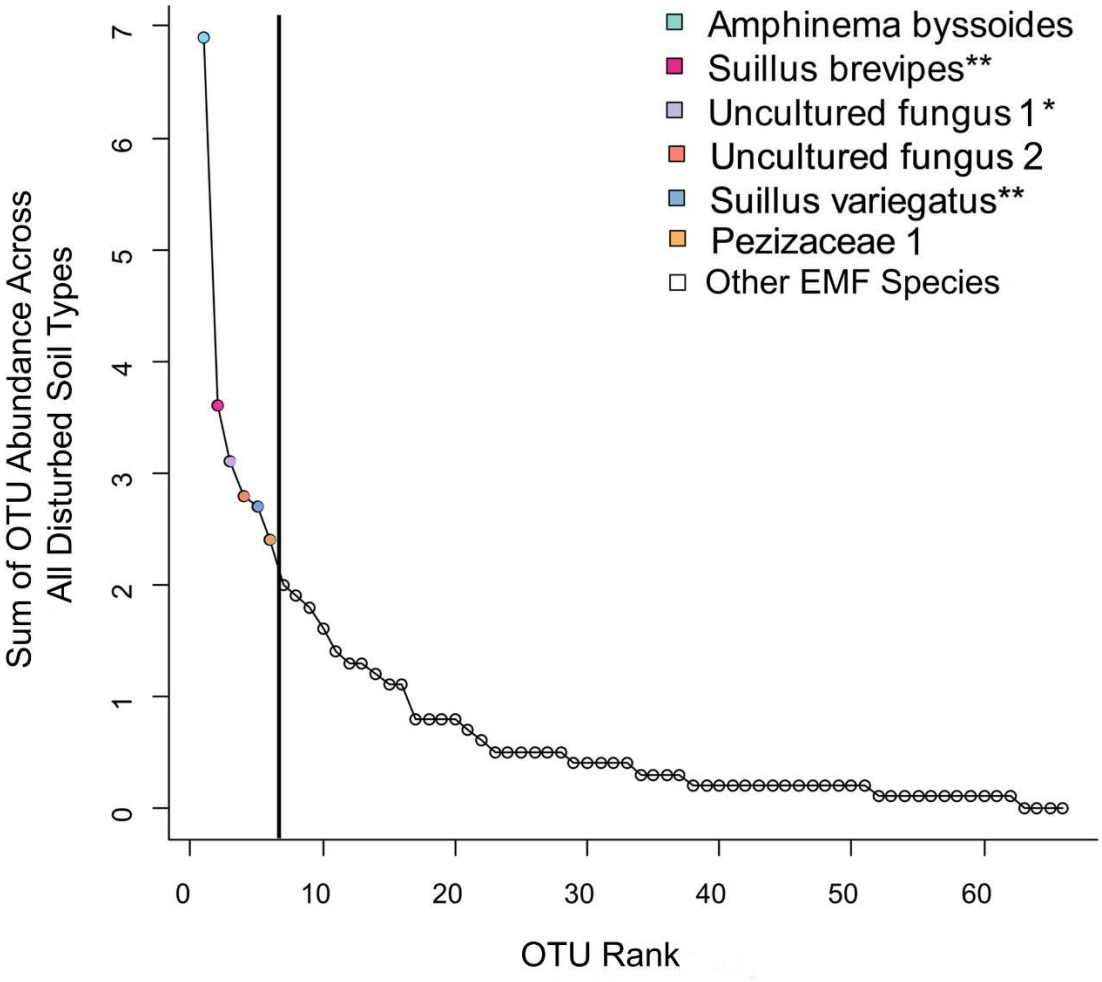

Amphinema byssoides
Suillus brevipes**
Uncultured fungus 1*
Uncultured fungus 2
Suillus variegatus**
Pezizaceae 1
Other EMF Species
Sum of OTU Abundance Across
All Disturbed Soil Types
0
1
2
3
4
5
6
7
0
10
20
30
40
50
60
OTU Rank

Figure 2-2. Principle coordinate analysis (PCoA) ordination of operational taxonomic units (OTU) of relative abundance of ectomycorrhizal fungi colonizing the roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) collected in 2015 grown in different soil types. Soil type includes three reconstructed soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining) and three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged at the surface soil plot or disturbance severity plot level. Each point on the ordination represents the centroid of the ectomycorrhizal fungal community on that species of planted seedling in each soil type. Points closer together signify more similarities in the fungal community composition. The bars represent standard error of each centroid point (n=3).

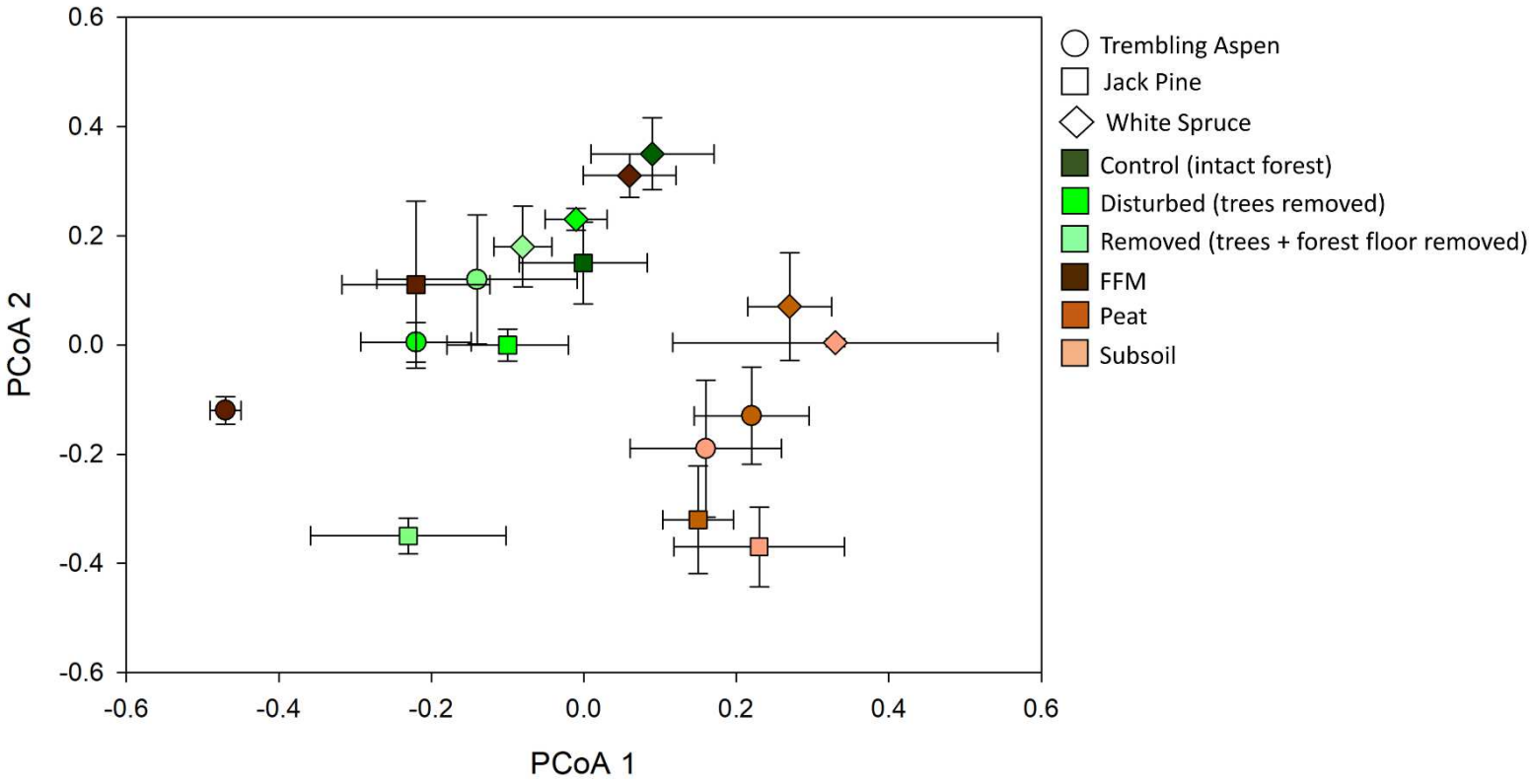
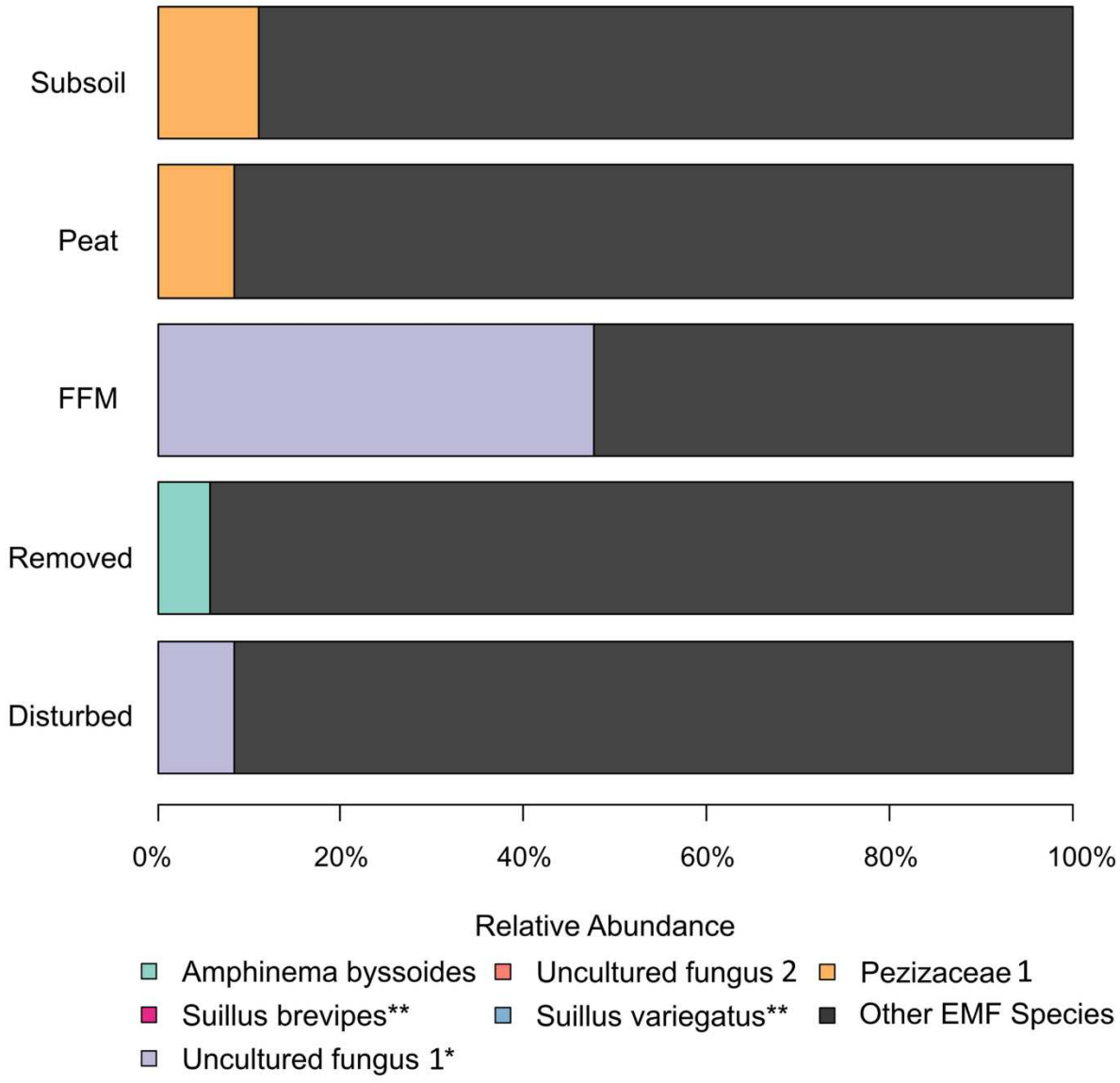
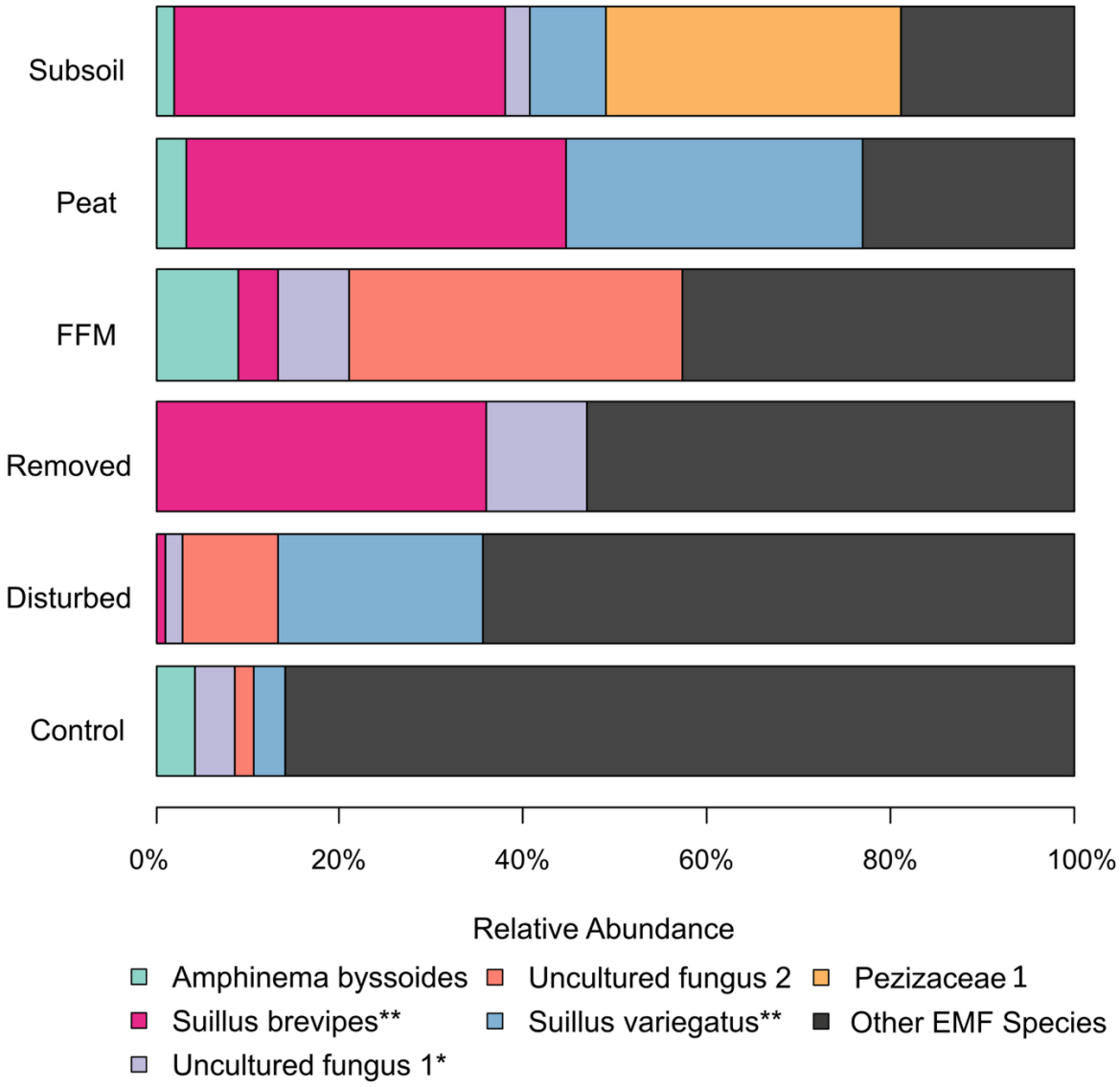

0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
PCoA 2
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
PCoA 1
Trembling Aspen
Jack Pine
White Spruce
Control (intact forest)
Disturbed (trees removed)
Removed (trees + forest floor removed)
FFM
Peat
Subsoil

Figure 2-3. Relative abundance of operational taxonomic units (OTU) of ectomycorrhizal fungi colonizing the roots of (a) *Populus tremuloides* (trembling aspen), (b) *Pinus banksiana* (jack pine) or (c) *Picea glauca* (white spruce) grown from 2012-2015 on three reconstructed soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining) and three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged at the surface soil plot or disturbance severity plot level. The common ectomycorrhizal fungi are represented by colored fills in the figures. Fungi considered to be the most common were chosen by a rank abundance curve (Figure 2-1). All other OTU not considered common are represented by a gray fill in the figures. *OTU identified in Genbank from 2012 survey (Hankin et al 2015) **OTU identified to same accession number as 2013 survey (Hupperts 2016)

(a)


Subsoil
Peat
FFM
Removed
Disturbed
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides
Uncultured fungus 2
Pezizaceae 1
Suillus brevipes**
Suillus variegatus**
Other EMF Species
Uncultured fungus 1*

(b)


Subsoil
Peat
FFM
Removed
Disturbed
Control
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides
Uncultured fungus 2
Pezizaceae 1
Suillus brevipes**
Suillus variegatus**
Other EMF Species
Uncultured fungus 1*

(c)

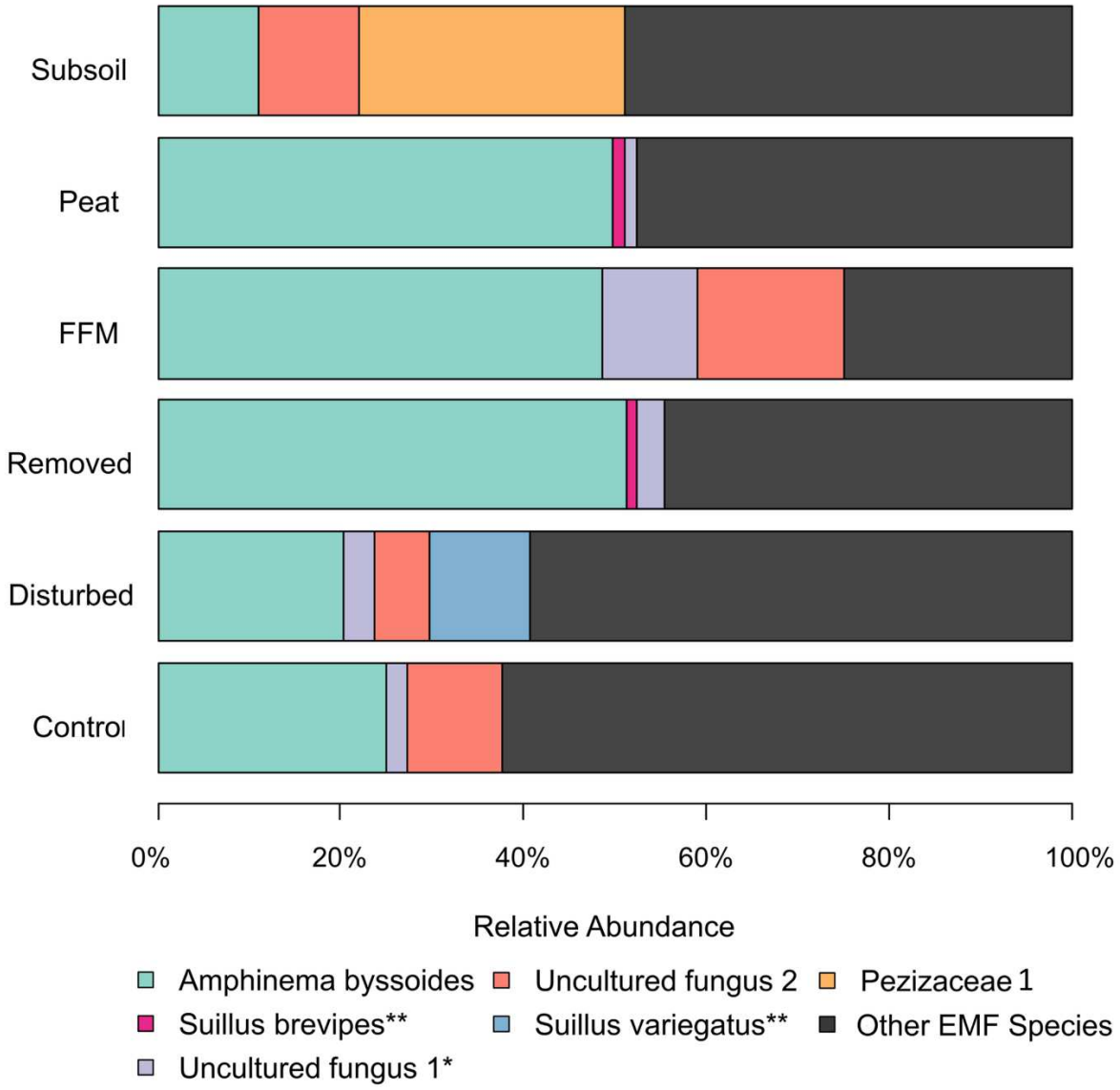

Subsoil
Peat
FFM
Removed
Disturbed
Control
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides
Uncultured fungus 2
Pezizaceae 1
Suillus brevipes**
Suillus variegatus**
Other EMF Species
Uncultured fungus 1*

Figure 2-4. Rank abundance curve representing all fungal operational taxonomic units (OTU) ranked from highest to lowest relative abundance on planted *Pinus banksiana* seedlings collected in 2015 and roots from naturally established *Pinus banksiana* trees/saplings collected in 2016 on three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling or tree/sapling and then averaged at the disturbance severity plot level. A natural break point for abundance was visually accessed by the slope of the curve; OTU left of the line were considered the most common fungi. **OTU identified to same accession number as 2013 survey (Hupperts 2016)

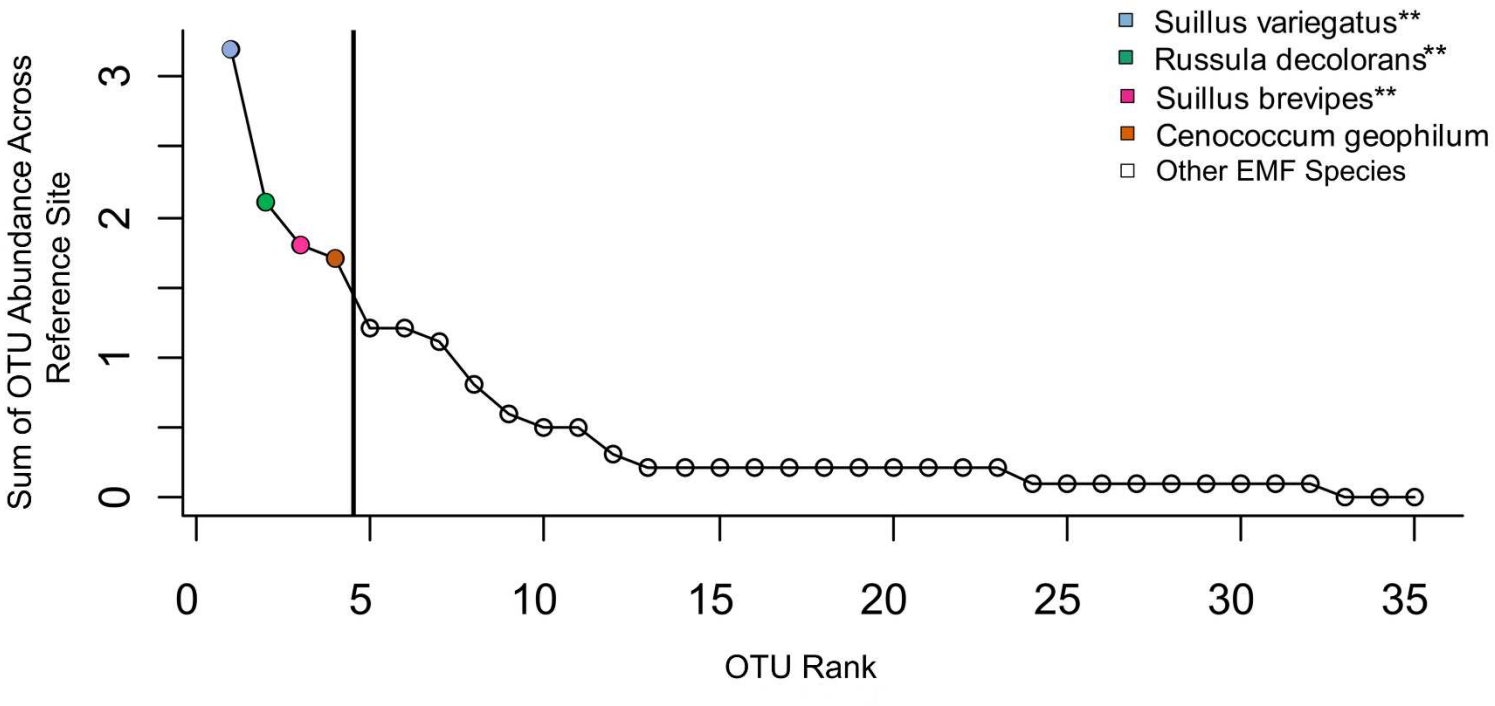
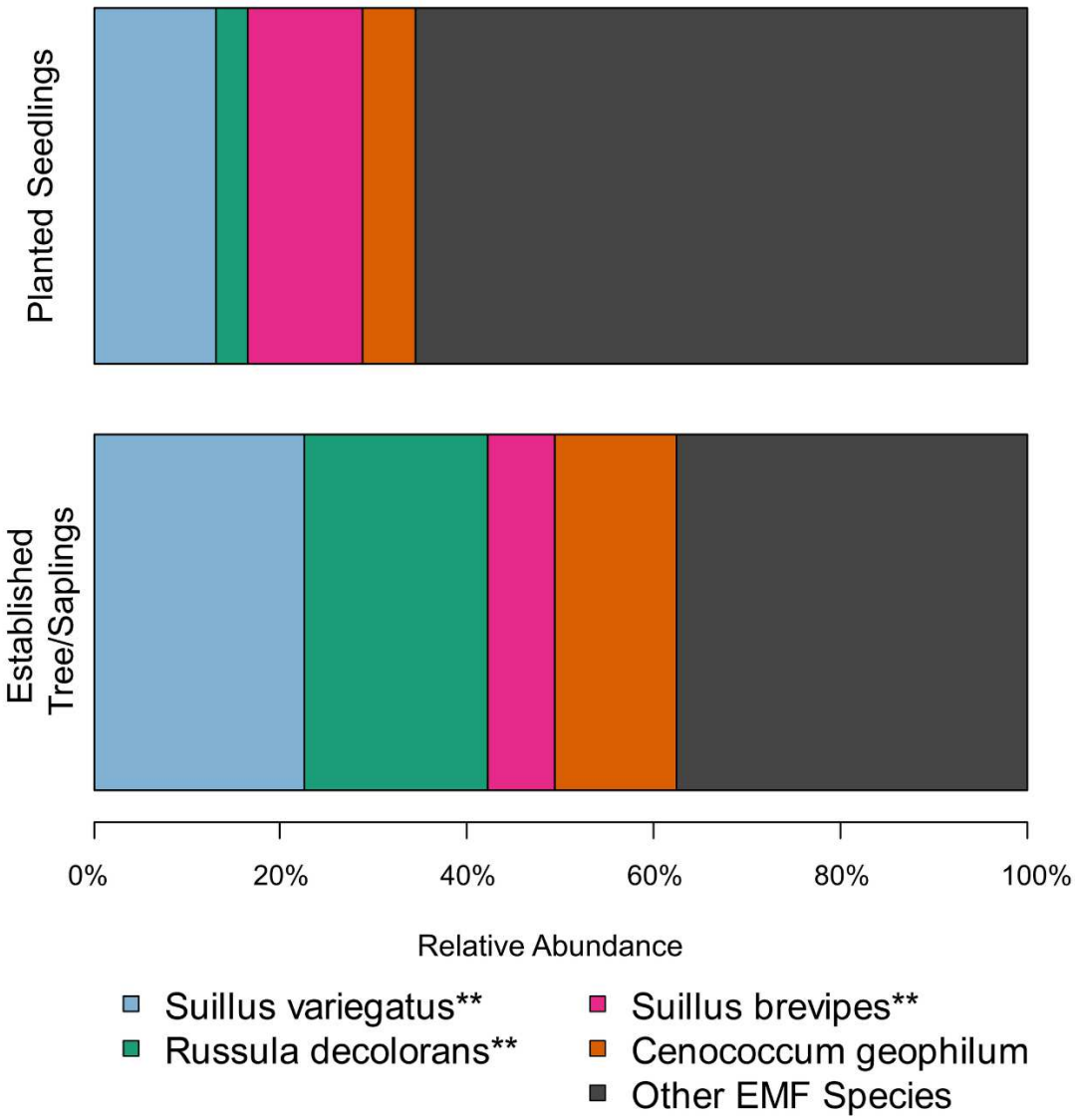
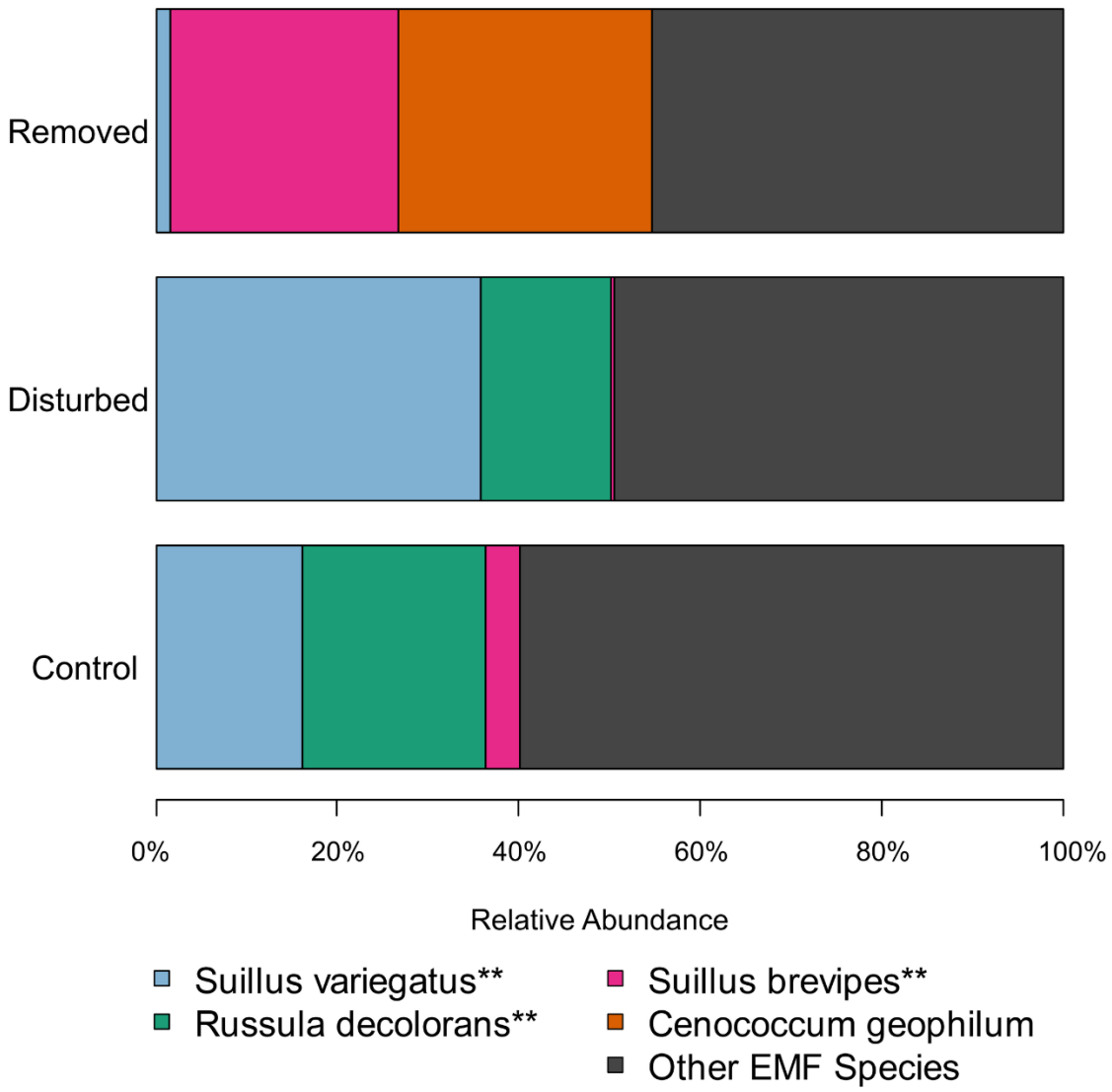


Figure 2-5. Relative abundance of operational taxonomic units (OTU) of ectomycorrhizal fungi assayed from roots of planted *Pinus banksiana* seedlings collected in 2015 and roots from naturally established *Pinus banksiana* trees/saplings collected in 2016 on three soils located in a Reference Site, which represented *Pinus banksiana* forest disturbed to varying extents (Control (intact forest), Disturbed (trees removed) and Removed (trees and forest floor removed)), located in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling or tree/sapling and then averaged at the disturbance severity plot level. Figure (a) represents differences in EMF relative abundance among the different soil types and (b) represents the differences in EMF relative abundance between planted seedlings versus established trees/saplings. The common ectomycorrhizal fungi are represented by colored fills in the figures. Fungi considered to be the most common were chosen by a rank curve (Figure 2-4). All other OTUs not considered common are represented by a gray fill in the figures. **OTU identified to same accession number as 2013 survey (Hupperts 2016)

(a)


Planted Seedlings
Established Tree/Saplings
0%
20%
40%
60%
80%
100%
Relative Abundance
Suillus variegatus**
Russula decolorans**
Suillus brevipes**
Cenococcum geophilum
Other EMF Species

(b)


Removed
Disturbed
Control
0%
20%
40%
60%
80%
100%
Relative Abundance
Suillus variegatus**
Suillus brevipes**
Russula decolorans**
Cenococcum geophilum
Other EMF Species

Chapter 3: Additive versus synergistic effects of planting a mix of tree species on the restoration of ectomycorrhizal communities in the boreal forest

3.1 Introduction

Ectomycorrhizal fungi (EMF) form symbiotic relationships with approximately 8000 plant species worldwide (Taylor and Alexander 2005). The symbiosis between EMF and trees in boreal forests is of particular importance because of the low nutrient availability in these ecosystems (van der Heijden, Bardgett and van Straalen 2008). Ectomycorrhizal fungi can access organic nutrients in the litter and humus of soils and trade these resources for photosynthetically derived carbon from their tree hosts (Frank 2005, Smith and Read 2008). Ectomycorrhizal fungi are functionally diverse and previous studies show that a rich EMF community can maintain ecosystem function (Courty et al. 2010a, Hawkins et al. 2015). For example, high EM fungal species richness can lead to increased plant productivity in low fertility substrates (Jonsson et al. 2001) and can also increase root biomass and phosphorus uptake (Baxter and Dighton 2001). Restoring ecosystem function is a primary goal of assisting in the recovery of degraded ecosystems, therefore, understanding factors underlying EMF community composition and richness are necessary to address this goal.

Soils and hosts have received the most attention in research on important factors affecting EM fungal communities. Soils, which are often correlated with aboveground tree composition, vary in physical, chemical and biological properties with direct and indirect influences on EM fungal communities (Bahram, Peay and Tedersoo 2015). The boreal forest characteristically has soils with a thin litter and organic horizon overlaying mineral horizons (Larson 1980). Accordingly, EM fungal communities and richness vary between the organic and mineral horizons (Rosling et al. 2003, Courty et al. 2008). Higher levels of EMF richness are associated

with the litter and organic horizon (Stendell et al. 1999, Jones et al. 2003, Hartmann et al. 2012). One reason for the differences in EMF composition between horizons is the difference in available nutrients, such as N and P, which have been shown to influence EMF composition (Bois et al. 2005, Leduc et al. 2013, Huang et al. 2014). Aboveground tree composition directly influences nutrient status in the organic horizon by differences in litter input (e.g. needles versus leaves), which can in turn influence EMF composition (Conn and Dighton 2000).

In addition to soils, host identity may also influence the composition of EM fungal communities. At one extreme, sometimes tree species host a select group of EMF, such as the high specificity of EMF in the genus *Alpova* for *Alnus* (Molina et al. 1992). Differences in EMF community composition are sometimes observed between broadleaf deciduous and coniferous host trees (Bills et al. 1986, DeBellis et al. 2006, Ishida et al. 2007). More commonly, trees form relationships with many different EMF and are considered generalists (Smith and Read 2008). Different species of trees may select similar fungal communities (Jones et al. 1997, Simard et al. 1997a, Horton and Bruns 1998, Hubert and Gehring 2008), and while EMF may be host-specific in monodominant stands in mixed-species stands they form relationships with a variety of tree species (Molina and Trappe 1982, Heslin et al. 1992, Kranabetter et al. 1999, Massicotte et al. 1999, Lang et al. 2011).

In restoration, a crucial question is which plant species are to be used in the revegetation of a site. A diverse mix of native species is usually desired, and while the influence of host identity and soil type on EM fungal communities has been shown in previous research (Ding et al. 2011, Huang et al. 2012, Hankin, Karst and Landhäusser 2015, Gaster, Karst and Landhäusser 2015), the putative benefits of planting trees as mixed or single-species had not been tested. Mixed-species stands may have additive effects on EMF community composition, in that the

fungal community is composed of fungi colonizing single tree species (Jones et al. 1997, Simard et al. 1997a, Hubert and Gehring 2008). The effect is additive, in that no EM fungal species unique to the mixed-species stands are found but rather was comprised of the combined fungal communities of multiple tree species. Alternatively, mixed-species stands may have synergistic effects on EMF community composition, harbouring EMF species that are not found in single-species stands and are unique to mixed species stands (Durall et al. 2006), which would lead to greater richness in mixed stands compared to richness of the EFM community in the pure stands when combined. Synergistic effects could be brought about by mixing characteristics of different tree species that in turn create unique properties not found in single-species stands. These characteristics include differences in litter and organic matter content, soil nutrient status, temperature and moisture (Larson 1980, Cavard et al. 2011) that can all in turn influence EM fungal communities (Bills et al. 1986, Last et al. 1987, Rumberger et al. 2004, Douglas et al. 2005, Walker et al. 2005, Matsuoka et al. 2016). Synergistic effects indicate that there may be advantages conferred to restoration by planting tree species in mixtures that cannot be attained through a combination of single-species stands alone. However, as reviewed by Cavard et al (2011), it is difficult to address the effect mixed-species stands have on the composition and richness of organisms because oftentimes only one type of single-species stand is compared to a mixed-species stand, stand age and canopy composition are confounded and stand characteristics often vary. Therefore, it has been challenging to discern between additive and synergistic effects that a mixed-species stand has on communities. Moreover, as soil type, host identity and stand composition are often confounded it has been difficult to extract the independent and interactive effects of these factors on EMF community composition and richness.

Reclamation sites provide an ideal platform to address the effects of mixed- versus single-species planting on EM fungal communities as soil type and host identity are often decoupled. Reclamation involves the placement of soils followed by revegetation. Soils are salvaged from the surrounding landscape and often planted with trees in novel plant-soil combinations not present on the existing landscape (i.e. 'novel ecosystems' *sensu* (Jackson and Hobbs 2009)). Working on a reclaimed oil sands mine it was asked whether mixed-species stands of trees have additive or synergistic effects on EMF community composition and species richness compared with single-species stands? As a secondary question, the importance of host identity and soil type on structuring EM fungal communities was also tested. This particular setting is ideal in that revegetation occurred simultaneously across all sites, and a variety of single-species stands were planted alongside mixed-species stands. Importantly, these study features circumvent previously identified confounding effects (Cavard et al. 2011).

3.2 Methods

3.2.1 General Description of Region

The study area is located in the Dry Boreal Mixedwoods region of Alberta, Canada. This region was covered by the Laurentide ice sheet 10,000 to 12,000 years ago, and left behind glaciofluvial and lacustrine deposits (Johnson and Miyanishi 2008). Due to the deposition of these sediments and disturbances that frequent the landscape, the region is a rich mosaic of uplands, lowlands and lakes. The Boreal Mixedwoods region is characterized by long harsh winters and short warm summers (Beckingham and Archibald 1996.). Depending on soil type, upland forests can range from mesic conditions, supporting a forest dominated by a mixture of trembling aspen (*Populus tremuloides* Michx) and white spruce (*Picea glauca* (Moench) Voss) or xeric conditions where coarser soils are dominated by jack pine (*Pinus banksiana* Lamb.)

(Johnson and Miyanishi 2008). In this region soils are young and characterized by a thin eluvial A horizon, a distinct Bm (Brunisols) or Bt horizon (Luvisols), and a C horizon. Lowland forests are dominated by organic soils and the tree species common to these forests include tamarack (*Larix laricina* (Du Roi) K. Koch) and black spruce (*Picea mariana* (Mill.) B.S.P.) (Johnson and Miyanishi 2008).

3.2.2 Forest Reclamation Site

Experimental plots were located on a large-landscape scale research study (36 ha, the Aurora Soils Capping Study hereafter 'Reclamation Site') in which a variety of vertical configurations of different surface soils and substrate types were tested for their use in forest reclamation of capping lean oil-sands overburden. The site was located on the Syncrude Canada Ltd.-Aurora mine lease, approximately 75 km north of Fort McMurray, Alberta, Canada (57°19'20"N, 111°30'24"W). The different surface soil materials tested in this study had been salvaged from areas slated for mining and directly placed (without stock-piling) in the winter of 2011/12 on the experimental site. Direct placement of the salvaged soil material is preferred as propagules contained in this material can lose their viability when stock-piled (Koch et al. 1996, Rokich et al. 2000, Macdonald et al. 2015b). The salvaged surface soils tested in this study were the same as those described in chapter 2. Each salvaged soil was placed last and positioned at the soil surface. For more information on the initial soil characteristics see Hankin et al (2015).

Each treatment plot with different surface soils was 1 ha in size and replicated three times. Each treatment plot contained four 25 × 25 m tree plots consisting of three single species plantings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) and a fourth plot with an even mixture of the tree species. The seedlings of all three species were grown commercially at the Smoky Lake Forest Nursery (Smoky Lake,

Alberta) from mixed open pollinated seed sources collected from several populations near Fort McMurray. Specifically, the seedlings were container-grown and 1-year old, aspen and spruce were grown in containers 6 cm in diameter and 15 cm deep while pine was grown in containers 4 cm in diameter and 12 cm deep. The seedlings were planted in May 2012 at a 1 m spacing equivalent to a density of ten thousand stems per hectare.

3.2.3 Molecular Identification

Root samples from planted seedlings were collected by starting at the base of the seedling and following lateral roots outward until approximately 300 fine roots were collected. For the single-species tree plots, five trees per tree plot were sampled for a total of 135 samples (5 planted seedlings × 3 species × 3 tree plots × 3 surface soils). Equivalently, for the mixed-species tree plots, five trees of each species were sampled from each plot for a total of 135 samples. All roots collected were placed on damp paper towels, sealed in bags and kept on ice for no more than 48 hours. Roots were then stored at -20°C until further processing.

Root samples were thawed at 2°C and gently washed by hand over a 1.2 mm sieve to remove adhering soil. Cleaned roots were cut into 1 cm fragments, placed in trays filled with water and thoroughly mixed. A subsample of the fragments was placed under a dissection microscope at 100 × magnification to identify the presence of fungi on the root tips, according to absence of root hairs, presence of a hyphae, mantle structure, color and texture (Goodman et al. 1998). Based on the findings from Chapter 2, a total of five ectomycorrhizal root tips per planted seedling for fungal DNA analysis.

Root tips were placed in 96 well plates and DNA template were extracted by adding 10μL of Sigma Extraction Buffer (Sigma Aldrich, St. Louis, Missouri, USA) to each well that each contained one root tip. The buffer and root tips were then incubated at 65°C for 10 minutes,

then 95°C for 10 minutes. After, 30µL of Neutralization Solution B was added. DNA template was then either immediately used for PCR or stored in a -20°C freezer until PCR could be performed.

Extractions were amplified using fungal specific primers ITS1-F (5'-cttggtcatttagaggaagtaa-3') for forward and ITS4 (5'- tcctccgcttattgatatgc -3') and reverse directions (Gardes and Bruns 1993) at 1.0µL of DNA extract, 6.5µL of autoclaved de-ionized water, 12.5µL of EconoTaq PLUS 2X Master Mix (Lucigen, Middleton, Wisconsin, USA), 2.5µL of 10µM ITS1-F and 2.5µL of 10µM ITS4. Specifically the ITS1-F binds on the SSU conservative region and the ITS4 binds on the LSU conservative region, with both primers amplifying the ITS1 and ITS2 region (Gardes and Bruns 1993). Thermal cycling conditions were as follows: initial denaturation of 95°C for 5 minutes followed by 40 cycles of (denaturation at 95°C for 1 minute and 30 seconds, annealing at 55°C for 1 minute and extension at 72°C for one minute and 30 seconds), and a final extension at 72°C for 10 minutes.

All PCR products at 5µl were visualized on a 1.7% agar run at 100 volts for 30-35 minutes. Only samples that produced clear single bands were used for further analysis. PCR products were cleaned enzymatically by Exo-SAP IT (New England Biolabs, Massachusetts, USA). After purification, bi-directional sequencing was performed with BigDye Terminator v3.1 (Applied Biosystems, Foster City, California, USA) using the ITS1-F/ITS4. Sequence reactions were cleaned using primers EDTA and ethanol and run on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

3.2.4 Bioinformatic Analysis

Sequences were edited with Geneious version 6.1.8 (Kearse et al. 2012). First, ends were trimmed with an error probability limit of 3%. Base pairs with a phred score below 20 were

changed to N. Complementary forward and reverse directions were assembled into contigs using the CAP3 assembler in the BioEdit version 7.2.5 software (Hall 1999). For sequences that did not form contigs, single directions were kept for the final assembly if there were overall less than 2% base pairs labelled as N. The resulting contigs and single direction sequences were then clustered into OTU (operational taxonomic units) using the CAP3 plugin in Geneious with the following settings: ≥ 97% identity, overlap percentage cutoff = 97, maximum overhang percentage length= 60, match score factor =5, clipping range = 6. OTUs were then run through the GenBank database (National Center for Biotechnology Information, Bethesda, Maryland) using BLASTn to identify the best match. Identity was assigned to an OTU if percent identity was ≥ 97 and query coverage was ≥ 80%.

3.2.5 Statistical Analysis

All statistics were run with R version 3.3.1 (R Development Core Team 2016). First, a measure of relative fungal OTU abundance for each planted seedling was taken and then averaged at the tree plot level. To determine whether stand composition, host identity or soil type influenced the community composition of EMF on planted seedlings, a perMANOVA was run using the *adonis* function in the *vegan* package with 9999 permutations (Oksanen et al. 2016). Stand composition referred to whether a seedling came from a single or mixed-species tree plot, host identity referred to the three species of planted seedlings: trembling aspen, jack pine and white spruce, and soil type referred to the three salvaged surface soils on the Reclamation Site: 'FFM', 'Peat' and 'Subsoil'. Multivariate homogeneity of dispersion was tested following perMANOVA to check for equal variance within predictor variables (a conservative alpha of 0.01 was used to avoid Type I error (Underwood 1997), stand composition p=0.4, host identity p=0.02, soil type p=0.01, host identity × soil type p=0.01) using the *betadisper* function in the

vegan package (Oksanen et al. 2016). Due to the low p-value found in the *betadisper* test for soil type and the interaction with host identity, some caution should be taken in interpreting the effect of soil type on fungal community composition as some variation could be caused by within group differences. To visualize the structure of the EMF community on the planted seedlings a rank abundance curve was made using the *rank abundance* function in the *BiodiversityR* package (Kindt and Coe 2005). This curve allowed for visualization of the EM fungal communities and dominance of OTUs. The curve was visually analyzed for changes in slope, in which fungal OTUs with the highest relative abundance values were considered the most common in colonizing the root tips. This information was then further used to look for differences in EM fungal communities between stand composition, host identity and soil types. Indicator species analysis was run using the *multipatt* function in the *indicspecies* package (De Caceres and Legendre 2009) to identify representative fungal OTUs found on the different species of planted seedlings within each soil type.

Stand composition, host identity or soil type were also tested for their influence on EMF species richness. The EMF species richness was calculated for host identity by counting the number of different fungal species found on each species of planted seedling across both tree plot types, representing a richness value for host identity and not stand composition. The EMF species richness was calculated for soil type by counting the number of different fungal species found in each soil type. Differences in species richness among the different species of planted seedlings and soil types were tested by creating generalized linear mixed models with a Poisson distribution using the *lme4* package (Bates et al. 2015) and run with a Wald Chisquare test in the *car* package (Fox and Weisberg 2011). Either host identity or soil type was set as a fixed effect and plot was set as a random effect to account for any site variation. Differences in species

richness by stand composition was tested in two different ways. First, mean species richness was compared for each species of planted seedling between tree plot types (e.g. mean species richness of trembling aspen in the single-species tree plots was compared to mean species richness of trembling aspen in the mixed-species tree plots) using a t-test. Second, mean species richness for the three single-species tree plots were individually compared to the mixed-species tree plots (e.g. mean species richness of trembling aspen in the single-species tree plots was compared to the mean species richness of the whole mixed-species tree plots) using a t-test. Assumptions were checked with a Shapiro-Wilks normality test and Bartlett's test for homogeneity of variance.

To test if mixed-species tree plots had additive or synergistic effects on EMF community composition and species richness, a measure of relative fungal abundance was calculated for each planted seedling and then averaged for the combined single-species tree plots and the mixed-species tree plots (Appendix VI). A perMANOVA was run using the *adonis* function in the *vegan* package with 9999 permutations (Oksanen et al. 2016). Multivariate homogeneity of dispersion was tested following perMANOVA to check for equal variance within predictor variables (a conservative alpha of 0.01 was used to avoid Type I error (Underwood 1997), stand composition p=0.3) using the *betadisper* function in the *vegan* package (Oksanen et al. 2016). The information from the previously made rank abundance curve was used to check for differences in EM fungal communities between stand composition. Species richness values were calculated in a similar fashion as the relative abundance, the number of different fungal species was counted for the combined single-species tree plots and the mixed-species tree plots. The mean species richness was then compared between the combined single-species tree plots and

the mixed-species tree plots with a t-test. Assumptions were checked with a Shapiro-Wilks normality test and Bartlett's test for homogeneity of variance.

3.3 Results

3.3.1 Response of ectomycorrhizal fungal communities to stand composition, soil type and host identity

Overall the fungal community on planted seedlings clustered into 46 operational taxonomic units (OTUs) (Table 3-1). Of the 46 OTUs identified on root tips of planted seedlings, four were most abundant on the root systems: *Amphinema byssoides* 1, *Suillus* 1, *Cenococcum geophilum* and Pezizaceae 1. The other 42 OTUs were found at relatively lower abundances (i.e. OTU frequency ≤ 4) (Figure 3-1).

Mean species richness of the EMF community was not significantly different among soil types or species of planted seedlings. Mean species richness also did not differ for each individual tree species between single and mixed-species tree plots (i.e., trembling aspen in the single-species tree plots had the same species richness as trembling aspen in the mixed-species tree plots). However, when comparing each individual single-species tree plots to the whole mixed-species tree plots, all three single-species tree plots had a significantly lower species richness compared to the mixed-species tree plots (Table 3-4).

The EMF community composition differed both among surface soil type and host identity, but not stand composition (i.e., trembling aspen in the single-species tree plots had similar communities to trembling aspen in the mixed-species tree plots) (Table 3-2). Soil type significantly influenced the selection by hosts for EMF in that fungal communities differed on each species of seedling depending on what soil type they were planted in. Generally, the 'FFM'

soil harboured different EM fungal communities than the ‘Peat’ and ‘Subsoil’ surface soils (Figure 3-2).

3.3.1.1 Trembling Aspen

Trembling aspen seedlings hosted EM fungal communities that were more similar between the ‘Peat’ and ‘Subsoil’ surface soils compared to the ‘FFM’ soil. Specifically, indicator species analysis revealed that *Thelephora terrestris* and *Cenococcum geophilum* were indicators for the ‘FFM’ soil and Uncultured fungus 2 was an indicator for the ‘Subsoil’ (Table 3-3). In accordance with the indicator species analysis, trembling aspen in the ‘FFM’ soil was heavily colonized by *Cenococcum geophilum*. Trembling aspen in the ‘Peat’ soil mainly hosted the less frequently found EMF species, but had a small abundance of the four more common EMF species on their roots as well. Similarly, trembling aspen in the ‘Subsoil’ mainly hosted the less frequently found EMF species, but had a small abundance of *Cenococcum geophilum* and Pezizaceae 1 (Figure 3-3a).

3.3.1.2 Jack Pine

Jack pine seedlings hosted a high abundance of *Suillus* 1 and Pezizaceae 1 and generally communities showed similarities between the ‘Peat’ and ‘Subsoil’ surface soils that differed from the ‘FFM’ soil. Specifically, indicator species analysis revealed that *Cenococcum geophilum* and Uncultured fungus 1 were indicators for the ‘FFM’ soil, *Suillus* 2 was an indicator for the ‘Peat’ soil and Pezizaceae 1 was in indicator for the ‘Subsoil’ (Table 3-3). Ectomycorrhizal fungi communities on jack pine in the ‘FFM’ soil differed from the ‘Peat’ and ‘Subsoil’ surface soils in that there was a higher abundance of *Cenococcum geophilum* and lower abundance of *Suillus* 1 and Pezizaceae 1. Jack pine in the ‘Peat’ and ‘Subsoil’ surface soils both

had a high abundance of *Suillus* 1. Jack pine in the 'Subsoil' also had a high abundance of Pezizaceae 1 (Figure 3-3b).

3.3.1.3 White Spruce

White spruce seedlings hosted a high abundance of *Amphinema byssoides* 1 and Pezizaceae 1 and generally fungal communities showed similarities between the 'FFM' and 'Peat' surface soils that differed from the 'Subsoil'. Specifically, indicator species analysis revealed that *Amphinema byssoides* 1 was an indicator for both 'FFM' and 'Peat' soils, *Cenococcum geophilum* was an indicator for 'FFM' soil and Pezizaceae 1 was an indicator for 'Subsoil' (Table 3-3). In accordance with the indicator species analysis, EM fungal communities on white spruce in the 'Peat' and 'FFM' soils were similar in that there was a high abundance of *Amphinema byssoides* 1. Additionally, white spruce in the 'FFM' soil also had a high abundance of *Cenococcum geophilum*. In contrast to the other two surface soils, white spruce in the 'Subsoil' soil had a high abundance of Pezizaceae 1 (Figure 3-3c).

3.3.2 Additive versus synergistic effects of mixed-species tree plots on ectomycorrhizal community composition and species richness

Mean species richness of combined single-species tree plots of trembling aspen, jack pine and white spruce was not significantly different from mixed-species tree plots (mean species richness ± SE, combined single = 10±0.5, mixed = 9±0.5, $p=0.64$). Ectomycorrhizal fungi community composition was also not significantly different between combined single-species tree plots and mixed-species tree plots (Table 3-5). Consequently, there was a similar abundance of the four most commonly found EMF species in combined single-species tree plots and mixed-species tree plots (Figure 3-4). Of note, several EMF species were found on only one tree species

in the single-species tree plots, but multiple tree species in the mixed-species tree plots (*Suillus* 2, Uncultured Fungus 9, Pezizales 1, *Russula* 1, *Thelephora* 1) (Table 3-1).

3.4 Discussion

Three-species of tree seedlings planted in single- and mixed-species plots across different types of salvaged surface soils were used to determine the importance of stand composition, host identity and soil type on the composition and richness of EM fungal communities, and whether mixed-species plots had overall additive or synergistic effects on EM fungal communities. Stand composition did not have a significant influence on EMF community composition as communities were similar for seedlings planted in the single- and mixed-species plots. Similarly, past research has shown a strong host effect in mixed-species stands on EMF community composition (Kernaghan et al. 2003b, DeBellis et al. 2006, Ishida et al. 2007, Ding et al. 2011). In regard to the tree species used in the current study, past research has shown trembling aspen to host different EM fungal communities compared to balsam fir, white spruce, balsam poplar, jack pine, paper birch and white birch in boreal mixed-species stands (Kernaghan et al. 2003b, DeBellis et al. 2006). In this study, jack pine and white spruce seedlings hosted different EM fungal communities, which is contrary to other studies, which have reported that lodgepole pine and white spruce (Kranabetter et al. 1999) as well as Engelmann spruce and lodgepole pine hosted similar EM fungal communities (Cullings et al. 2000). One possible explanation for the differences in EM fungal communities observed between the two-coniferous species in this study, is that the seedlings were planted in reconstructed soil, and of different types. The interaction between host identity and soil type may have been driven by different carbon allocation strategies of fast versus slower growing trees species (Hobbie 2006) and their response

to different soil conditions such as C:N rations, nitrogen levels and pH (Hobbie, Macko and Williams 2000, Hogberg, Hogberg and Myrold 2007, Hogberg et al. 2010) .

Seedlings were in their fourth growing season and some EMF from the first growing season were still present on the roots (Table 3-1) (Hankin et al. 2015). However, the more abundant EMF species included *Cenococcum geophilum*, a ubiquitous species that is often found on disturbed sites (Byrd et al. 2000, Douglas et al. 2005, Heinonsalo et al. 2007), *Amphinema byssoides*, a common ruderal species (Kernaghan et al. 2003a), Pezizaceae 1, a family that has been found in another similar forest reclamation site (Bois et al. 2005) and may prefer mineral soils (Korkama et al. 2006) and *Suillus* 1, which is a genus normally associated with pine trees and disturbed sites (Visser 1995, Jonsson et al. 1999, Ashkannejhad and Horton 2006, Hartmann et al. 2012, Leduc et al. 2013). These EMF are representative of an early successional disturbed site but, intriguingly seedling preference for these EMF taxa varied by host and soil type. For example, trembling aspen and white spruce had higher abundances of *Cenococcum geophilum* found on their roots in the 'FFM' soil compared to jack pine. White spruce and jack pine had higher abundances of Pezizaceae 1 in the 'Subsoil' compared to trembling aspen. Similar to other studies, white spruce was colonized by *Amphinema byssoides* (Danielson 1991, Lazaruk et al. 2005, Gagne et al. 2006) compared to the other two hosts. The influence of soil type on host preference for EMF species could have important implications if the communities function differently (Jones et al. 2010, Walker et al. 2016). However, it was beyond the scope of this study to investigate functional differences in the fungal community, so it is currently unknown if the differences in EMF community composition are responsible for impacting tree growth or development.

There were no significant differences found in EMF species richness among surface soils which is aligned with other studies (Kernaghan et al. 2003b, Douglas et al. 2005, Matsuoka et al. 2016). Also, each species of seedling hosted on average a similar number of EMF, similar to past work that compared EMF richness on multiple host tree species (Jones et al. 1997, Horton and Bruns 1998, Durall et al. 2006). Consequently, for each species of planted seedling, having neighboring trees of a different species did not influence EMF species richness. However, overall species richness was higher in the mixed-species tree plots when compared individually to the three single-species plots, in agreement with past work showing mixed forest stands to have higher levels of species richness compared to single species stands (Bills et al. 1986, Heslin et al. 1992, Rumberger et al. 2004).

Similar to Durall et al (2006), there were very few EMF associated with only the mixed-species plots. Therefore, overall mixed-species tree plots had additive effects on the fungal community in that the EMF community was comprised of similar fungal species found in the combined single-species plots. To my knowledge, this was the first study to compare EMF community composition between single- and mixed-tree stands controlling for stand age, soil type and site history. Although there was a strong host effect on EM fungal communities in mixed-plots, over time as the seedlings age interactions between their root systems could lead to an influence on their fungal communities. Currently, some patterns are already emerging. For example, comparable to past work, some of the EMF showed an intermediate range of host tree preference (Massicotte et al. 1999, Lang et al. 2011), which could lead to possible resource sharing between tree species in the mixed-species plots (Simard and Durall 2004). Along the same lines, a few of the EMF only found on one host species in the single-species tree plots were

then found on another in the mixed-species plots, which showed that neighboring trees could have an influence on fungal community composition (Bogar and Kennedy 2013).

Despite identifying the influence of stand composition, host identity and soil type on EMF community composition, there was still variation left unexplained (43%) (Table 3-2). A likely possibility is that unmeasured soil factors played a role in structuring fungal communities. There was a difference in properties among the salvaged surface soils such as texture, available nutrients, C:N ratio, salinity, electrical conductivity and pH (Hankin et al. 2015). It has been established in past research that different properties of reclaimed mine soils can influence EM fungal communities (Munzenberger et al. 2004, Bois et al. 2005, Huang et al. 2014). Looking forward, it would be valuable to know if any EM fungal species correlate with these unmeasured soil factors and also if there is an interaction between them and the previously measured factors of host identity and stand composition.

Tables

Table 3-1. Operational taxonomic units from the best BLAST in the NCBI database and the corresponding UNITE species hypothesis (SH), assembled from quality filtered sequences from amplified fungal rDNA. A Reclamation Site with reconstructed soils were assayed for ectomycorrhizal fungi with seedlings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) planted in single- and mixed-species tree plots grown in 2012-2015. The OTUs are ranked from most to least abundant in the table based on a rank abundance curve (Figure 3-1). The 'Single' and 'Mixed' columns indicate which species of seedling the OTU was found on in the single- and mixed-species plots. *OTU identified in Genbank from 2012 survey (Hankin et al 2015)

Best Match	OTU Rank	Blast ID	Query Length	Max Score	Query Cover	% ID	UNITE SH	UNITE Accession	Single	Mixed
Amphinema byssoides 1	1	KP814511	625	1155	98%	100%	*Amphinema*	SH197944	PS	APS
Suillus 1	2	JQ711787	715	1299	99%	99%	*Suillus*	SH176743	PS	AP
Cenococcum geophilum	3	LC095188	1029	1681	99%	97%	*Cenococcum geophilum*	SH214459	APS	APS
Pezizaceae 1	4	JN704828	592	1068	98%	99%	Pustularia	SH222144	APS	APS
Uncultured fungus 1	5	KP889629	665	1229	99%	100%	Fungi	SH203891	PS	PS
Uncultured fungus 2	6	LC013889	591	1075	88%	99%	Fungi	SH189869	AS	APS
Thelephora terrestris	7	HM189958	689	1266	99%	99%	*Thelephora terrestris*	SH184510	APS	APS
Uncultured fungus 3 *	8	KJ938035	636	1175	99%	100%	Fungi	SH194156	PS	PS
Pezizales 1	9	JN704819	620	1140	98%	99%	Pezizales	SH212010	A	AP
Suillus 2	10	JQ711950	714	1267	99%	99%	*Suillus*	SH176741	P	PS
Uncultured fungus 4 *	11	KJ938040	690	1271	99%	99%	Tuber	SH188859	APS	APS
Hebeloma 1	12	FJ378789	711	1314	99%	100%	Cortinariaceae	SH215994	APS	APS
Uncultured fungus 5 *	13	KJ938039	525	952	97%	99%	*Cenococcum*	SH199612	AP	APS
Uncultured fungus 6 *	14	KJ938033	658	1181	99%	99%	*Rhizopogon pseudoroseolus*	SH221091	P	P
Uncultured fungus 7	15	GU566255	577	1038	99%	99%	Fungi	SH205326	AS	
Uncultured fungus 8	16	AJ875374	644	1157	99%	99%	Fungi	SH193724		A
Thelephoraceae 1	17	JN704829	678	1192	99%	99%	Thelephoraceae	SH189381	APS	A
Russula 1	18	KF002778	709	1247	99%	98%	*Russula*	SH218421	A	AP
Geopora	19	GU327416	648	1070	100%	97%	Geopora	SH213655	A	A
Wilcoxina 1	20	EU668262	635	1168	99%	99%	Pyronemataceae	SH194158	PS	S

Uncultured fungus 9	21	KF617463	611	1118	96%	99%	Fungi	SH211927	S	PS
Meliniomyces 1	22	FN565279	568	1050	100%	100%	*Meliniomyces bicolor*	SH214265	APS	P
Leucocortinarius bulbiger	23	KC984861	711	1282	100%	99%	*Hebeloma*	SH215995	P	P
Uncultured fungus 10 *	24	KJ938030	613	1127	99%	99%	Fungi	SH197943	S	S
Thelephorales 1	25	KF000547	669	1205	98%	99%	Thelephorales	SH177808		A
Uncultured fungus 11	26	KF617396	839	1472	99%	98%	Fungi	SH214265		A
Tomentella	27	JX630694	654	1175	99%	99%	Thelephoraceae	SH184538	A	
Thelephora 1	28	KT334743	651	1149	98%	98%	Thelephoraceae	SH184510	P	AP
Thelephorales 2	29	KF000514	662	1197	100%	99%	Thelephorales	SH189413	A	
Amphinema byssoides 2	30	KP814527	618	1109	99%	99%	*Amphinema*	SH197943	S	S
Inocybe jacobi	31	HQ604812	605	1085	99%	99%	*Inocybe jacobi*	SH211892	A	A
Pulvinula	32	JN704812	609	1114	99%	99%	Pulvinula	SH204543	P	P
Uncultured fungus 12*	33	KJ983041	649	1199	100%	100%	Fungi	SH184552		AP
Uncultured fungus 13 *	34	KJ938034	689	1232	99%	99%	Thelephoraceae	SH184510		A
Uncultured fungus 14	35	KM596883	700	1242	98%	98%	Fungi	SH221083	P	
Laccaria proxima	36	KU685717	700	1229	99%	98%	*Laccaria proxima*	SH179278		AS
Uncultured fungus 15	37	KC965383	664	1201	99%	99%	Thelephoraceae	SH205652		A
Wilcoxina 2	38	DQ320129	567	983	99%	98%	*Wilcoxina*	SH194157	S	
Inocybe lacera	39	HQ604430	692	1253	99%	99%	*Inocybe lacera*	SH201230		A
Tomentellopsis	40	KP403093	672	1182	100%	98%	Thelephoraceae	SH184845	P	
Hebeloma ingratum	41	KT217496	688	1232	97%	99%	*Hebeloma ingratum*	SH215994	A	

Helotiales	42	FJ475791	556	1013	93%	99%	Phialocephala	SH204986		S
Uncultured fungus 16	43	EU292251	548	992	88%	99%	*Amphinema*	SH197944		S
Suillus variegatus	44	JQ711926	697	1218	98%	98%	*Suillus variegatus*	SH176741	P	
Pyronemataceae	45	KR019795	610	1074	98%	98%	Pyronemataceae	SH194157	S	
Russula laccata	46	HQ604844	690	1236	96%	99%	*Russula laccata*	SH218421	A	

A=Trembling aspen, P=Jack pine, S=White spruce

Table 3-2. Results of permutational multivariate analysis of variance testing the effects of stand composition, host identity, soil type and their interaction on ectomycorrhizal community composition (n=3). Soil type includes three salvaged surface soils (Forest Floor Material, Peat and Subsoil used in reclamation of an overburden landform constructed through oil sands mining). The Reclamation Site was located in northern Alberta, Canada and soils were assayed for fungi by planted seedlings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) in single- and mixed-species plots grown in 2012-2015.

	DF	**Sum Sq**	**Mean Square**	***F***	**R^2**	***P-value***
Soil type	2	4.5723	2.28613	8.8530	0.21260	**0.001**
Host identity	2	3.7400	1.86999	7.2415	0.17390	**0.001**
Stand composition	1	0.2549	0.25486	0.9870	0.01185	0.447
Soil type × Host identity	4	1.9217	0.48043	1.8605	0.08936	**0.002**
Soil type × Stand Composition	2	0.3846	0.19232	0.7447	0.01788	0.810
Host identity × Stand Composition	2	0.3672	0.18360	0.7110	0.01707	0.865
Soil type × Host identity × Stand Composition	4	0.9696	0.24241	0.9387	0.04509	0.570
Residuals	36	9.2964	0.25823		0.43226	
Total	53	21.5067			1.00000	

DF: degrees of freedom, Sum Sq: sum of squares

Table 3-3. Indicator species analysis (α=0.05) using operational taxonomic units (OTUs) of ectomycorrhizal fungi from roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) grown in single- and mixed-species plots from 2012-2015 on a Reclamation Site with three surface soils (Forest Floor Material (FFM), Peat, Subsoil) in northern Alberta, Canada.

Host identity	**Soil type**	**OTU**	**Stat**	***P-value***
Trembling Aspen	FFM	*Thelephora terrestris*	1.000	0.0001
		Cenococcum geophilum	0.804	0.0149
	Subsoil	Uncultured fungus 2	0.777	0.0407
Jack Pine	FFM	*Cenococcum geophilum*	0.868	0.0015
		Uncultured fungus 1	0.816	0.0139
	Peat	*Suillus* 2	0.755	0.028
	Subsoil	Pezizaceae 1	0.959	0.0001
White Spruce	FFM	*Cenococcum geophilum*	0.913	0.002
	Subsoil	Pezizaceae 1	0.913	0.0021
	FFM + Peat	*Amphinema byssoides* 1	0.973	0.0014

Table 3-4. Differences in mean species richness ± standard error for ectomycorrhizal fungal communities among three different surface soils (Forest Floor Material (FFM), Peat, Subsoil) on a Reclamation Site in northern Alberta, Canada (n=3, χ^2=1.1862, p=0.5526), three different species of planted seedlings (n=9, χ^2=0.882, p=0.6434), single-species plots versus mixed-species plots for each species of planted seedling (n=9, trembling aspen: t-test, T=0, p=1, jack pine: t-test, T= -0.17961, p=0.8597, white spruce: t-test, T=0, p=1) and single-species plots versus the combined mixed-species plots (n=9, trembling aspen: t-test, T=7.6238, p<0.0001, jack pine: t-test, T=6.5959, p<0.0001, white spruce: t-test, T=6.1063, p<0.0001).

	Species Richness
Soil type	
FFM	12 ± 0.3
Peat	16 ± 1.2
Subsoil	14 ± 0.3
Host identity	
Trembling Aspen	6 ± 0.5
Jack Pine	6 ± 0.5
White Spruce	7 ± 0.6
Stand Composition	
Single	
Trembling Aspen	4 ± 0.5 (a)
Jack Pine	4 ± 0.5 (a)
White Spruce	4 ± 0.7 (a)
Mixed	
Trembling Aspen	4 ± 0.4
Jack Pine	4 ± 0.3
White Spruce	4 ± 0.4
Combined	9 ± 0.5 (b)

Letters indicate significant differences with α=0.05

Table 3-5. Results of permutational multivariate analysis of variance testing for additive versus synergistic effects on EMF community composition. Significant differences in the ectomycorrhizal community composition were compared between combined single-species plots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) and mixed-species plots that contained all three species (n=9) grown from 2012-2015 on a Reclamation Site with three surface soils (Forest Floor Material (FFM), Peat, Subsoil) in northern Alberta, Canada.

	DF	**Sum Sq**	**Mean Square**	***F***	**R^2**	***P-value***
Stand Composition	1	0.1817	0.18172	0.60844	0.03663	0.77
Residuals	16	4.7786	0.29866		0.96337	
Total	17	4.9603			1.00000	

DF: degrees of freedom, Sum Sq: sum of squares

Figures

Figure 3-1. Rank abundance curve representing all fungal operational taxonomic units (OTU) ranked from highest to lowest abundance on roots of planted seedlings of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) grown in single- and mixed-species tree plots from 2012-2015 on a Reclamation Site with three different surface soils (Forest Floor Material (FFM), Peat Subsoil) in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling was taken and then averaged at the tree plot level. A natural break point for abundance was visually accessed by the slope of the curve; OTU left of the line were considered the most common fungi.

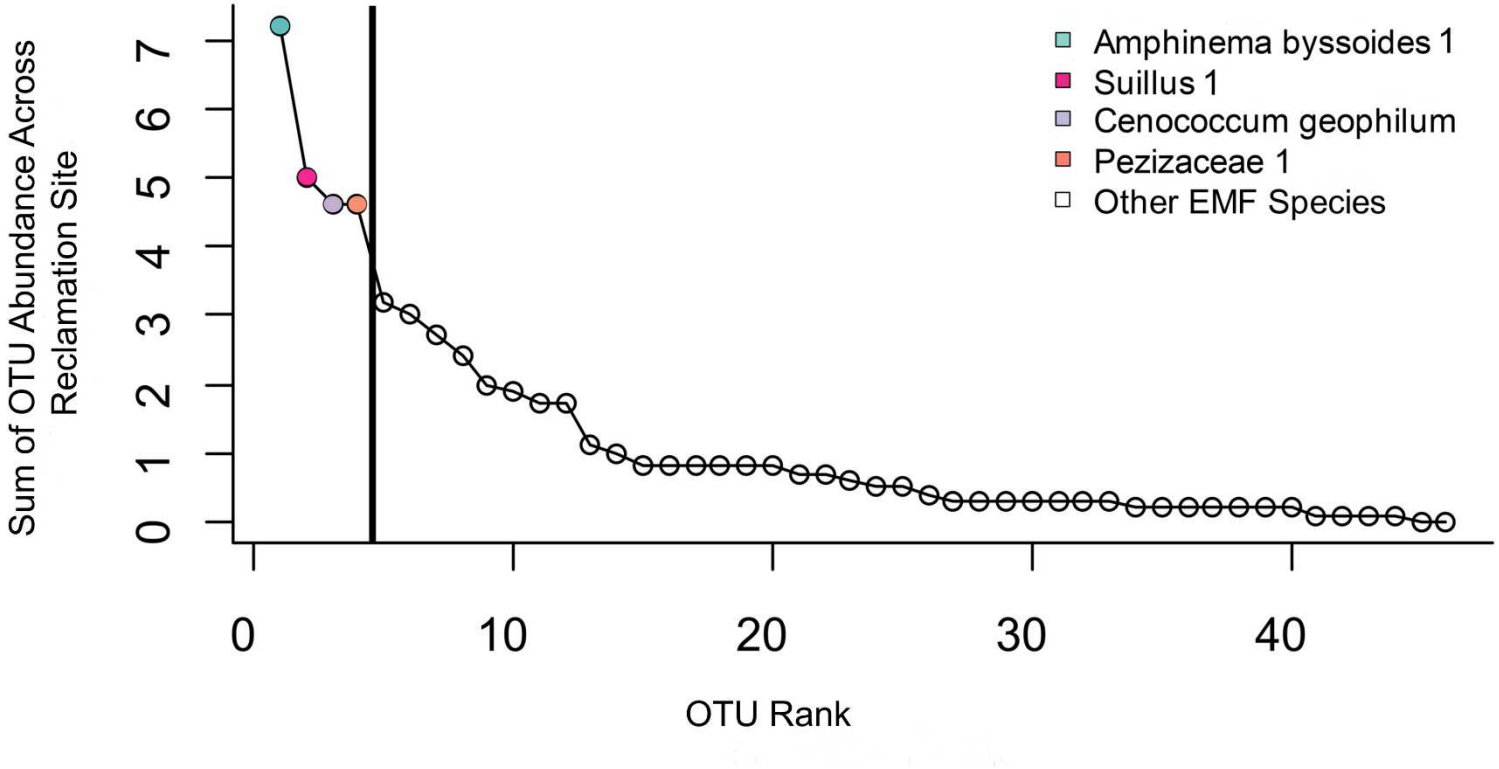


Figure 3-2. Principle coordinate analysis (PCoA) ordination of operational taxonomic units (OTU) of relative abundance of ectomycorrhizal fungi colonizing the roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) grown from 2012-2015 in single- and mixed-species plots on a Reclamation Site with three different surface soils (Forest Floor Material (FFM), Peat Subsoil) in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged at the tree plot level. Each point on the ordination represents the centroid of the ectomycorrhizal fungal community on that species of planted seedling in each soil type. Points closer together signify more similarities in the fungal community composition. The bars represent standard error of each centroid point (n=6).

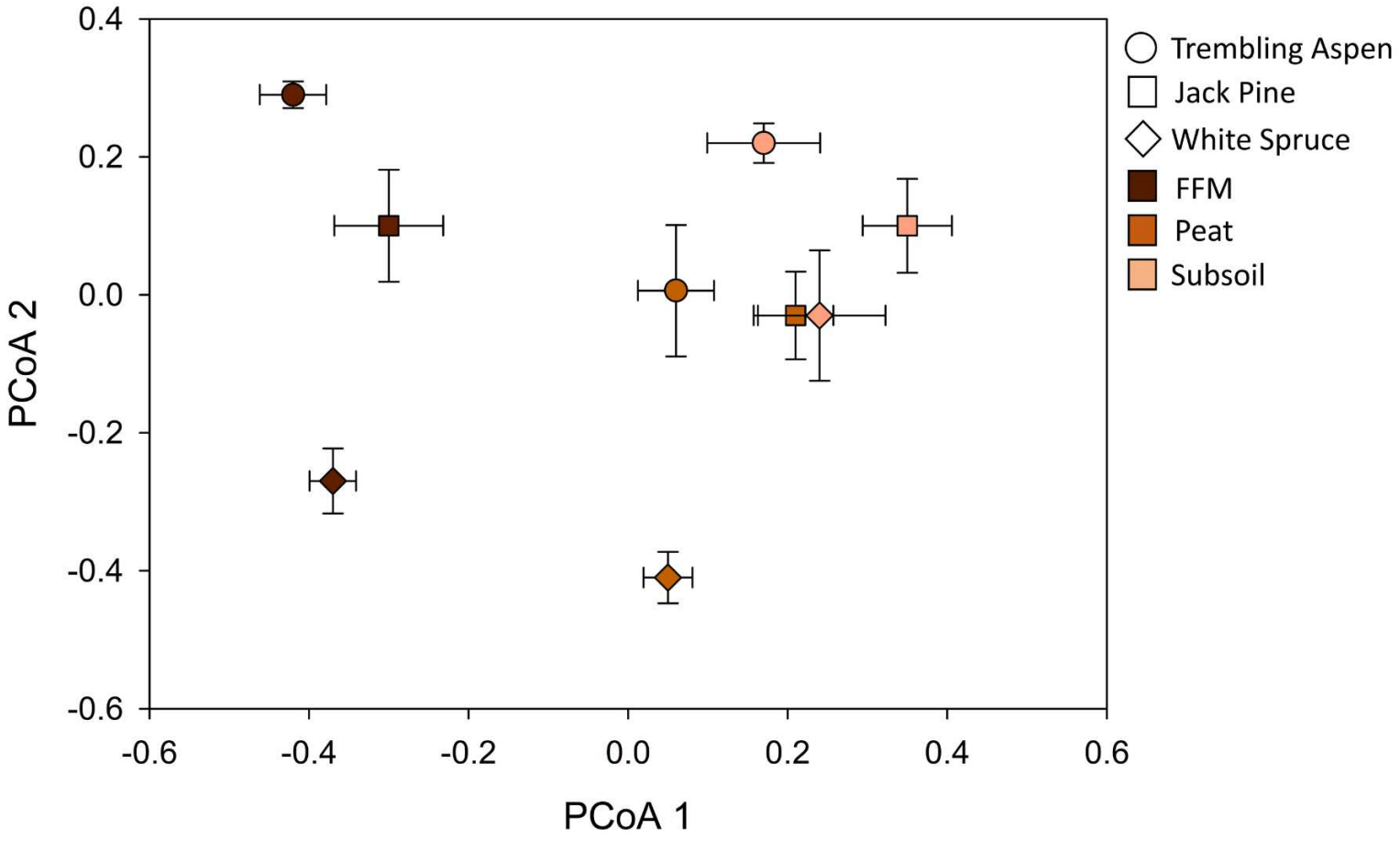
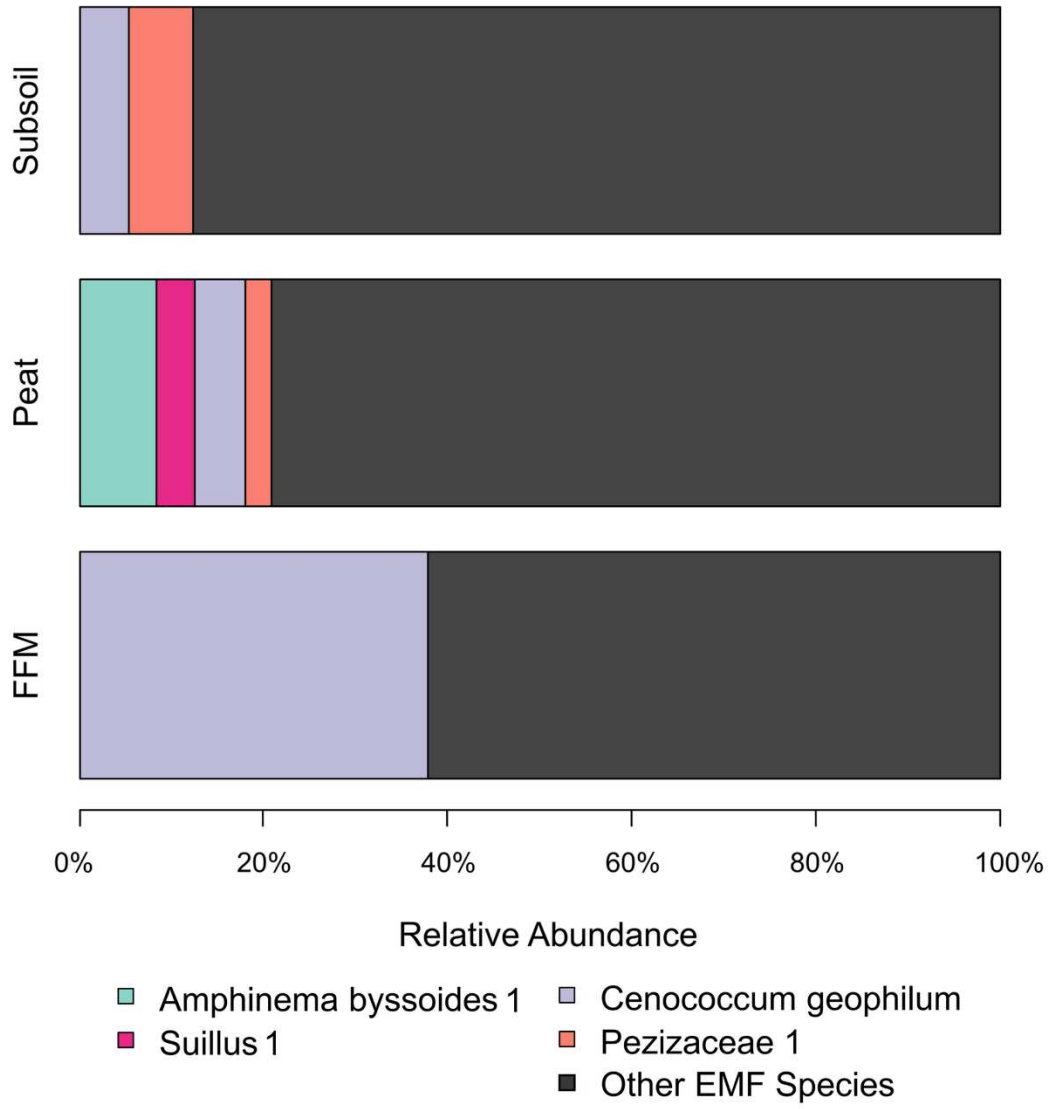
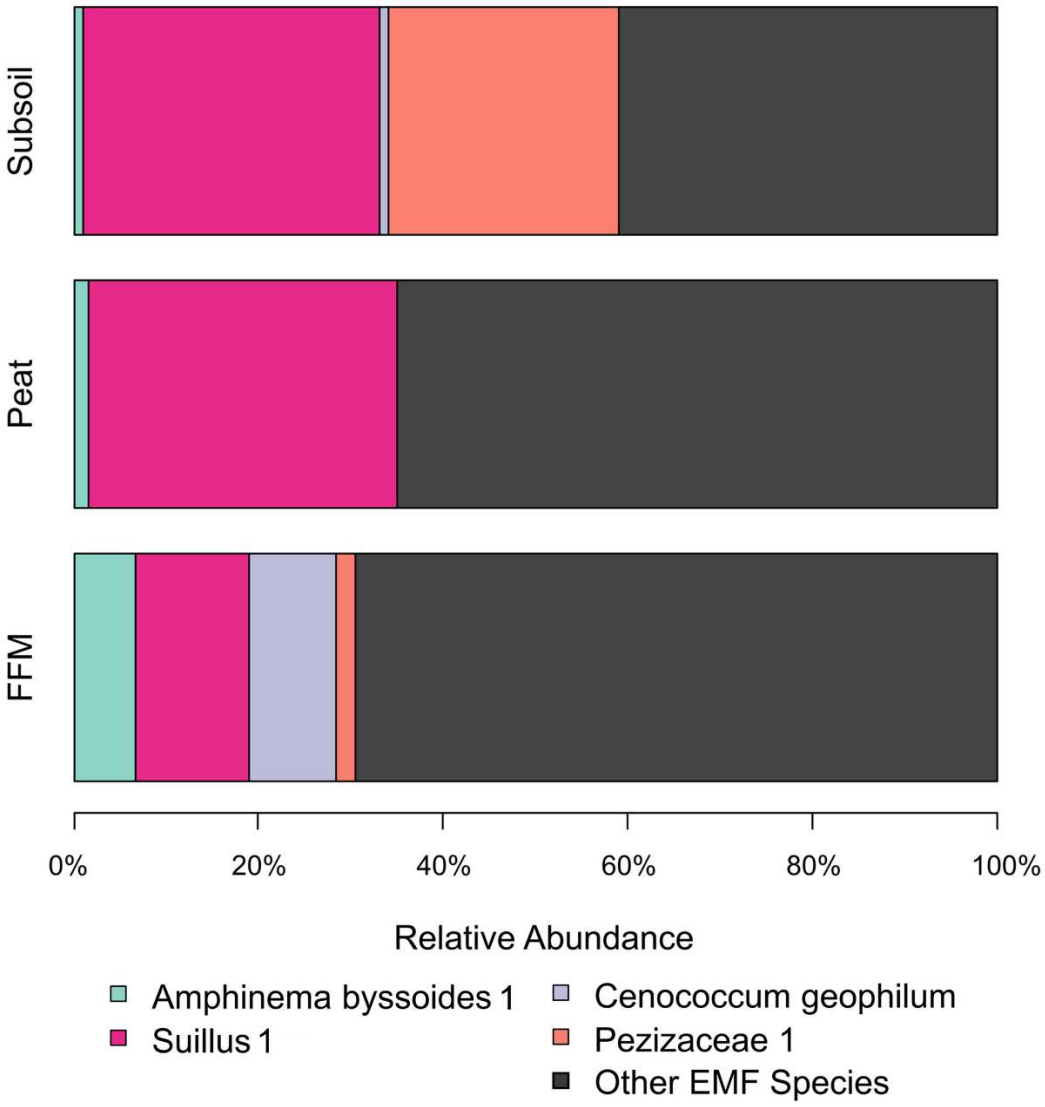


Figure 3-3. Relative abundance of operational taxonomic units (OTU) of ectomycorrhizal fungi colonizing the roots of (a) *Populus tremuloides* (trembling aspen), (b) *Pinus banksiana* (jack pine), or (c) white *Picea glauca* (white spruce) from 2012-2015 in single- and mixed-species plots on a Reclamation Site with three different surface soils (Forest Floor Material (FFM), Peat Subsoil) in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged at the tree plot level. The common ectomycorrhizal fungi are represented by colored fills in the figures. Fungi considered to be the most common were chosen by a rank abundance curve (Figure 3-1). All other OTU are represented by a gray fill in the figures.

(a)


Subsoil
Peat
FFM
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides 1
Suillus 1
Cenococcum geophilum
Pezizaceae 1
Other EMF Species

(b)


Subsoil
Peat
FFM
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides 1
Suillus 1
Cenococcum geophilum
Pezizaceae 1
Other EMF Species

(c)

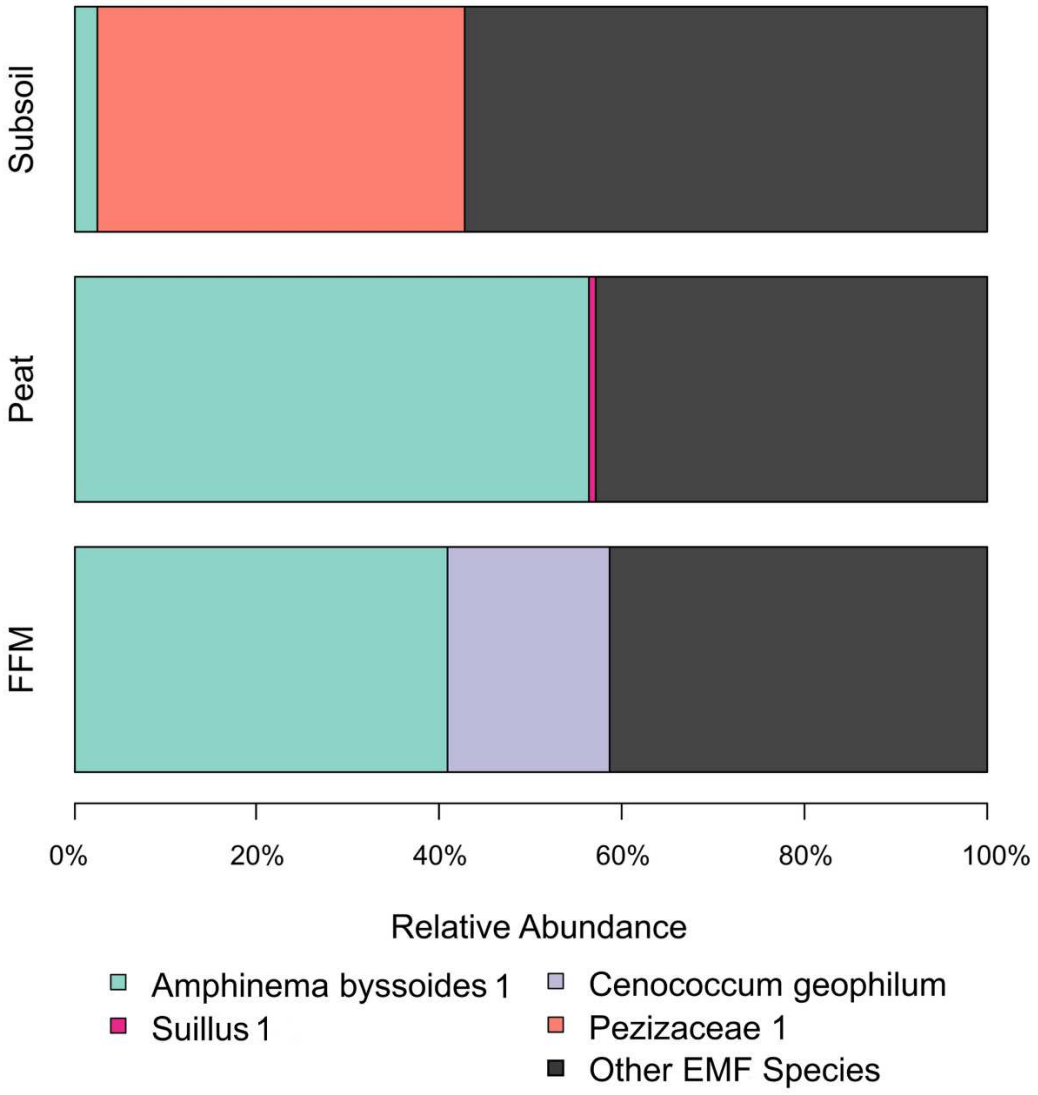
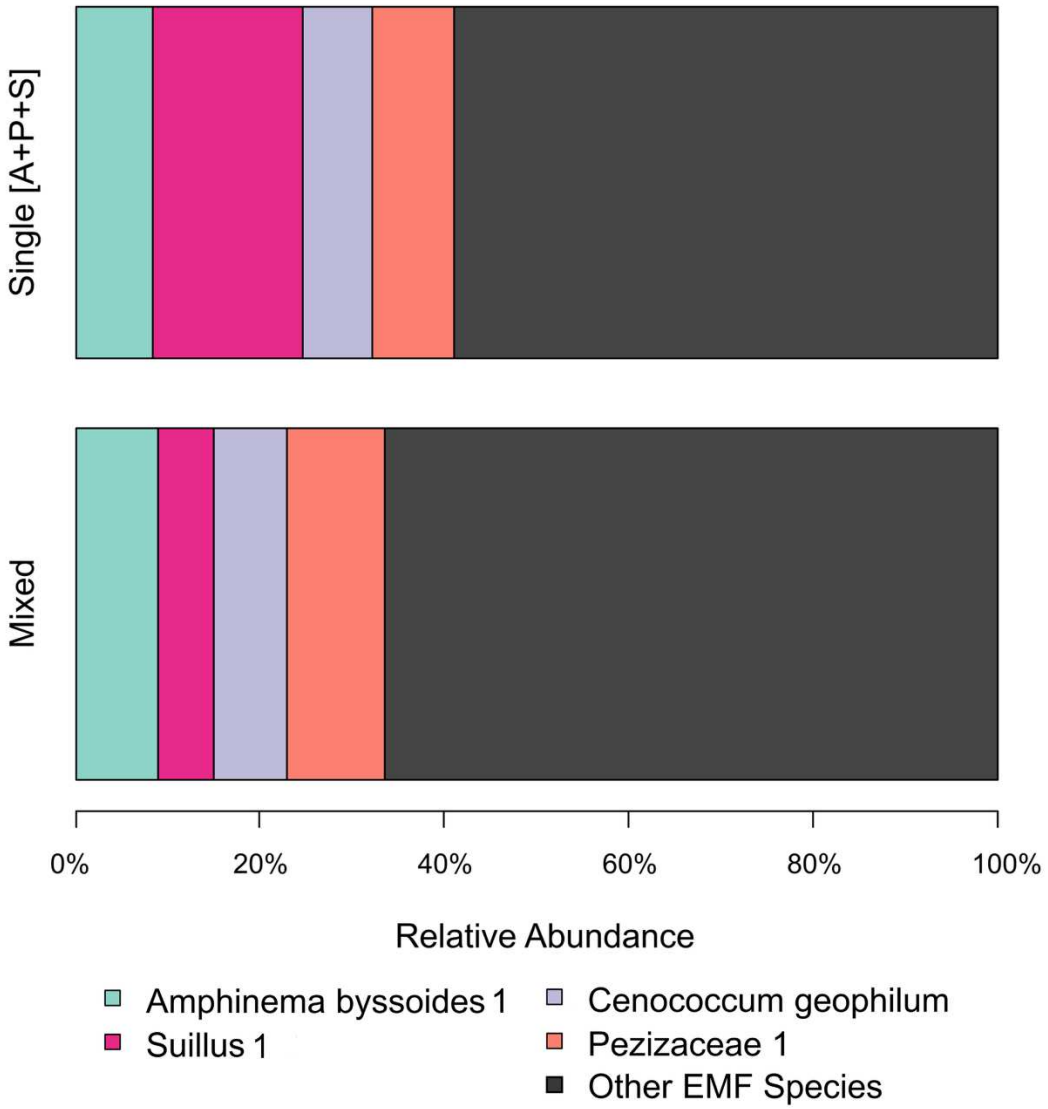

Subsoil
Peat
FFM
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides 1
Suillus 1
Cenococcum geophilum
Pezizaceae 1
Other EMF Species

Figure 3-4. Relative abundance of operational taxonomic units (OTU) of ectomycorrhizal fungi in the combined single-species plots on the roots of *Populus tremuloides* (trembling aspen), *Picea glauca* (white spruce) and *Pinus banksiana* (jack pine) compared to mixed-species plots grown from 2012-2015 on a Reclamation Site with three different surface soils (Forest Floor Material (FFM), Peat Subsoil) in northern Alberta, Canada. Relative abundance of fungal OTUs was calculated for each planted seedling and then averaged for combined single-species plots and the mixed-species plots. The common ectomycorrhizal fungi are represented by colored fills in the figure. Fungi considered to be the most common were chosen by a rank abundance curve (Figure 3-1). All other OTU are represented by a gray fill in the figure.


Single [A+P+S]
Mixed
0%
20%
40%
60%
80%
100%
Relative Abundance
Amphinema byssoides 1
Suillus 1
Cenococcum geophilum
Pezizaceae 1
Other EMF Species

Chapter 4: General discussion and synthesis

The primary objective of this thesis was to understand underlying ecological factors and the possible interactions between them in affecting EM fungal communities in the boreal forest. Specifically, EM fungal communities were assayed with different species of tree seedlings on soils from (1) an oil sands reclamation site constructed with different salvaged surface soils and (2) a site that experienced a gradient of harvest disturbances to address the influence of disturbance severity, soil type and host identity on EMF community composition (Chapter 2). Also, an oil sands reclamation site was used to explore the possible interaction between different soil types and stand composition on EM fungal communities and if mixed-species stands had overall additive or synergistic effects on EMF richness and composition (Chapter 3).

4.1 Research summary

Overall differences in EMF community composition were mainly driven by an interaction between host identity and soil type; fungal communities differed on each species of planted seedling depending on what soil type they were planted in (Chapters 2 & 3). Specifically, trembling aspen hosted different EM fungal communities in the 'FFM' surface soil compared to the 'Peat' and 'Subsoil' surface soils. Jack pine followed a similar pattern to trembling aspen, while white spruce hosted different EM fungal communities in the 'Subsoil' surface soil compared to the 'Peat' and 'FFM' surface soils. Similar research found that jack pine and hybrid poplar hosted different EM fungal communities in overburden versus peat material; EM fungal communities on the jack pine were strongly correlated to potassium levels while hybrid poplar were correlated to phosphorus, magnesium and potassium levels (Bois et al. 2005). Likewise, a strong host effect was seen for EM fungal communities on Masson pine and white oak in a manganese mine site, but communities were also highly correlated with levels of copper,

phosphorus and cadmium (Huang et al. 2014). Findings from this thesis suggest that because salvaged soils on the forest reclamation site had different properties (Hankin et al. 2015), it is possible that nutrient status along with texture, C:N ratio, salinity, electrical conductivity and pH are also a driving factors in structuring the EM fungal communities on the planted seedlings and warrants further investigation. Additionally, in Chapter 3, EM fungal richness levels were similar for all three-tree species and soil types, implying that in terms of richness one species of tree or soil type did not outperform each other on this forest reclamation site. Taken together, results from both studies imply that planting a variety of tree species in different salvaged soils can lead to a richer EMF community compared to planting one species of tree in one type of salvaged soil.

In Chapter 2, it was not only observed that type of salvaged soil could influence EM fungal communities, but further that these communities differed in soils that experienced a gradient of harvest disturbances. Of interest is the fact that the 'FFM' surface soil hosted similar fungal communities to some of the harvest disturbances compared to the other salvaged soils, although this was dependent upon the species of planted seedling. Trembling aspen EM fungal communities in the 'FFM' soil shared some similarities to the 'Disturbed' soil, while similarly jack pine EM fungal communities in the 'FFM' soil shared similarities to the 'Control' forest and 'Disturbed' soil. Surface soils, such as 'FFM', could host EM fungal communities similar to a site that experienced a lower severity disturbance. This makes 'FFM' a good candidate for reclamation of sites disturbed by oil sands mining. The 'FFM' soil was also shown to have a positive impact on understory diversity (Jones 2016). Curiously, white spruce hosted a high abundance of the EMF species *Amphinema byssoides* across all disturbed soils, with the exception of the 'Subsoil' surface soil. It is probable over a longer span of time other EMF

species will colonize white spruce (Danielson 1991). The similarities in EM fungal communities seen on white spruce regardless of soil type could also be due to its a slow growth; slow-growing tree species might allocate carbon in a different manner to its fungal partners compared to fast-growing tree species (Hogberg et al. 1999, Taylor et al. 2003). Tree carbon allocation to EMF increases at slower growth rates and lower nutrient supply rates (Hobbie 2006). How shifts in carbon allocation affect the composition of EM fungal communities should be investigating further avenue of inquiry. Ultimately, it is valuable to know that white spruce seems to be less sensitive to disturbance, making it a useful species to use for reclamation of disturbed sites.

In contrast to the strong effect of soil type, disturbance severity and host identity on EM fungal communities, (Chapter 3), stand composition did not have a pronounced influence on EMF community composition on planted seedlings, which is similar to results found by (Jones et al. 1997). Additionally, mixed-species tree plots had overall additive effects on EMF richness and composition. A similar conclusion was drawn for understory plant composition in mixed-species forest stands (Cavard et al. 2011). In the case of this study, the additive effects seen in the mixed-species tree plots were derived from a strong host identity effect in that each planted seedling had a preference for a certain EMF and planting in mixtures did not influence this. Thus, these findings suggest that, in terms of overall EMF richness and composition, differences in the identity of neighboring trees does not seem to influence EM fungal communities on a focal tree. This study investigated EM fungal communities on young seedlings, but it is possible that the non-pronounced effect of stand composition will persist with time (DeBellis et al. 2006, Ishida et al. 2007). However, the forest reclamation site does provide an opportunity to test if the influence of stand composition on EMF community composition changes with time and if new fungal species colonize the mixed-species plots over time not seen in the single-species plots

(synergistic effects). This information would provide managers with the knowledge to assess additional benefits planting in mixtures could have for overall EM fungal richness levels.

Based on the findings from Chapter 2, planted seedlings recovered similar EM fungal communities as to what is found on roots of naturally established trees. Specifically, EMF colonizing seedlings of jack pine were similar to those colonizing fine roots of established saplings and trees of the same species. These similarities in fungal communities could have important implications for the seedlings growth and development. A common mycorrhizal network (CMN) can occur when two or more root systems are interconnected by mycorrhizal fungal hyphae (Simard and Durall 2004). Because of CMN, adult trees can play a large role in seedling establishment and survival, with no net negative effects to their own nutrient and water uptake (Simard and Durall 2004, Selosse et al. 2006). Therefore, because older trees can act as both a refuge of fungi to colonize seedlings as well as provide an opportunity to share resources, seedlings in disturbed sites with intact trees have an advantage for development. Considering the nature of surface mining, it is impossible to preserve older trees to help reclaim sites. However, if there are seedling recruitment issues over time, underplanting seedlings might be a valuable practice as the trees age.

4.2 Limitations and future directions

Taken together, this thesis demonstrates that tree species may host different fungal communities based on both the severity of a disturbance and what type of soil is used to reconstruct a site after a disturbance and that this interaction is a stronger driving factor for structuring fungal communities compared to stand composition. This work is essential in that it creates a starting point to measure EMF community trajectory over time across different types of disturbances and emphasizes the importance of studying the influence of both biotic and abiotic

factors on fungal community composition. While the research presented in this thesis takes important steps forward in understanding the interaction between multiple factors in structuring EM fungal communities, there were still limitations that future work should try to address and expand upon. Below are a few key points:

Measure both biotic and abiotic factors: The interaction of many components that make up forest ecosystems makes it difficult to singularly assess which are the most essential to measure in relation to EMF community composition. For example, the salvaged soils from the forest reclamation site in this thesis had different properties, and all these small-scale characteristics might have caused some of the variation in the EM fungal communities. Therefore, when considering soil properties, it might be valuable to measure the influence of factors such as texture, water holding capacity, bulk density and microbial communities alongside more commonly seen measurements of nutrient status, organic matter content and pH. Aboveground components, such as tree and understory composition, could interact with soil properties via influencing litter components, nutrient status, light and moisture levels and therefore should also be taken into consideration.

Compare EMF community functionality across disturbances: The work presented in Chapter 2 showed that EM fungal communities could differ between a harvest and mining disturbance, depending on species of host tree. However, this study was limited to measuring fungal communities between anthropogenic disturbances on two sites. It would be valuable to compare EM fungal communities on oil sands reclamation sites to other natural disturbances, such as fire, because past work has found differences in EMF community composition between harvest and fire disturbances (Dahlberg et al. 2001, Smith et al. 2005, Barker et al. 2013). Additionally, this study did not investigate if changes in EMF community composition affected

enzymatic activities of the fungi, which could have important consequences for nutrient mobilization and uptake. Ectomycorrhizal fungi are able to use a variety of extracellular enzymes to break down organic matter and acquire valuable nutrients, such as nitrogen and phosphorus, but the ability to use these enzymes can vary among EMF species (Smith and Read 2008). For example, it was found that both fire and harvest disturbances changed EMF community composition and individual enzymatic function of EMF species on Douglas fir seedlings, but at the plot level overall enzymatic function was comparable to an undisturbed control site (Jones et al. 2010). This indicates that disturbed EM fungal communities can still function in a similar way to undisturbed communities. Comparatively, EMF enzyme profiles differed between spruce seedlings on a forested versus harvested site, but overall biomass of the seedlings did not change (Walker et al. 2016). Although biomass was not different between disturbed and undisturbed seedlings, the difference in enzyme profiles implies that this trend may change with time and warrants further investigation. Perhaps functional redundancy in EM fungal communities signifies that changes to composition or richness may not have overall negative effects, but more research over longer study periods is needed to confirm this. It also lends support to the idea that while it is important to measure EMF richness, it is also imperative to consider the presence of all essential functional groups within the community (Cardinale et al. 2012). Future work should strive to measure differences in EMF community composition, enzyme function, functional genes and subsequently, the effect on tree productivity using different ages and species of host trees over time. Particularly, this information could be valuable for forest reclamation sites, to assess forest recovery and function (i.e. nutrient mobility, carbon storage).

Role of common mycorrhizal networks (CMN): It was previously mentioned the role that CMN can play in seedling establishment from receiving resources from adult trees (Chapter 2).

However, there is considerable less knowledge about the roles these networks can play between seedlings of the similar ages. Although it was beyond the scope of this thesis, advantages or disadvantages of these networks could provide some context for level of preference certain genus or species of trees show for fungal partners. In some cases, it could be advantageous for different trees to host similar EM fungal communities as the CMN allow for nutrient, water and carbon transfer between trees (Simard and Durall 2004). Past studies have shown that bi directional transfer of carbon isotopes is possible between trees of different species (Simard et al. 1997b, Philip, Simard and Jones 2010). However, whether these networks are beneficial over long periods of time remains to be tested as it could lead to one tree species benefiting more than the other. A hypothesis that needs further examination is that the CMN could still be advantageous if one tree species is fast growing and has carbon it can allocate to a slower growing species, similar to an adult tree supplying resources to a seedling. On the other hand, there has been speculation that an advantage to different tree species hosting different EM fungal communities is to compete for resources and avoid possible parasitism that could come from CMN (Cullings et al. 2000, Selosse et al. 2006, Tedersoo et al. 2011). This may be one explanation for the strong driving effect of host seen in the mixed-species tree plots in Chapter 3 and in past work on mixed-species stands. Again, relating back to functional diversity of different EMF species, if each EMF community on the different tree species function in the same way, the growth and development should not be hindered from not forming CMN. Looking forward, forest reclamation sites like the one present in this thesis, provide an intriguing opportunity to address some of these unknowns about the role of CMN in forest function. Do CMN form between trees in single-species stands? In consequence, is tree growth and development similar in single versus mixed-species stands? Do the EM fungal communities on

the trees function in the same way in single versus mixed-species stands? Addressing these types of questions on forest reclamation sites can help to develop management practices for planting seedlings and importantly, linking belowground function to aboveground productivity.

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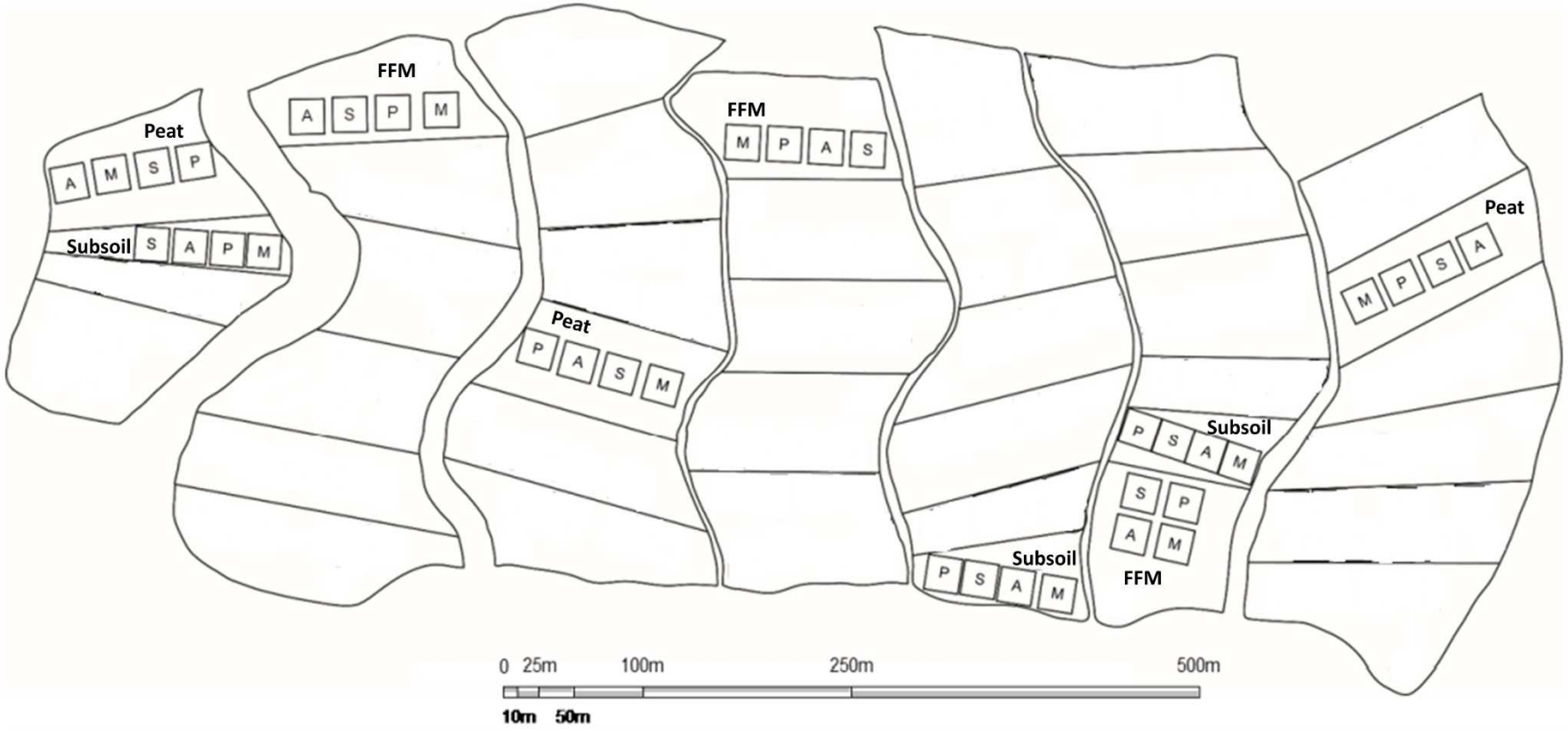
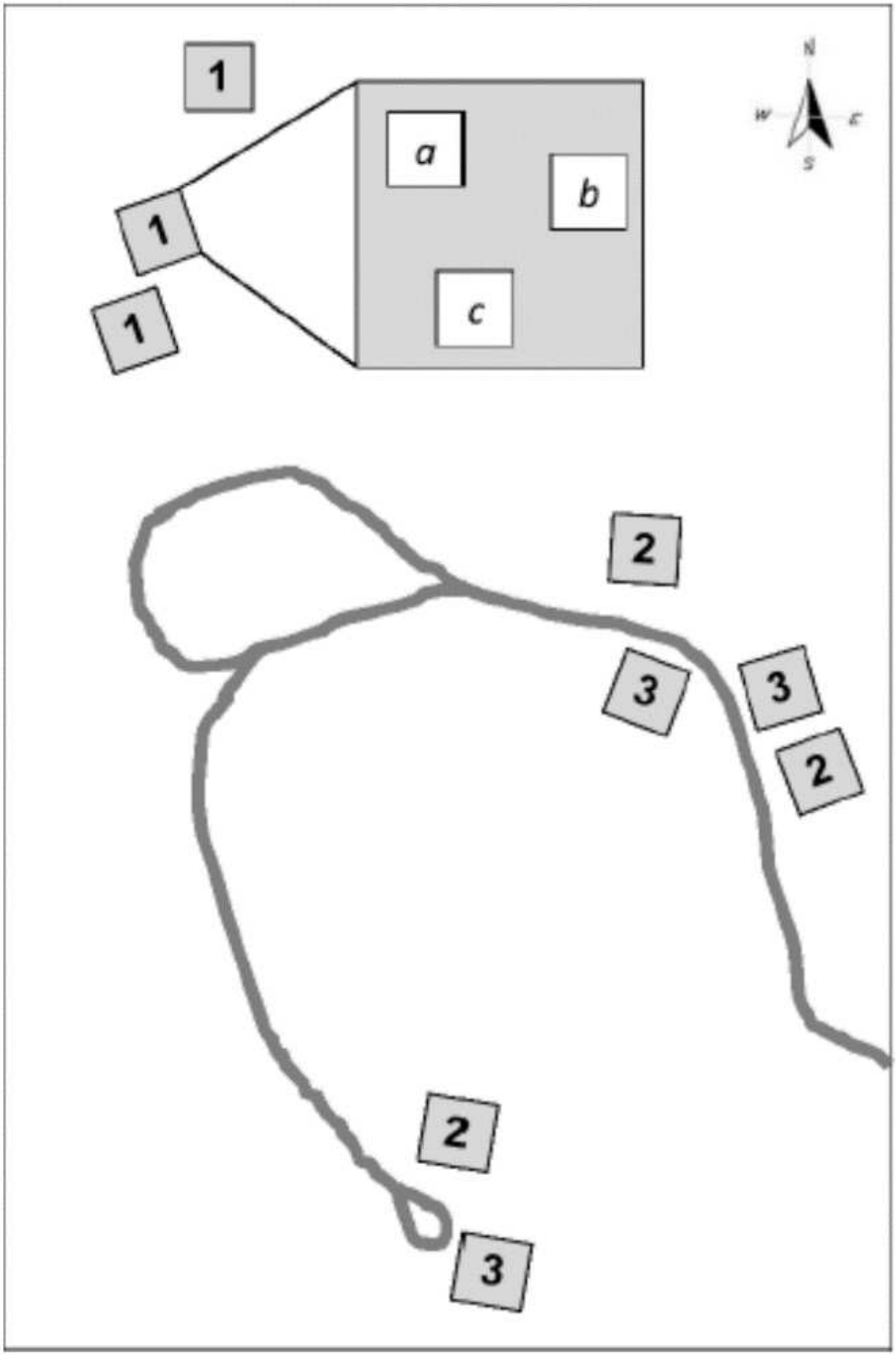
Appendices

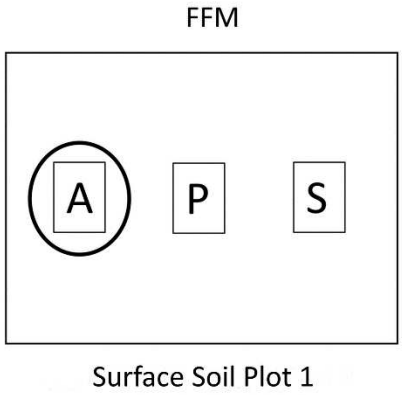
Appendix I. Layout of the Reclamation Site, showing surface soil plots of Forest Floor Material ('FFM'), 'Peat' and 'Subsoil' (n=3). Each surface soil plot contains four 25 x 25 m tree plots. Three of the tree plots were planted with single species, trembling aspen, jack pine or white spruce and one plot with a mixture of all three species. All tree plots were planted in May 2012 at 10,000 stems per hectare and a subset of these seedlings were used to assay the ectomycorrhizal community. Tree plots are labeled as A (trembling aspen), P (jack pine), S (white spruce) or M (mixture of all three) (Hankin et al 2015).


Peat
A M S P
Subsoil S A P M
FFM
A S P M
Peat
P A S M
FFM
M P A S
Subsoil
P S A M
S P
A M
FFM
Subsoil
P S A M
Peat
M P S A
0 25m 100m 250m 500m
10m 50m

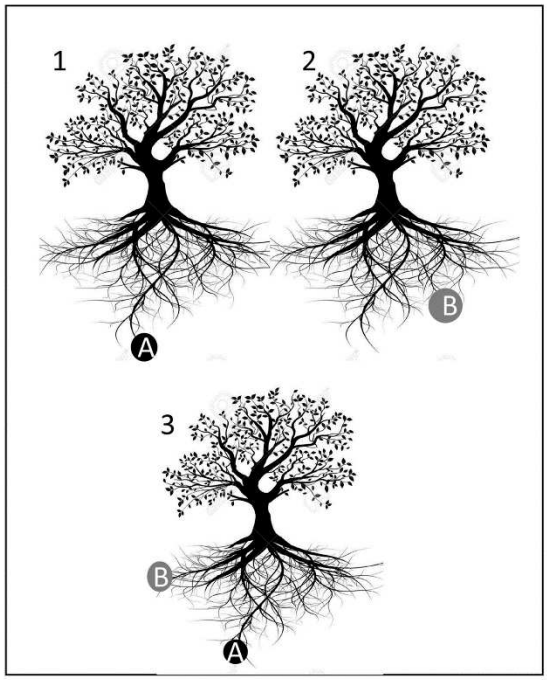
Appendix II. Layout of the Reference Site used to compare EM fungal communities to those assayed on the Reclamation Site. The Reference Site was located approximately 5 km northeast of the Reclamation Site on the Syncrude Canada Ltd.- Aurora mine site. The Reference Site included forests varying in extent of disturbance severity: (1) a 'Control' site which consisted of an intact jack pine forest, (2) a 'Disturbed' site which was clearcut in the past, but still had an organic forest floor intact and (3) a 'Removed' site that had the same clearcut but also had the organic forest floor removed. Each disturbance severity had plots (1-3) that were replicated three times contained three 2.5 x 2.5 m tree plots (a-c) separated by at least 2 m. In May 2012, each tree plot was planted with 6-8 seedlings of trembling aspen, jack pine and white spruce from the same seedling stock as the Reclamation Site.


1
a
b
1
c
1
N
W
E
S
2
3
3
2
2
3

Appendix III. Example of how EMF relative abundance values were calculated at the Reclamation Site. These values were used a way of measuring abundance of the EMF community to use for statistical tests looking for differences in the community composition between two testing sites. Values were calculated in this way to account for variation in sampling level, site design and successful root tip amplification. First, each OTU was assigned a proportional value per individual planted seedling. Second, the proportional values were pooled for each OTU then divided by the total number of planted seedlings in the surface soil plot.



Surface Soil Plot 1



Tree Plot

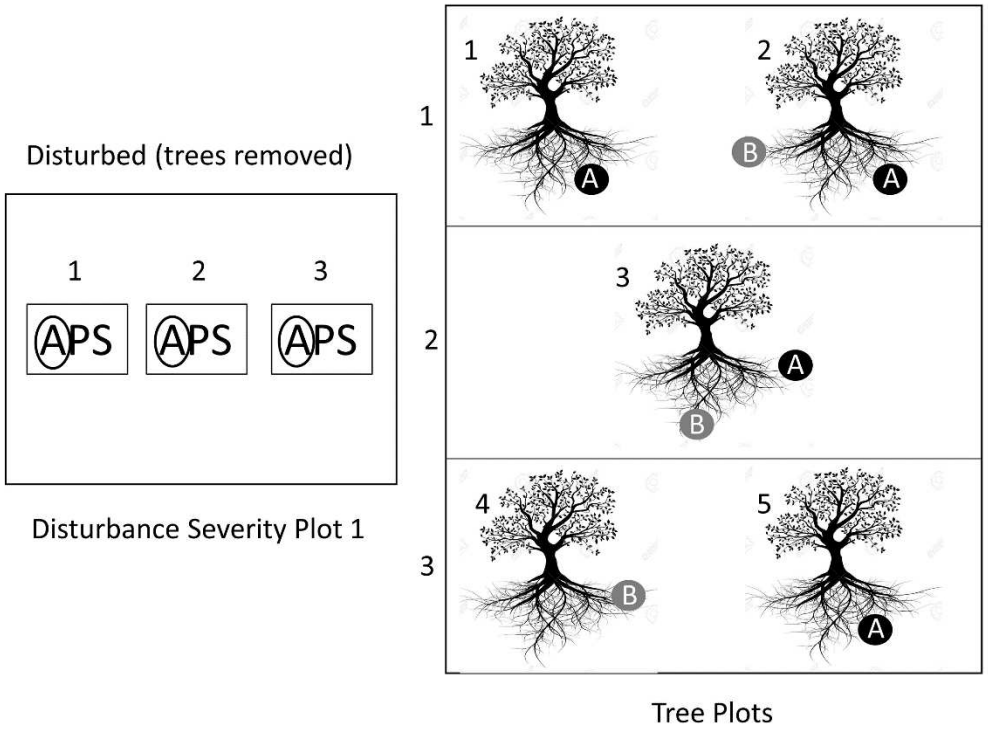
Seedling 1:
OTU A= 1.00

Seedling 2:
OTU B=1.00

Seedling 3:
OTU A=0.5
OTU B=0.5

Surface Soil Plot 1:
OTU A=(1.00+0.5)/3=0.5
OTU B=(1.00+0.5)/3=0.5

Appendix IV. Example of how EMF relative abundance values were calculated at the Reference Site. These values were used a way of measuring abundance of the EMF community to use for statistical tests looking for differences in the community composition between two sites. Values were calculated in this way to account for variation in sampling level, site design and successful root tip amplification. First, each OTU was assigned a proportional value per individual planted seedling. Second, the proportional values were pooled for each OTU then divided by the total number of planted seedlings in the disturbance severity plot. The tree plot level was ignored so that these values would be equivalent to those calculated for the Reclamation Site.



Tree 1:
OTU A= 1.00

Tree2:
OTU A=0.5
OTU B=0.5

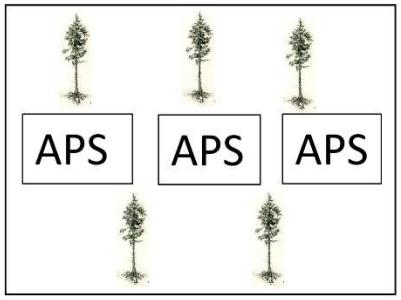
Tree 3:
OTU A=0.5
OTU B=0.5

Tree 4:
OTU B=1.00

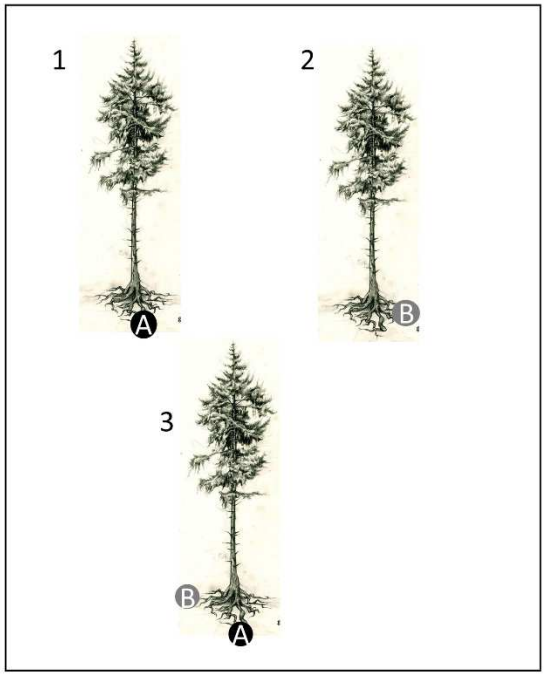
Tree 5:
OTU A=1.00

Disturbance Severity Plot 1
(ignoring tree plots):
OTU A=(1.00+0.5+0.5+1.00)/5=0.6
OTU B=(0.5+0.5+1.00)/5=0.4

Appendix V. Example of how EMF relative abundance values were calculated for the established jack pine trees/saplings at the Reference Site. These values were used a way of measuring abundance of the EMF community to use for statistical tests looking for differences in the community composition between host types. Values were calculated in this way to account for variation in sampling level, site design and successful root tip amplification. First, each OTU was assigned a proportional value per individual established tree or sapling. Second, the proportional values were pooled for each OTU then divided by the total number of established trees or saplings in the disturbance severity plot.



Disturbance Severity Plot 1



Established Jack Pine in
Disturbance Severity Plot 1

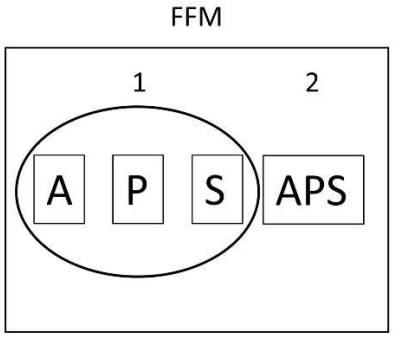
Tree 1:
OTU A= 1.00

Tree 2:
OTU B=1.00

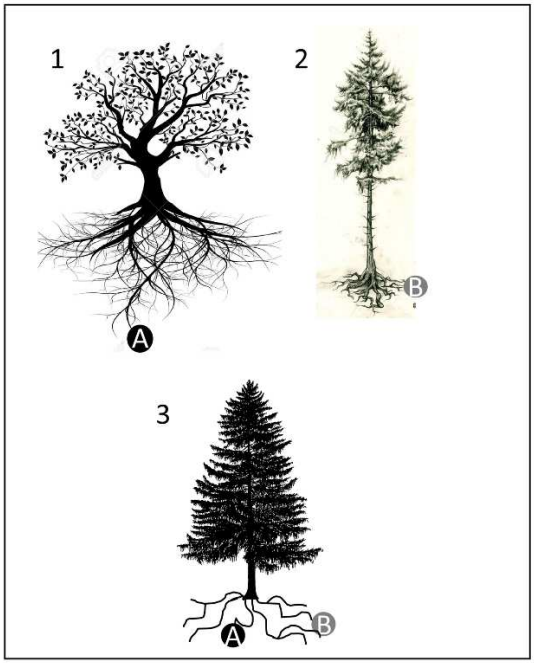
Tree 3:
OTU A=0.5
OTU B=0.5

Disturbance Severity Plot 1:
OTU A=(1.00+0.5)/3=0.5
OTU B=(1.00+0.5)/3=0.5

Appendix VI: Example of how EMF relative abundance values were calculated to determine whether mixed-species tree plots had additive or synergistic effects on EMF community composition and species richness. Within each surface soil plot, the three single-species tree plots were combined and treated as one plot and compared to the mixed-species tree plots. First, each fungal OTU was assigned a proportional value per individual planted seedling. Second, the proportional values were pooled for each OTU then divided by the total number of planted seedlings in the tree plot.



Surface Soil Plot 1



A+P+S Plot 1

Tree 1:
A= 1.00

Tree 2:
B=1.00

Tree 3:
A=0.5
B=0.5

A+P+S Plot 1:
A=(1.00+0.5)/3=0.5
B=(1.00+0.5)/3=0.5