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The introduction of nursery seedlings and their fungi to a spruce-fir forest in Newfoundland

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Abstract

A few species of boreal trees form symbiotic relationships with many ectomycorrhizal (ECM) fungi that allow trees to thrive on nutrient poor soils in Canada. A small number of ECM species grow on tree seedlings raised in nurseries for reforestation of clearcut sites. The native ECM fungal community composition of a mixed spruce-fir forest in Newfoundland was determined through next generation sequencing. With the introduction of nursery seedlings, the transfer of native ECM fungi to seedlings and nursery-established ECM fungi to roots was investigated over 20 months. ECM community composition was found to be similar to that of other boreal forests in Canada. Seven taxa likely transferred from roots to seedlings but none were transferred from seedlings to roots. Maintaining the composition of native ECM communities is key to forest health and this study suggests that reforestation practices in Canada do not alter native ECM community composition within the studied time-frame.

Keywords: ectomycorrhizal (ECM) fungi, boreal forest, forestry, clearcut, ectomycorrhizal (ECM) community composition, nursery ectomycorrhizal (ECM) fungi, native ectomycorrhizal (ECM) fungi, DNA sequencing, next generation sequencing

Summary for Lay Audience

Mutually beneficial relationships between fungi and plant roots, termed mycorrhizal associations, are found in over 90% of terrestrial plants. There are different types of mycorrhizal fungi, including ectomycorrhizal (ECM) fungi, which form underground connections with tree roots in forest stands and are of importance in Canada for their production of edible mushrooms such as chanterelles (*Cantharellus*) and supporting boreal tree species by performing functions such as nutrient and water uptake. The assemblage of ECM fungi in a given area is referred to as a community and the composition of a community may be influenced by the plant community, soil moisture, temperature, acidity, climate and other ecological factors. Previous studies have determined the ECM community composition of boreal forests in Canada, but there are currently no published studies of Newfoundland forests.

In Canada, tree seedlings raised in nurseries are often planted for the purpose of reforestation in forest sites that have been clearcut and have their own ECM community composition, with many of the same species found in nurseries globally. Little is known about the effect that ECM fungi established on nursery seedlings have on natural forest ECM fungi once the seedlings are planted.

By sequencing fungal DNA found on roots, my project determined the natural community composition of ECM fungi of a mixed spruce-fir forest in Newfoundland, and with the introduction of nursery seedlings to the same forest, the transfer of natural ECM fungi to seedlings and nursery-established fungi to natural roots was investigated over 20 months. ECM community composition was found to be similar to that of other boreal forests in Canada. Seven ECM fungi likely transferred from natural roots to seedlings, and no ECM fungi transferred from seedlings to natural roots. Due to the important role of ECM fungi in supporting forests, maintaining the composition of natural ECM communities is key and this study suggests that reforestation with nursery raised seedlings in Canada does not alter natural ECM community composition within a short time-frame.

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1 Introduction

1.1 Mycorrhizal associations

Symbiotic interactions between fungi and plant roots, termed mycorrhizal associations, are found in over 90% of terrestrial plants (Wang and Qiu, 2006) and are a popular topic of interest in scientific research because of their potential contribution to the evolution of terrestrial plants (Pirozynski and Malloch, 1975) and their potential benefits to plant growth (Menge, 1983, Peterson et al., 1983). Different mycorrhizal types have evolved independently of each other over time, with the four most common types being arbuscular mycorrhizae, ericoid mycorrhizae, orchid mycorrhizae and ectomycorrhizae (Strullu-Derrien et al., 2018).

1.2 Ectomycorrhizal associations

Ectomycorrhizae (ECM) are found in only three percent of seed-producing plants but they are mostly associated with trees and other woody plants which occupy much of the global land surface and provide economic value as timber (Smith and Read, 2008). Ectomycorrhizal associations were discovered as early as the late 1800s (Frank, 2005) and can be recognized by four main structural features: a mantle of fungal tissue covering the mycorrhizal root tip, hyphae growing between the epidermal and cortical cells of the root to form a structure called a Hartig net, hyphae obtaining nutrients and water by growing into the surrounding soil, and the absence of intracellular penetration of root cells (Smith and Read, 2008). In most cases all four features can be found but since different ECM fungi likely independently evolved from

saprotrophic fungi on multiple occasions, some ectomycorrhizal associations lack the mantle structure or occasionally penetrate root cortical cells (Smith and Read, 2008). For example, the fungus *Wilcoxina mikolae* often penetrates the root cortical cells of *Pinus* and *Larix*, but does not penetrate root cortical cells of *Abies*, *Picea*, or *Tsuga* (Mikola, 1988). Evidence from the fossil record shows that ECM associations have existed for at least 50 million years (LePage et al., 1997, Dighton, 2009), with some scientists hypothesizing that ECM associations date back more than 130 million years based on geographic and phylogenetic origins of plants which form ECM associations (Smith and Read, 2008).

It was been previously estimated that between five and six thousand species of fungi form ectomycorrhizae (Molina et al., 1992) but with the introduction of sequencing techniques such as next generation sequencing (NGS), a more recent estimate is between 20,000 and 25,000 (Rinaldi et al., 2008). Of the known fungal species that participate in ECM associations, most are in the phyla Basidiomycota and Ascomycota, and few are in the Mucoromycota phyla (Dighton, 2009, Tedersoo et al., 2018). These fungal species form ECM associations with approximately six to seven thousand plant species (Tedersoo and Brundrett, 2017), and in Canada, typically woody perennial plants and shrubs in the families Betulaceae, Cistaceae, Fagaceae, Pinaceae, and Salicaceae (Dighton, 2009, Brundrett and Tedersoo, 2020). While many ECM fungal species are generalists, some are specialists associating with fewer plant species such as fungal species in the genus *Rhizopogon*, which only form associations with plants in the Pinaceae family (Harley and Smith, 1983).

1.3 The function of ectomycorrhizae

The known function of ectomycorrhizae in ecosystems is nutrient and water acquisition. Since fungal hyphae produce greater surface area than plant roots, the hyphae of the fungal partner can scavenge the soil and acquire water that the plant roots cannot reach and nutrients, in particular nitrogen, that plant roots cannot absorb (Molina et al., 1992, Teste et al., 2009). In addition, many ECM fungal species can access complex organic forms of N that are not available to plants (Abuzindah and Read, 1989, Read et al., 1989). The acquired resources

are traded with the plant partner at the Hartig net and in return, the fungal partner receives photosynthates for energy in proportions as great as 13% of the plant partner's total fixed carbon (Hawkins et al., 2023).

1.4 Ectomycorrhizal fungi as a network and community

Tree individuals form associations with multiple fungal species simultaneously on different root tips with the number of connections seemingly depending on tree species and age. Seedlings of *Tsuga diversifolia* connect with up to 40 ECM fungal species (Yoshida et al., 2014) and mature individuals of *Populus* spp. connect with up to 200 ECM fungal species (Bahram et al., 2011). Since trees can host many ECM fungal species, forests with a low diversity of host trees can still support a high diversity of ECM fungi. For example, a study of a boreal forest consisting of mostly *Pinus banksiana* was found to support 58 different ECM fungal taxa (Danielson, 1984). Each individual of an ECM fungal species forms a network that connects plants of different species (Molina et al., 1992) and of any age, with one study showing that one individual of *Pseudotsuga menziesii* was connected to nearly 50 other individuals of the same tree species through 13 individuals of *Rhizopogon vinicolor* and 14 individuals of *Rhizopogon vesiculosus* (Beiler et al., 2010), both of which are false truffles.

1.4.1 Potential functions of ectomycorrhizal networks

Recent debate has risen within the scientific community on the extent of the function of ECM networks. Studies using microsatellite techniques have shown that ECM networks may connect most trees within forests (Lian et al., 2006, Beiler et al., 2010, Beiler et al., 2012, Beiler et al., 2015, Dorp et al., 2020) but scientists have raised question to the transferability of these results to all forests given that the studies have been limited to only one hectare of the world's four billion hectares, and two tree species and three fungal species (Karst et al., 2023).

Further, recent studies have claimed that nutrients and water are transferred between trees

connected by an ECM network which can improve seedling function but citing research that does not support that claim. Simard et al. (1997) showed that a net transfer of carbon between *Betula papyrifera* and *Pseudotsuga menziesii* took place, suggesting that this occurred through the ECM network, but alternative explanations such as soil transfer are possible (Karst et al., 2023). In Teste et al. (2009), performance of *Pseudotsuga menziesii* var. *glauca* was shown to improve when connected to ECM fungi but the sampling design of the study may play a role in the results, given that control seedlings that were not connected to the network were placed in mesh bags, reducing soil availability for resource extraction (Karst et al., 2023). Song et al. (2015) showed transfer of carbon from *Pseudotsuga menziesii* to *Pinus ponderosa*; however, this study was not performed in the field and therefore cannot be used to draw conclusions about the function of ECM networks in forests. There are also recent claims that older trees will supply resources through the ECM network preferentially to their kin. In a thesis study (Asay, 2013), kin seedlings were found to have a higher percentage of colonized root tips than non-kin when planted adjacent to older kin seedlings in a greenhouse suggesting evidence of kin recognition but no peer-reviewed field study has been published supporting the same findings. ECM networks may have extensive roles in supporting forests but the full extent of their function is not fully understood.

1.4.2 Factors influencing ectomycorrhizal community composition

The assemblage of fungal species found on a particular plant individual or in a given area is referred to as an ECM fungal community. It is not known how the composition of any given ECM community is determined, but many factors have been shown to predict ECM community composition, including host plant phylogeny, environmental conditions, and host tree age. Host specificity of ECM fungal species ranges from high specificity, where a fungus associates with specific genera or families of trees to low specificity, where a fungus associates with hosts across different orders of trees. For example, *Cantharellus betularum* is known to associate only with *Betula* (Thorn et al., 2020), species of *Rhizopogon* are known to associate mostly with Pinaceae (Molina et al., 1999), and some species of *Laccaria* are known to associate with both gymnosperms and angiosperms (Molina et al., 1992).

Tedersoo et al. (2013) found that a higher percentage of variation in ECM fungal community composition in mixed conifer-broadleaf forests in Japan could be explained by host plant phylogeny rather than specific host plant identity, suggesting that host plant phylogeny is the most influential factor in ECM fungal community composition. Global analysis using datasets from many studies revealed that of all factors, host plant phylogeny had the strongest effect on ECM community composition (Tedersoo et al., 2012). In a study in Siberia, the ECM community composition of forests dominated by *Larix cajanderi*, *Betula pendula*, and *Pinus sylvestris* was also shown to be characterized by host plant phylogeny, with certain ECM fungal species within the genera *Cortinarius* and *Tricholoma* more likely to be found on Pinaceae hosts and other fungal species within the genera *Russula*, *Laccaria* and *Inocybe* more likely to be found on angiosperms in the understory despite the low host specificity that they generally exhibit (Miyamoto et al., 2022). This suggests that host plant phylogeny has a stronger effect on ECM community composition regionally rather than globally.

It has been hypothesized that environmental conditions such as temperature, soil pH, soil moisture, soil nutrient content, host plant age, and host plant species richness influence ECM community composition. ECM community composition of *Fagus orientalis*, *Carpinus betulus*, *Quercus castaneifolia* and *Betula pendula* in Iran was most significantly affected by temperature, suggesting that some ECM fungal species have specific temperature preferences (Bahram et al., 2011). For example, species of *Inocybe* are often the dominant component of ECM communities in arctic environments on host plants within different families, including Pinaceae, Betulaceae, Salicaceae, and Rosaceae, implying an increased ability of *Inocybe* spp. to tolerate cold temperatures (Miyamoto et al., 2022, Geml et al., 2012, Timling et al., 2012, Blaalid et al., 2014).

Soil pH has been shown to correlate with ECM community composition with different host trees and across multiple continents, including with *Pinus sylvestris*, *Picea abies*, and *Betula pubescens* in Sweden (Toljander et al., 2006), *Pinus sylvestris* in Germany (Cox et al., 2010), and *Pinus contorta* and *Pinus albicaulis* in California, USA (Glassman et al., 2017). Soil pH was one of the strongest predictors of ECM fungal diversity in a study of forested sites across each of the 11 biomes (Tedersoo et al., 2014). Soil moisture was correlated with ECM

community composition of *Salix* spp. in Minnesota, USA (Erlandson et al., 2016), and of *Pinus sylvestris* in Scotland (Jarvis et al., 2013) and soil nutrient content including available nitrogen, phosphorus, and sodium were correlated to ECM community composition of *Pinus sylvestris*, *Pinus contorta*, *Pinus albicaulis*, and *Salix* spp. in Europe, California, USA, and Minnesota, USA (Cox et al., 2010, Glassman et al., 2017, and Erlandson et al., 2016).

Host plant age was shown to be a strong predictor of ECM community composition in a study of *Pinus contorta* and *Pinus albicaulis* in California, USA (Glassman et al., 2017), and ECM community composition between three different aged forests of *Pinus sylvestris* in China was significantly different with diversity increasing with stand age (Guo et al., 2020). One study found that ECM community composition of *Corylus avellana* were significantly affected by host plant age, while the ECM community composition of *Alnus glutinosa* and *Crataegus monogyna* were more affected by soil properties (Boeraeve et al., 2018). Studies have shown that some genera including *Russula* and *Piloderma* increase in abundance and frequency as stand or host age increases (Twieg et al., 2007, Visser, 1995, Smith et al., 2002, Smith et al., 2000), and other taxa including *Rhizopogon vinicolor* and *Laccaria* spp. have been shown to decrease in abundance and frequency as stand or host age increases (Twieg et al., 2007, Nara et al., 2003). Studies which have determined that taxa decrease in abundance and frequency as stand or host age increases have found less consistent results than studies which have determined that some taxa increase in abundance as stand or host age increases, likely a result of many studies taking place at secondary successional sites with previously established inoculum in the soil.

Additionally, species richness of host plants was a predictor of ECM fungal richness in a study of forested sites across each of the 11 biomes (Tedersoo et al., 2014). Many studies show that multiple factors are strong predictors of the ECM community composition, suggesting that many factors are involved in determining the ECM community composition simultaneously (Glassman et al., 2017, Guo et al., 2020, Tedersoo et al., 2014).

1.4.3 Methods of determining ectomycorrhizal community composition

Throughout most of the 20th century, macroscopic and microscopic morphological features of ECM root tips were most commonly used to identify ECM fungal species (Agerer, 1986, Agerer, 1991, Malloch and Thorn, 1985, Goodman et al., 1998). Surveys of fruiting bodies were also used to infer which ECM fungal species would be found on nearby trees but this technique was shown to exclude many ECM fungi in comparison to other methods (Menge and Grand, 1978, Dahlberg et al., 1997, Gardes and Bruns, 1996) and was therefore rarely used as the sole identification technique in studies. ECM fungal species have also previously been identified through isolation and culturing, allowing more ECM fungal species to be identified compared to fruiting body and morphological identification techniques. Fruiting body and morphological identification studies have likely overlooked many ECM fungal species due to the dependence of ECM fungi on weather conditions and season to produce fruiting bodies, the inability of some ECM fungi to grow in pure culture (Zak and Marx, 1964, Lamb and Richards, 1970), and the uniformity in morphological features of different ECM fungi.

Molecular identification techniques gained popularity throughout the 1990s and early 2000s, including restriction fragment length polymorphism (RFLP) (Iwanski et al., 2006), direct PCR amplification from individual washed ECM root tips (Gardes and Bruns, 1993), DNA cloning and sequencing (Geml et al., 2012), and pyrosequencing (Blaalid et al., 2014). Geml et al. (2012) used DNA cloning and sequencing to identify ECM fungi at a site in Norway where a previous morphological identification study had been done (Vare et al., 1992) and found many fungal taxa that had not been identified in the previous study, including mostly fungi with rare or inconspicuous fruiting bodies. Although DNA cloning and sequencing outperforms morphological identification, it is both a labour-intensive and costly technique and produces a smaller number of clones per sample (2-12) in comparison to NGS (Lynch and Thorn, 2006). Currently, NGS is one of the most popular methods for ECM fungal identification, providing more cost-efficient and less time-consuming sequencing for a high volume of samples in comparison to other sequencing methods such as Sanger sequencing (Shendure and Ji, 2008). NGS generates millions of reads from many different fungi and the investigator can then attempt to

match them with known sequences in a database.

1.4.4 Limits of ectomycorrhizal community research

The nuclear ribosomal internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) was selected as the universal fungal barcode (Schoch et al., 2012) and data from either ITS1 or ITS2 obtained using NGS through the Illumina MiSeq platform are often used to infer ECM community composition (Taylor et al., 2016). Throughout the DNA extraction, amplification and sequencing processes, many artifacts and biases are introduced due to varying DNA extraction efficiency among fungal taxa (Feinstein et al., 2009), primer selection (Jumpponen, 2007), varying amplification efficiency among taxa (Polz and Cavanaugh, 1998), varying copy numbers of the tandemly repeated nuclear ribosomal operon (Maleszka and Clark-Walker, 1993, Ganley and Kobayashi, 2007), and varying amplicon length among fungal taxa (Ihrmark et al., 2012). Some artifacts and biases can be avoided by using primers designed to amplify ITS2, a smaller portion of the ITS region with less length variation than ITS1 (Nilsson et al., 2019). These primers have been shown to produce more diverse amplicon communities than longer amplicons (Ihrmark et al., 2012, Taylor et al., 2016), but ECM community composition and abundance remain affected by primer biases, varying amplicon length, and varying copy number.

1.4.5 Introduced fungi influencing ectomycorrhizal community composition

It has been estimated that over 200 species of ECM fungi have been introduced on host plants to non-native habitats accidentally and intentionally, with varying effects on native ECM community composition (Vellinga et al., 2009). *Amanita phalloides* is a generalist ECM fungal species native to Europe which has been introduced to Africa, Australia, New Zealand, and North America through the planting of various non-native host plants (Pringle et al., 2009). In most ecosystems, *Amanita phalloides* has only been found associating with the introduced host plants, but it has been found associating with native *Eucalyptus* in Africa and Australia (Pringle

and Vellinga, 2006) and more recently, on native *Quercus garryana* in British Columbia (Berch et al., 2016). Introduced fungi that persist only on introduced host plants do not alter native ECM community composition but introduced fungi that spread from introduced host plants and associate with native host plants do alter native ECM community composition. Other ECM fungi have been moved from native to non-native habitats intentionally for cultivation of fruiting bodies (i.e.; in the case of *Tuber*; Berch and Bonito, 2016) and to promote growth and survival of host trees in commercial forestry settings (Vozzo and Hacskaylo, 1971, Boyle and Hellenbrand, 1991, Menkis et al., 2007, Theodorou and Bowen, 1973).

1.5 Commercial forestry and ectomycorrhizal networks

1.5.1 Inoculation of seedlings

Intentional inoculation of host trees with non-native ECM fungi began in the early 1800s with the cultivation of *Tuber* in southern France (Hall et al., 1998). Joseph Talon developed a system to cultivate *Tuber*, a fungus that produces highly prized edible truffles, by planting acorns near oak trees. When the acorns grew into seedlings, he would transplant them to a new location and find fruiting bodies in that new location shortly after. With a more thorough understanding of ectomycorrhizal associations, it is now understood that oak trees form ectomycorrhizal associations with *Tuber*, thus planted acorns would develop root systems that become inoculated as a result of their close proximity to oak trees (Hall et al., 1998).

In the early 1970s, scientists developed methods to inoculate seedlings in nurseries with ECM fungal species believed to increase seedling growth and survival including *Rhizopoogon roseolus*, *Suillus cothurnatus*, *Pisolithus tinctorius*, *Paxillus involutus*, and *Hebeloma longicaudum* (Smith and Read, 2008, Boyle and Hellenbrand, 1991, Menkis et al., 2007, Theodorou and Bowen, 1973, Vozzo and Hacskaylo, 1971). Increased seedling performance was seen in some cases; for example an increased tolerance to drought was found in seedlings of *Picea mariana* inoculated with *Laccaria laccata*, *Paxillus involutus*, *Pisolithus tinctorius*, and *Hebeloma longicaudum* (Boyle and Hellenbrand, 1991) and an increase in survival and growth was found in

seedlings of *Pinus sylvestris* and *Picea abies* inoculated with *Cenococcum geophilum* and the ectomycorrhizal morphotype *Piceirhiza bicolorata* (Menkis et al., 2007; the latter now known as members of the *Hymenoscyphus ericae* clade; Vrålstad et al., 2000). Other studies found no change in seedling performance (Rincón et al., 2001, Alvarez and Trappe, 1983, Cram et al., 1999) or decreased seedling performance (Rincón et al., 2001, Alvarez and Trappe, 1983, Hung and Trappe, 1987, Stenström et al., 1990) with the inoculation of ECM fungi.

ECM fungal species have varying persistence once seedlings are outplanted. Some species have shown long persistence: *Pisolithus* spp. inoculated on seedlings of *Eucalyptus* persisted for three years (Dell et al., 2002), *Laccaria bicolor* inoculated on seedlings of *Pseudotsuga menziesii* persisted for three years (Battista et al., 2002), *Suillus collinitus* inoculated on seedlings of *Pinus halepensis* persisted for four years (El Karkouri et al., 2006), and *Amanita muscaria* inoculated on seedlings of *Pinus radiata* persisted for 30 years, although these seedlings were planted in Australia where *Pinus radiata* is non-native, limiting the number of other compatible ECM fungal species (Sawyer et al., 2001). Other species have shown shorter persistence: *Rhizopogon* spp. inoculated on seedlings of *Pseudotsuga menziesii* did not persist past two years (Battista et al., 2002). The variation in results of both improved seedling performance and ECM fungal species persistence is likely explained by a multitude of factors including tree species, fungal species, soil, and climate (Menkis et al., 2007).

1.5.2 Weedy ectomycorrhizal fungal species

Studies on the effects of ECM fungal inoculation on seedling performance are complicated by the presence of weedy ECM fungal species which can act as potential competitors for inoculant species. Weedy ECM fungal species are species which colonize tree seedlings in tree nurseries and have a large global span, presumably because they are ruderal species meaning they have a short vegetative phase, followed by high spore production and low host specificity (Colpaert et al., 1999). All studies characterizing weedy ECM fungal community composition of nursery seedlings have been performed in European nurseries but within Europe, little variation is seen between nurseries. Common weedy ECM fungal species include Basidiomycota such

as *Thelephora terrestris* and *Laccaria* spp. and Ascomycota such as *Wilcoxina mikolae* and *Cenococcum geophilum* (Trocha et al., 2006, Rudawska and Leski, 2021, Iwanski et al., 2006, Colpaert et al., 1999, Aučina et al., 2014, Stenström et al., 2014, Pietras et al., 2013). In a study of *Pinus sylvestris* seedlings derived from four different nurseries in Poland, 13 fungal taxa were found, with two to eight fungal taxa found in each nursery (Iwanski et al., 2006). When 23 nurseries in Poland were surveyed, 29 different fungal taxa were found on *Pinus sylvestris* seedlings, with three to 13 fungal taxa found in each nursery and five taxa found in two or fewer nurseries (Rudawska and Leski, 2021). Additionally, significant differences were found between communities on *Pinus sylvestris* seedlings of different ages, with one-year-old seedlings primarily colonized by Ascomycota and two-year-old seedlings primarily colonized by Basidiomycota (Rudawska and Leski, 2021). In contrast, a study of *Picea abies* seedlings derived from 16 different nurseries in Poland found no significant differences between ECM community composition of one, two, three and four-year old seedlings (Rudawska et al., 2006). Iwanski et al. (2006) found that while seedling age may affect the quantity of certain fungal taxa, it does not seem to affect the presence of fungal taxa on one and two year old seedlings grown in nurseries.

More than half of the forest area harvested in Canada is reforested by outplanting nursery seedlings (Natural Resources Canada, 2020). Many studies on ECM fungi in commercial forestry settings thus far have focused on the effect that outplanting seedlings has on the weedy ECM fungal species associated with seedlings, but the effect that outplanting seedlings and thus their weedy ECM fungal species has on native ECM fungal communities has been greatly understudied. Dahlberg and Stenström (1991) investigated whether native ECM fungi had an effect on nursery seedlings of *Pinus sylvestris* outplanted in young clearcuts, old clearcuts and forests of *Pinus sylvestris* and *Picea abies*, finding that native ECM fungal species colonized almost all seedlings one year after outplanting, increasing from 9-24% ECM root colonization in year one to 28-42% ECM root colonization in year two. However, ECM fungal identification in this study was done using morphological techniques and likely missed a large portion of the ECM community on seedlings. Additionally, the effects that the seedlings had on the native ECM fungal community were not studied in Dahlberg and Stenström (1991) and have not yet

been investigated for any forest ecosystem globally.

There is a remarkable lack of research on ECM fungi given the known and potential functions of ECM networks. There are large gaps in ECM community composition knowledge in certain geographical areas and thus with certain host tree species. Boreal forests represent nearly half of all forests worldwide with 28% found in Canada; however, there has been very little research on ECM community composition in this ecologically and economically valuable region of Canada. Some studies have investigated the ECM community composition of common tree species including *Abies balsamea* (Kernaghan and Patriquin, 2015, DeBellis et al., 2006), *Pinus banksiana* (DeBellis et al., 2006) (Danielson, 1984), *Populus tremuloides* (Visser et al., 1998, DeBellis et al., 2006), *Picea mariana* (Thormann et al., 1999, Robertson et al., 2006, Reithmeier and Kernaghan, 2013), *Larix laricina* (Thormann et al., 1999), and *Picea glauca* (Mah et al., 2001, Kernaghan and Patriquin, 2015) in Canada's boreal forest, but some of the studies have used morphological or other outdated identification techniques and there are geographical gaps that have not been studied. In Newfoundland, an island in eastern Canada which is more than 50% forested, there has been no published investigation of the ECM community composition associated with common boreal tree species including *Abies balsamea*, *Picea glauca*, and *Picea mariana*. Further, the effect that weedy ECM fungal species may have on native ECM fungi has yet to be investigated for any tree species globally. In Newfoundland alone, over 300 million seedlings have been planted since 1974 (Thomas Howe Demonstration Forest, 2020), with no insight on the effect that these seedlings and their associated weedy ECM fungal species have on native ECM communities in forests.

1.6 Research question and objectives

1.6.1 Research questions

a. What is the ECM community composition of a typical boreal forest in Newfoundland?
b. How do nursery seedlings and their weedy ECM species affect native ECM community composition in Newfoundland?

1.6.2 Objectives

The goals of this project were to determine the ECM community composition of a typical boreal forest in Newfoundland and determine whether the introduction of nursery-grown tree seedlings and their weedy ECM fungal species to a boreal forest, a common practice in Canada's forestry industry for reforestation, has an effect on the native ECM community composition found in the forest. The knowledge from this project can be used to inform Canada's current reforestation practices, and potentially lead to further research on how reforestation practices can be improved to preserve the native ECM communities of Canada's boreal forests.

In a mixed balsam fir, black spruce, white spruce and white birch forest near Gander Bay, Newfoundland, Canada, nursery-grown balsam fir, black spruce and white spruce seedlings were planted. Through DNA extraction, amplification and NGS of root and seedling samples collected approximately 12 months and 20 months after seedlings were planted, I:

a. Determined the ECM community composition native to a mixed balsam fir, black spruce, white spruce and white birch stand in a boreal forest in Newfoundland.
b. Determined how the ECM community of a mixed balsam fir, black spruce, white spruce and white birch stand changed temporally after the introduction of nursery-grown tree seedlings and their weedy ECM fungal species.
c. Determined how the ECM community of nursery-grown tree seedlings changed temporally after being introduced to a mixed balsam fir, black spruce, white spruce and white birch stand.

2 Methods

2.1 Site description

The experiment was conducted in an approximately 80 x 40 m forested plot located 70 m above sea level near the Northeastern coast of Newfoundland and Labrador near Gander Bay North (49.39 N 54.57 W) (Figure 2.1).

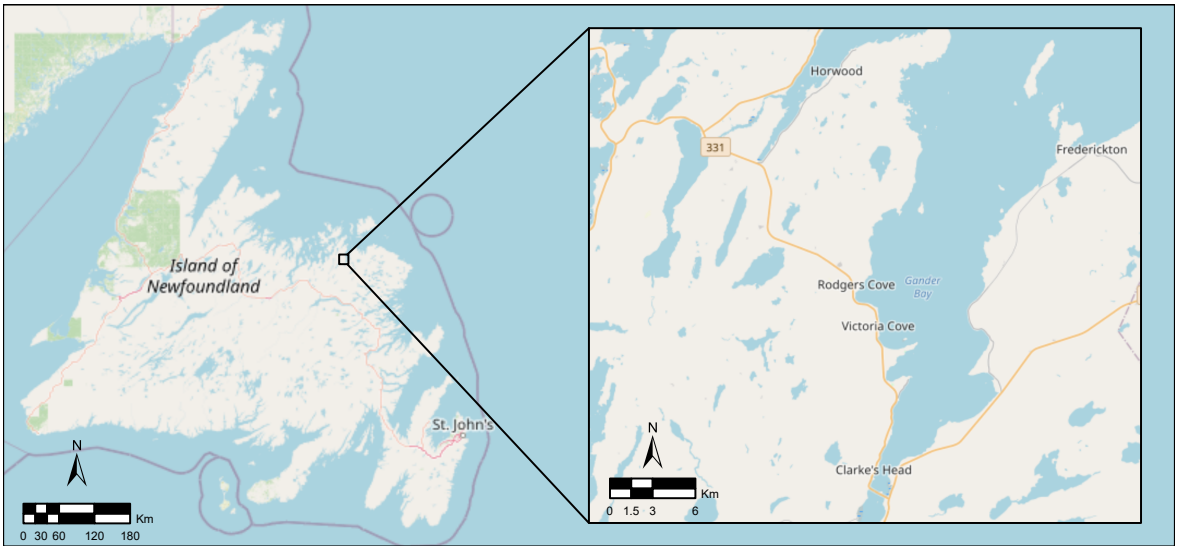

Figure 2.1: Location of experimental site near Gander Bay North, Newfoundland. Created using ArcGIS Pro.

The Damman forest type of the site is Dryopteris-Hylocomium-balsam Fir, characterized by 30-50% fern coverage, a feathermoss understory dominated by *Hylocomium*, and well drained to somewhat moist orthic podzol on sandy loam or loamy sand (Meades and Moores, 1994).

The overstory as determined through point-quarter sampling in September 2021 is composed of approximately 75% balsam fir (*Abies balsamea* (L.) Mill.), 12.5% white spruce (*Picea glauca* (Moench) Voss), 7.5% black spruce (*Picea mariana* Britton, Stearns & Poggenb.), and 5% white birch (*Betula papyrifera* Marshall) and has a density of 3581.53 stems/hectare (See Appendix Table A.1 and Figure A.1 for full metrics determined through point-quarter sampling). For the duration of the study, the mean monthly total precipitation was 76.3 mm (minimum of 25.5 mm and maximum of 134.8 mm), the mean temperature was 6.0 °C (min. -19.1 °C and max. 32.9 °C), the mean humidity was 87.9% (min 21.4% and max. 100.0%), and the mean soil temperature was 6.1 °C (min. -1.6 °C and max. 18.8 °C) (See Appendix Figure A.2 and Figure A.3). Additional data pertaining to overstory composition, understory composition and soil chemical properties are provided in the Appendix.

2.2 Seedling planting

In November 2020, three patches of black spruce and three patches of white spruce seedlings acquired from Wooddale Provincial Tree Nursery (located in Peterview, Newfoundland) were planted in the experimental site. In June 2021, three patches of balsam fir seedlings acquired from Wooddale Provincial Tree Nursery were planted. Each patch was six to eight meters in diameter and consisted of 30-50 seedlings (Figure 2.2).

Hardware cloth with a 0.65 cm opening was placed around white spruce seedling patches immediately after planting to provide protection from grazing by snowshoe hares. Before planting, eight to sixteen randomly selected seedlings of each tree species were stored for later molecular analyses to determine ECM associates acquired in the nursery.

2.3 Seedling and root harvest

In September and October 2021, approximately four to 12 months after planting, and in June and July 2022, approximately 12-16 months after planting, eight to sixteen seedlings from each

of the nine patches were harvested. Seedlings were harvested by removing the entire seedling from the ground, removing non-target plant roots, washing with 0.1 M sodium pyrophosphate, cutting and placing 0.1 g in 750 μL of DNA/RNA Shield (Zymo) to be stored until lab analyses began. In June and July 2022, seedlings were placed back in the ground after a small portion of roots were harvested. In September and October 2021, five 1 x 1 m plots were created around each seedling patch. Two plots were placed within the patch (A and B), one plot was placed on the outside edge of the patch (C), one plot was placed 1m away from the patch (D) and one plot was placed 2m away from the patch (E) (Figure 2.3). In September and October 2021 and June and July 2022, five root sections approximately 3-15 cm from nearby mature trees were collected from each plot at a depth of 5-10 cm, washed with 0.1 M sodium pyrophosphate, cut and 0.1 g was stored in 750 μL of DNA/RNA Shield (Zymo) until lab analyses began. Trowels and sampling tools were washed in freshly prepared 0.5% sodium hypochlorite and rinsed in distilled water between each sampling plot.

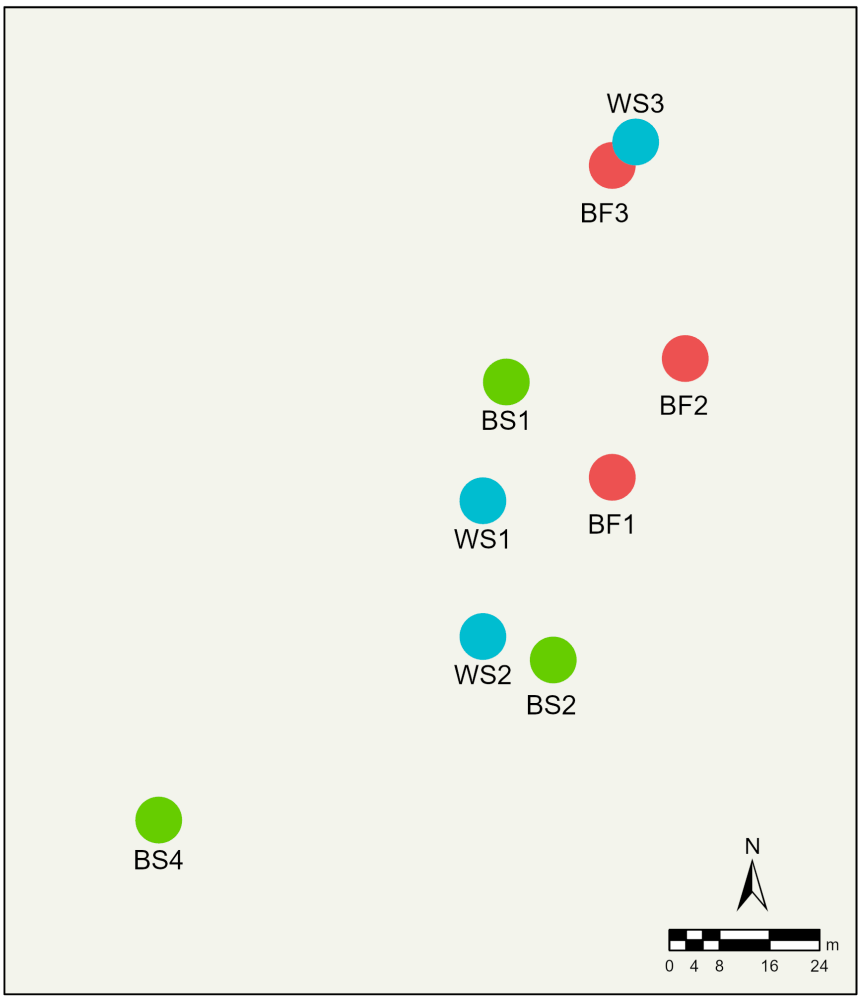

Figure 2.2: Location of seedling patches at experimental site. Black spruce patches labelled as BS1, BS2, and BS4. White spruce patches labelled as WS1, WS2, and WS3. Balsam fir patches labelled as BF1, BF2, and BF3. Patch sizes shown are approximate.

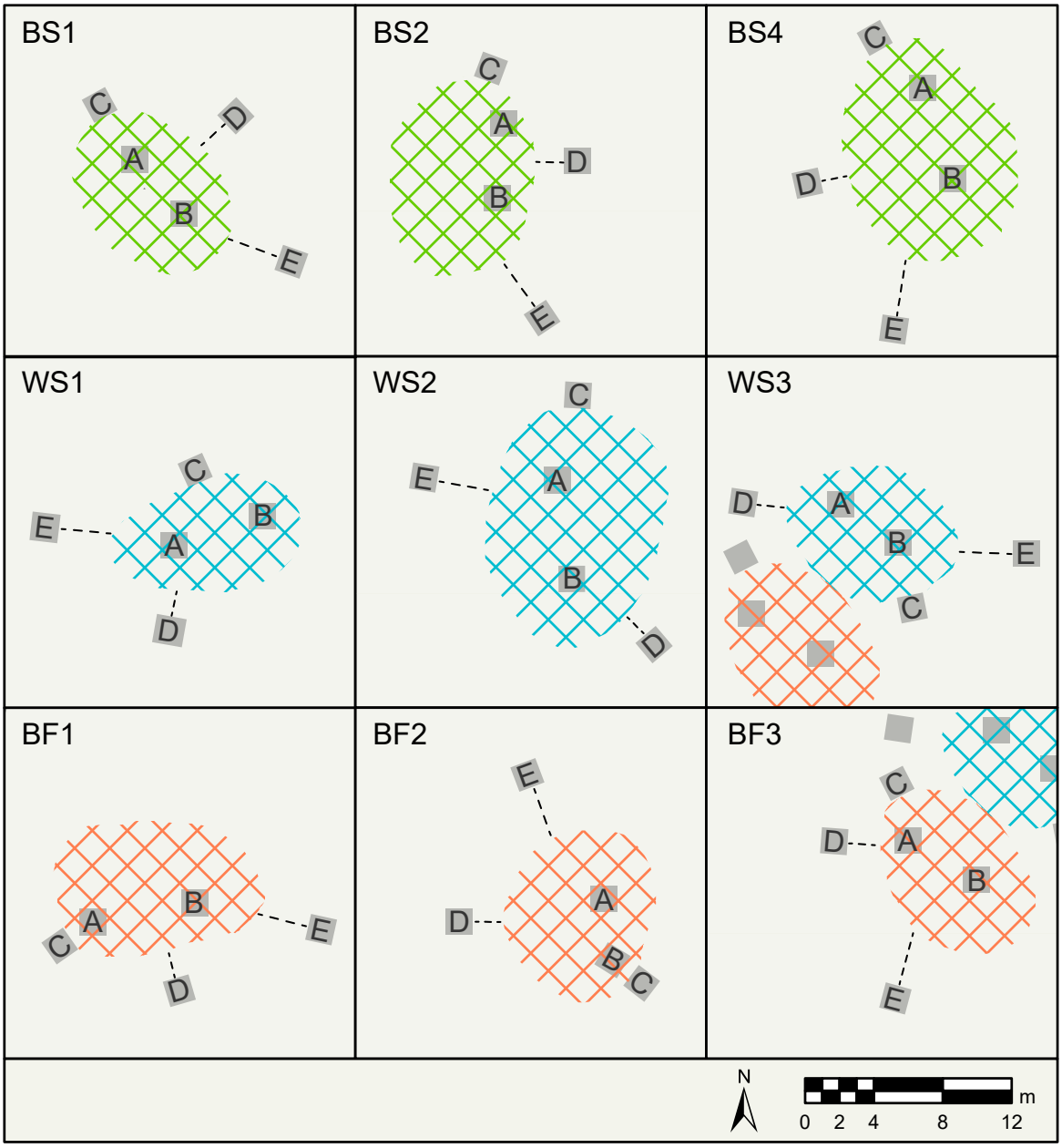

Figure 2.3: Location of plots A-E within each patch. Plots A and B are located within the patch, plot C is located on the edge of the patch, plot D is located 1 m away from the patch and plot E is located 2 m away from the patch. BF represents balsam fir, BS represents black spruce, and WS represents white spruce.

2.4 DNA extraction, amplification and sequencing

2.4.1 Seedling and root extraction, ITS2 amplification and sequencing

Once transported back to the lab at Western University, genomic DNA was extracted from 223 seedlings and 442 roots using the Quick-DNA Plant/Seed Miniprep Kit (Zymo). Primers gITS7 (5'-GYGAATCATCGARTCTTTG-3') (modified from Ihrmark et al., 2012) and ITS4ngs (5'-TCCTCCGCTTAKTGATATGC-3') (modified from Tedersoo et al., 2014) with eight base-pair barcodes and Illumina adaptors were used to amplify a portion of the ITS2 (internal transcribed spacer) region, a widely accepted DNA barcode of fungi (~350-400 bases in most fungi) (Schoch et al., 2012). ITS2 PCR products were assessed using gel electrophoresis or capillary electrophoresis and successful products were submitted to the sequencing facility of London Regional Genomics Centre (Western University, London, ON) to obtain sequences using Illumina MiSeq 2x300 paired-end sequencing.

2.4.2 Root psbA/trnH and rbcLa amplification and sequencing

Primers psbA3-F (5'-GTTATGCATGAACGTAATGCTC-3') and trnH-R (5'-CGCGCATGGT GGATTCACAAATC-3') (Sang et al., 1997) were used to amplify the plant chloroplast trnH-psbA intergenic spacer of roots collected from plots (443-636 bases) to identify the host tree corresponding to each sample. For samples that would not amplify using psbAF and trnHR, primers rbcLa-F (5'-ATGTCACCACAAACAGAGACTAAAGC-3') and rbcLa-R (5'-CTTCT GCTACAAATAAGAATCGATCTC) (Kress and Erickson, 2007) were used to amplify the ribulose- 1, 5-biphosphate carboxylase/oxygenase A (rbcLa) chloroplast gene (561 bases). In addition, a representative of each tree species of seedling acquired from Wooddale Nursery was amplified using psbA3-F/trnH-R and rbcLa-F/rbcLa-R to confirm identity. PCR products were assessed using gel electrophoresis or capillary electrophoresis and successful products were cleaned using the BioBasic EZ-10 Spin Column PCR Products Purification Kit, then submitted to the sequencing facility of London Regional Genomics Centre (Western University, Lon-

don, ON, Canada) or Eurofins Genomics (Louisville, KY, USA) to obtain sequences through Sanger sequencing. For samples that amplified non-target DNA in addition to the targeted plant DNA, primers rbcLa-F and rbcLa-R with eight base-pair barcodes and Illumina adaptors were used to amplify the rbcLa gene region and 262 bases in the forward direction were sequenced using next generation sequencing using Illumina MiSeq 2x300 paired-end sequencing at London Regional Genomics Centre. Sequences generated through Sanger sequencing at London Regional Genomics Centre and Eurofins Genomics were cleaned and assembled with SeqEd v.1.03 and BLASTn of GenBank was used to assign taxonomy. Representative sequences of the trnH-psbA intergenic spacer region and rbcLa gene region from collected roots and seedlings acquired from Wooddale Nursery were submitted to GenBank (accession #OQ319473-77 and #OQ344479-85 with detailed descriptions provided in Appendix Table A.3).

2.4.3 Illumina MiSeq sequence processing

ITS2 and rbcLa FASTQ files received from London Regional Genomics Centre from Illumina MiSeq were separated by gene region using a custom BASH script (Weerasuriya, 2021). Each region was split into a separate file and processed separately. Files were demultiplexed and primers were removed using a custom BASH script (Gloor and Macklaim, 2015) modified to primer and barcode lengths. Using the DADA2 pipeline in R (Callahan et al., 2017), quality plots were generated to determine parameters for quality filtering. Sequences were then dereplicated, forward and reverse reads were paired, chimeras were removed, and amplicon sequence variants (ASVs) were determined. No further processing was done for rbcLa sequences since plant identity was determined through ASVs. For ITS2 reads, ASVs were clustered into OTUs using vsearch in QIIME2 based on a 99.5% similarity threshold. Taxonomy was assigned using the UNITE database for ITS2 sequences (Kõljalg et al., 2013) and rbcL reference library for rbcLa sequences (Bell, 2021). OTUs with a genus (or other taxonomic rank) name not followed by a species name or 'sp.' indicates an OTU which could include multiple unknown species, whereas OTUs with a genus name followed by 'sp.' indicates an OTU which was identified as one unknown species. Sequences of abundant or otherwise important OTUs were run through a BLASTn search of GenBank and sequence identities were

updated if BLASTn results returned a high percent identity to a different identification (See Appendix Table A.4).

2.5 Statistical analysis

Statistical analyses were done in R version 4.2.0. The tidyverse package (Wickham et al., 2019), readxl package (Wickham and Bryan, 2023), writexl (Ooms, 2023), phyloseq package (McMurdie and Holmes, 2013) and microViz package (Barnett et al., 2021) were used to import and organize data, and produce line graphs, Venn diagrams and bar plots to visualize data. With the vegan package (Oksanen et al., 2022), PERMANOVA tests (using adonis) using Bray-Curtis Dissimilarity indices were performed to detect community compositional differences. The indicspecies package (De Cáceres and Legendre, 2009) was used to determine indicator OTUs using the Indicator Value Index (IndVal) (Dufrêne and Legendre, 1997) with 9999 permutations.

3 Results

3.1 Root samples outside of seedling patches

Of the 180 collected root samples from D and E plots, 130 were identified as balsam fir (BF), 36 as black spruce (BS), 12 as white spruce (WS) and two as white birch (WB) using plant psbA/trnH and rbcLa sequences (Table 3.1). After clustering and removal of low abundance (<0.1%) and non-target reads, a total of 1,341,970 reads and 1630 OTUs were generated from fungal ITS2 sequences (Table 3.2). An average of 31.3 ±18.9 OTUs were generated per sample, with a minimum of one and a maximum of 99 OTUs per sample. Samples collected in fall 2021 generated 969,692 reads and 1255 OTUs and samples collected in spring 2022 generated 372,378 reads and 656 OTUs, with 281 OTUs shared between seasons. Samples of BF generated 922,309 reads and 1254 OTUs, BS generated 331,908 reads and 574 OTUs, WS generated 78,260 reads and 227 OTUs and WB generated 9493 reads and 37 OTUs. Of the 1630 total OTUs, 19 were shared between all tree species, 935 were unique to BF, 297 were unique to BS, 72 were unique to WS, and four were unique to WB (Figure 3.1). Among OTUs unique to each of the four tree species, none had an abundance greater than 1%. One OTU of BF, 198 OTUs of BS, 46 OTUs of WS and three OTUs of WB had an abundance greater than 0.01%. A species accumulation curve was generated and does not reach a horizontal asymptote (Figure 3.2).

Table 3.1: Count of root samples of each tree species collected in each season. Identification determined through plant psbA/trnH and rbcLa sequences. BF represents balsam fir, BS represents black spruce, WS represents white spruce and WB represents white birch.

	Tree Species				
Season	**BF**	**BS**	**WS**	**WB**	**Total**
Fall 2021	63	20	6	1	90
Spring 2022	67	16	6	1	90
Total	130	36	12	2	180

Table 3.2: Read count of root samples after filtering, denoising, and chimera and non-target read removal in dada2 pipeline of fungal ITS2 sequences.

	Input Reads	**Filtered Reads**	**Denoised Reads**	**Non-Chimeric Reads**	**Target Reads**
Sum	9,051,773	2,422,513	2,351,089	1,358,180	1,341,970

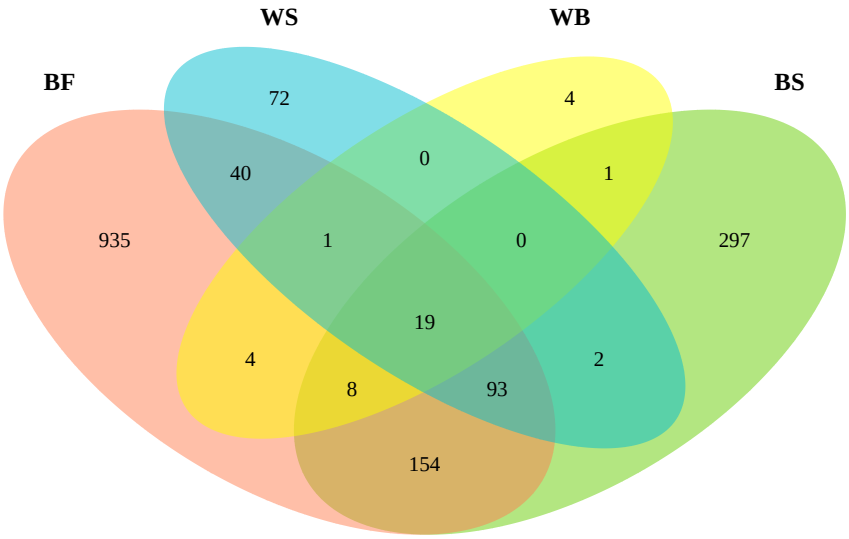

Figure 3.1: Venn diagram showing operational taxonomic units shared between tree species. OTUs generated using fungal ITS2 sequences.

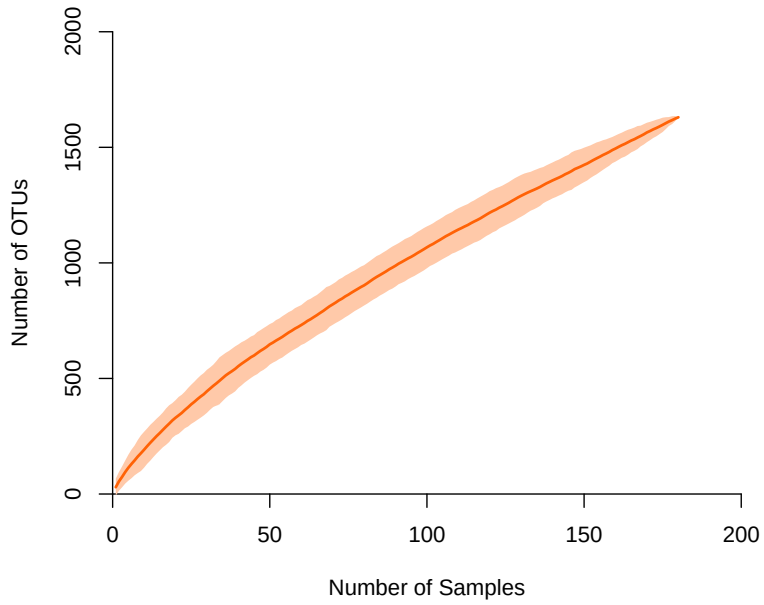

Figure 3.2: Species accumulation curve of root samples. Cumulative number of unique OTUs generated using fungal ITS2 sequences from each sample in a randomized order. Shaded area represents a 95% confidence interval.

3.1.1 Phylum level comparisons

Relative abundance of Basidiomycota within samples ranged from 12.7% to 100.0%, relative abundance of Ascomycota ranged from 0.0% to 87.3%, and relative abundance of other phyla ranged from 0.0% to 3.4% (Figure 3.3).

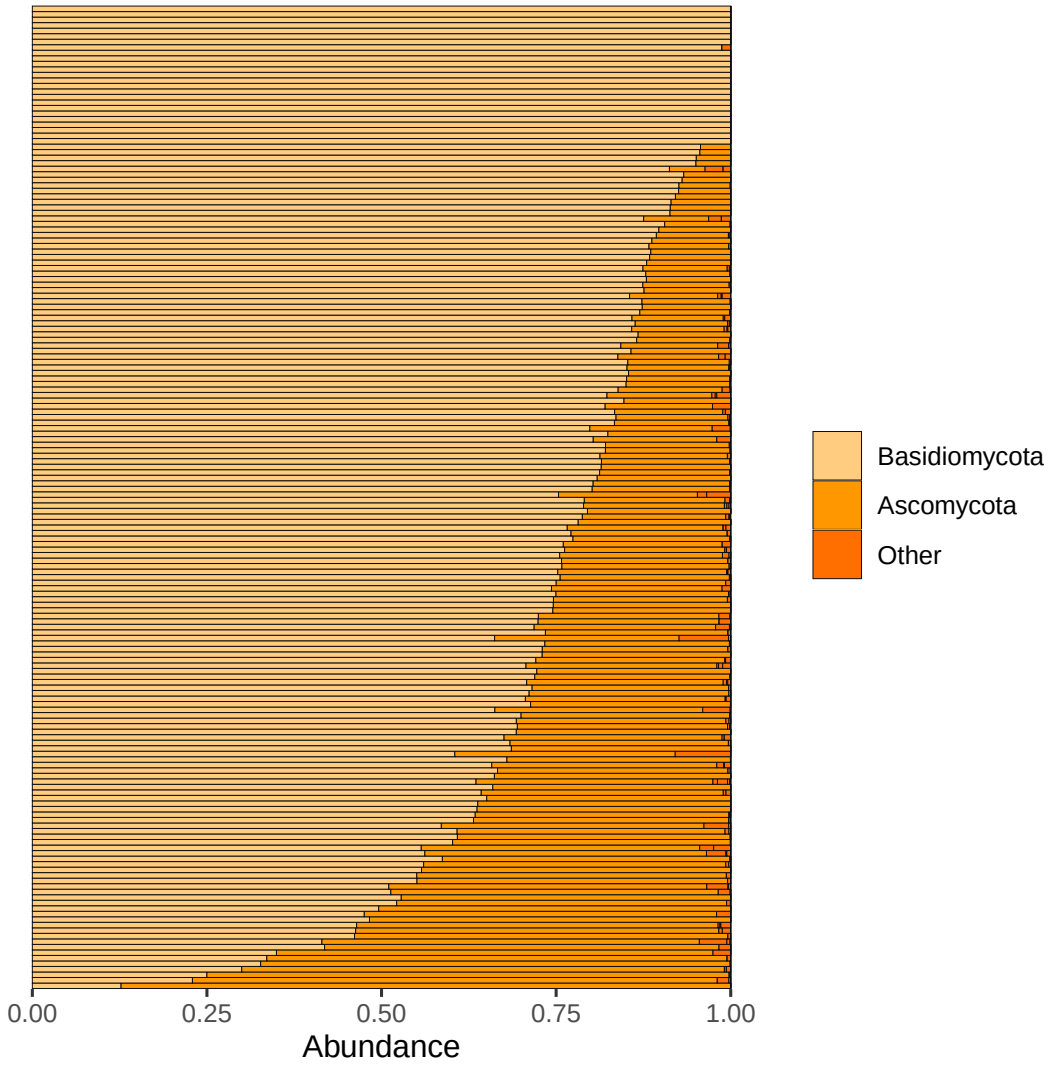

Figure 3.3: Relative abundances of operational taxonomic units in samples identified to phylum level. Each row represents one sample.

3.1.2 Comparisons between tree species

A PERMANOVA revealed that there were significant differences between the community composition of BF, BS and WS ($F_{2,175}$=1.32, p=0.014) (Table 3.3A). A pairwise PERMANOVA revealed that there were no significant differences between the community composition of BF and BS (p=0.081), BF and WS (p=0.219), or BS and WS (p=0.570) with Bonferroni adjusted p-values (Table 3.3B).

Table 3.3: PERMANOVA results of community compositional differences between balsam fir, black spruce and white spruce. **A)** PERMANOVA summary statistic based on Bray-Curtis dissimilarity indices of 178 samples. White birch samples were removed due to low sample size. **B)** Pair-wise comparisons of PERMANOVA with Bonferroni adjusted p-values. BF represents balsam fir, BS represents black spruce, and WS represents white spruce.

A)

	df	Sum of Sqs	Mean Sqs	*F value*	R^2	*p*
Tree species	2	1.1	0.552	1.32	0.0149	0.014
Residuals	175	72.9	0.416		0.9851	
Total	177	74			1	

B)

	BF	BS	WS
BF			
BS	0.081		
WS	0.219	0.570	

An ordination created using NMDS comparing all tree species had an average stress value above 0.2 and thus was not included following standards of Clarke (1993). Observed species richness, Fisher's diversity index and effective number of species calculated using Shannon diversity index (e^H) are shown in Table 3.4.

Table 3.4: Observed species richness, Fisher's diversity index and effective number of species calculated using Shannon diversity index (e^H) of each tree species. BF represents balsam fir, BS represents black spruce, and WS represents white spruce. White birch was not included due to low sample size.

Tree species	Observed	Fisher	e^H
BF	1254	142.95	148.55
BS	574	67.53	144.30
WS	227	28.69	69.95

3.1.3 Seasonal comparisons

A PERMANOVA revealed that there were significant differences between the community composition of fall 2021 and spring 2022 samples ($F_{1,176}$=4.43, p=0.001) (Table 3.5); however, the NGS runs containing spring 2022 samples had an average %Q30 (percent of bases with quality

score >30) of 73.8% and an average of 1542 reads per sample, while NGS runs containing fall 2021 samples had an average %Q30 of 79.4% and an average of 12,537 reads per sample. Due to the uncertain cause of the calculated significant difference, no further PERMANOVA tests to detect seasonal differences were performed on the dataset.

Table 3.5: PERMANOVA results of community compositional differences between samples collected in fall 2021 and spring 2022. Based on Bray-Curtis dissimilarity indices of 178 balsam fir, black spruce and white spruce samples.

	df	Sum of Sqs	Mean Sqs	*F value*	R^2	*p*
Season	1	1.81	1.81	4.43	0.0245	0.001
Residuals	176	72.17	0.41		0.9755	
Total	177	73.99			1	

An ordination created using NMDS comparing all tree species had an average stress value above 0.2 and thus was not included following standards of Clarke (1993). Observed species richness, Fisher's diversity index and effective number of species calculated using Shannon diversity index (e^H) are shown in Table 3.6.

Table 3.6: Observed species richness, Fisher's diversity index and effective number of species calculated using Shannon diversity index (e^H) for each season.

Season	Observed	Fisher	e^H
Fall 2021	1263	143.18	199.88
Spring 2022	661	78.03	94.65

3.1.4 Balsam fir community composition

The five most abundant OTUs of BF across both seasons were *Russula montana* (OTU1), *Mycena* cf. *cinerella* (OTU3), *Clavulina coralloides* (OTU8), *Amphinema* sp. (OTU2), and *Russula decolorans* (OTU10) (Figure 3.4).

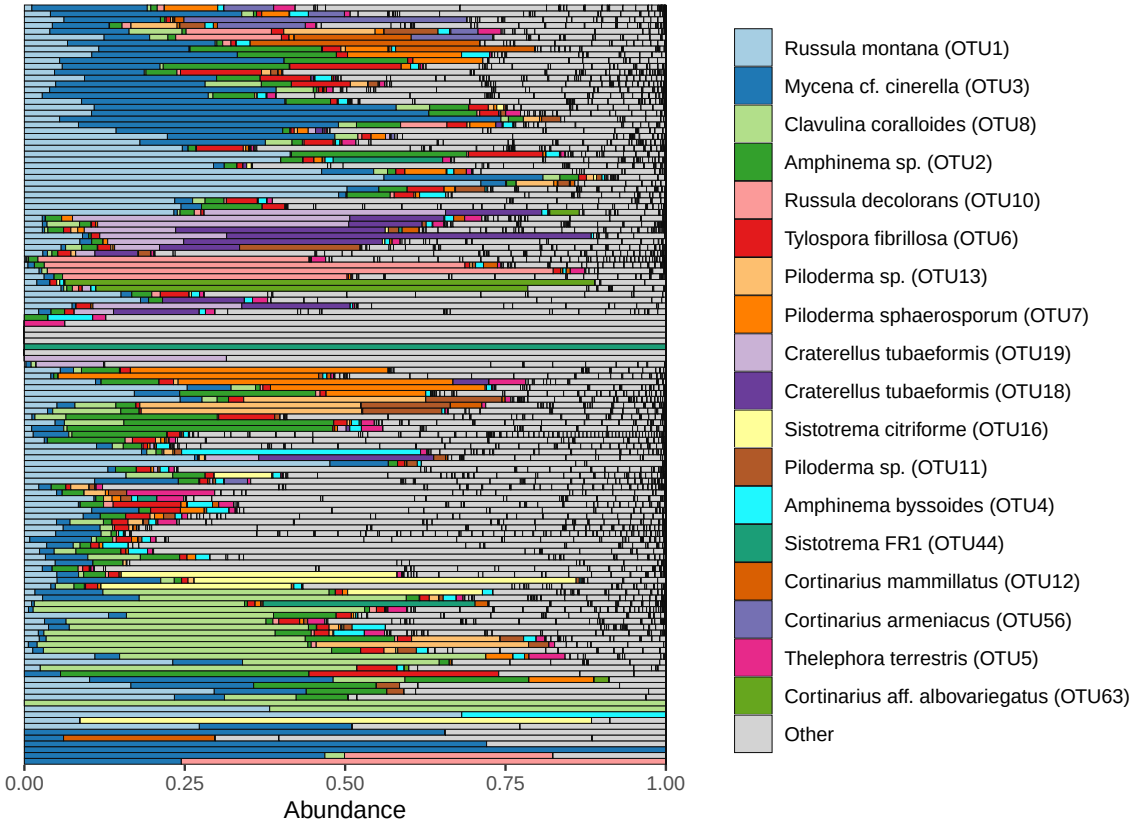

Figure 3.4: Relative abundances of operational taxonomic units in balsam fir samples. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each row represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

3.1.5 Black spruce community composition

The five most abundant OTUs of BS across both seasons were *Russula montana* (OTU1), *Piloderma sphaerosporum* (OTU7), *Cortinarius mammillatus* (OTU12), *Mycena* cf. *cinerella* (OTU3), and *Amphinema* sp. (OTU2) (Figure 3.5).

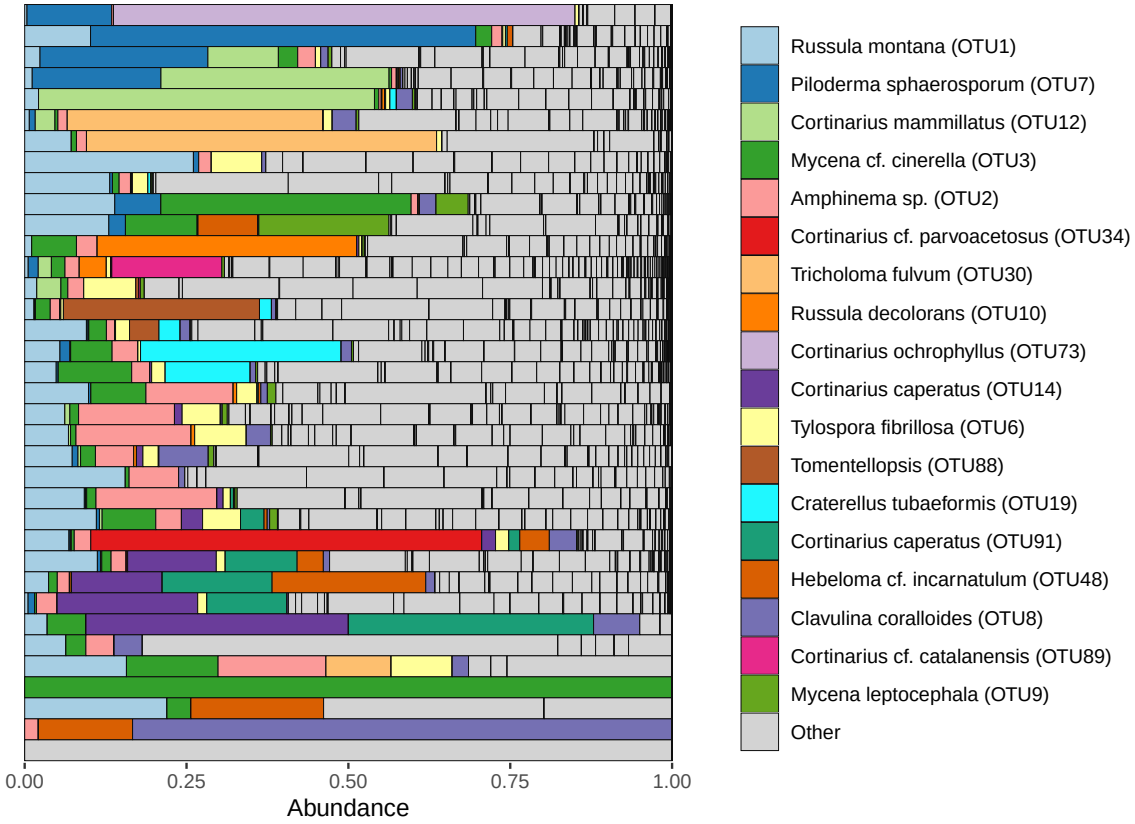

Figure 3.5: Relative abundances of operational taxonomic units in black spruce samples. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each row represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

3.1.6 White spruce community composition

The five most abundant OTUs of WS across both seasons were *Russula montana* (OTU1), *Amphinema* sp. (OTU2), *Clavulina coralloides* (OTU43), *Piloderma* sp. (OTU50) and *Mycena* cf. *cinerella* (OTU3) (Figure 3.6).

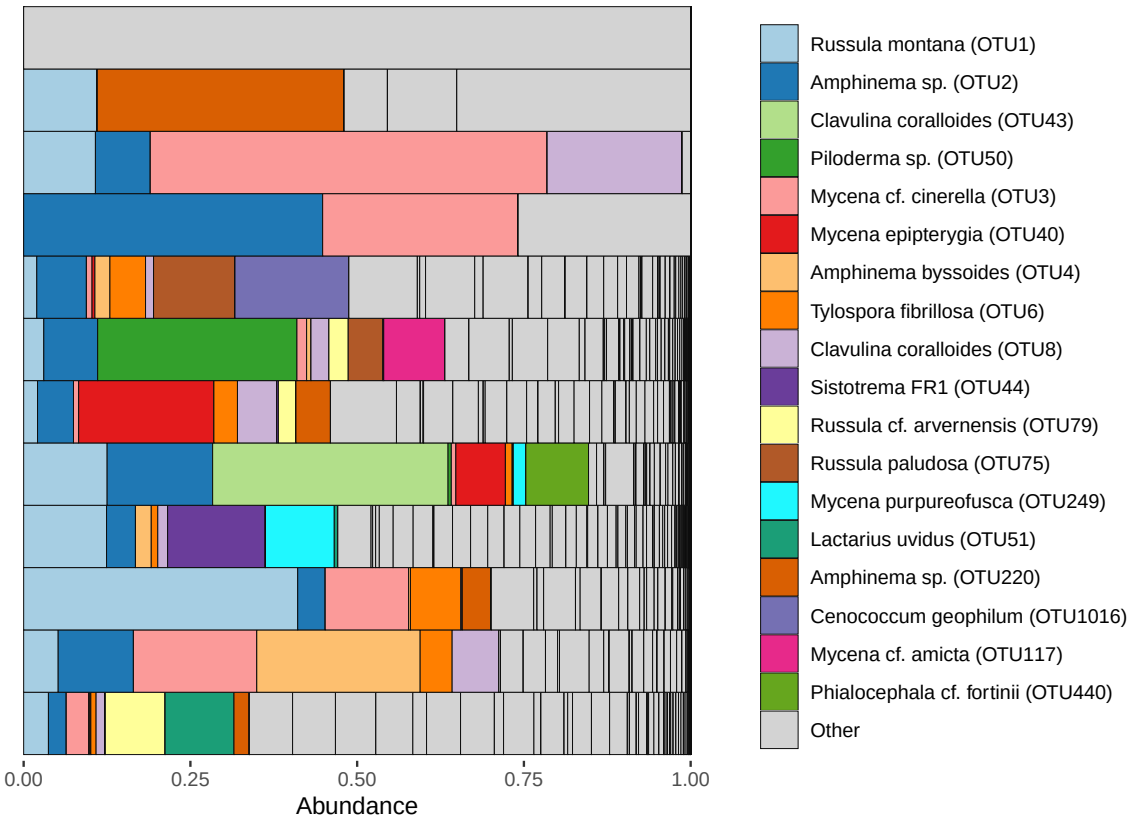

Figure 3.6: Relative abundances of operational taxonomic units in white spruce samples. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each row represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

3.1.7 White birch community composition

The five most abundant OTUs of WB across both seasons were *Mycena epipterygia* (OTU40), *Amphinema* sp. (OTU2), *Russula montana* (OTU1), *Tomentella* cf. *cinereoumbrina* (OTU366) and *Tylospora fibrillosa* (OTU6) (Figure 3.7).

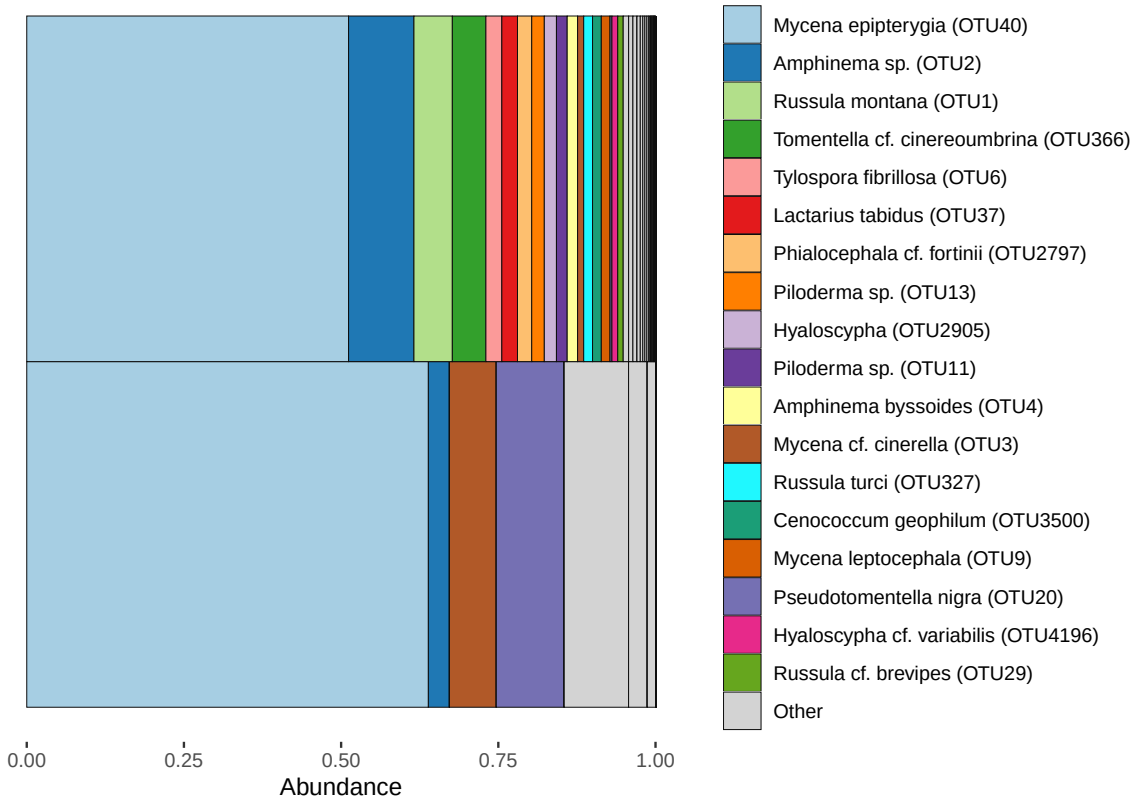

Figure 3.7: Relative abundances of operational taxonomic units in white birch samples in fall 2021 and spring 2022. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each row represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

3.1.8 Comparisons of 18 most abundant OTUs between tree species

Four OTUs were found in high abundance on all four tree species (Figure 3.8), including *Amphinema* sp. (OTU2), *Mycena* cf. *cinerella* (OTU3), *Russula montana* (OTU1), and *Tylospora fibrillosa* (OTU6) (Table 3.7). Five OTUs were found only on BF, eight OTUs were found only on BS, ten OTUs were found only on WS and nine OTUs were found only on WB were (Table 3.8).

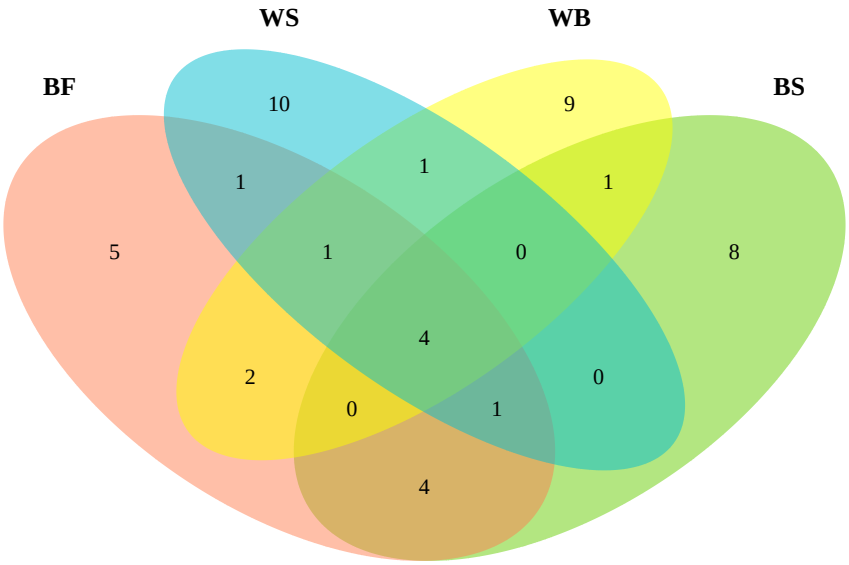

Figure 3.8: Venn diagram showing top 18 operational taxonomic units shared between tree species. BF represents balsam fir, BS represents black spruce, WS represents white spruce and WB represents white birch.

Table 3.7: Presence of most abundant operational taxonomic units in each tree species. Top 18 OTUs of each species shown. Black box denotes presence of OTU on roots of tree species. Confidence of OTU identification can be found in Appendix Table A.4.

		OTU	**Presence**			
Class	**Family**	**Genus & Species**	**BF**	**BS**	**WS**	**WB**
Agaricales	Cortinariaceae	*Cortinarius acutus* (OTU63)	■			
		Cortinarius armeniacus (OTU56)	■			
		Cortinarius caperatus (OTU14)		■		
		Cortinarius caperatus (OTU91)		■		
		Cortinarius cf. *catalanensis* (OTU89)		■		
		Cortinarius cf. *parvoacetosus* (OTU34)		■		
		Cortinarius mammillatus (OTU12)	■	■		
		Cortinarius ochrophyllus (OTU73)		■		
	Hymenogastraceae	*Hebeloma* cf. *incarnatulum* (OTU48)		■		
	Mycenaceae	*Mycena* cf. *amicta* (OTU117)			■	
		Mycena cf. *cinerella* (OTU3)	■	■	■	■
		Mycena epipterygia (OTU40)			■	■
		Mycena leptocephala (OTU9)		■		■
		Mycena purpureofusca (OTU249)			■	
	Tricholomataceae	*Tricholoma fulvum* (OTU30)		■		
Atheliales	Tylosporaceae	*Amphinema* sp. (OTU2)	■	■	■	■
		Amphinema sp. (OTU220)			■	
		Amphinema byssoides (OTU4)	■		■	■
	Pilodermataceae	*Piloderma* sp. (OTU11)	■			■
		Piloderma sp. (OTU13)	■			■
		Piloderma sp. (OTU50)			■	
		Piloderma sphaerosporum (OTU7)	■	■		
	Tylosporaceae	*Tylospora fibrillosa* (OTU6)	■	■	■	■
Cantharellales	Hydnaceae	*Clavulina coralloides* (OTU43)			■	
		Clavulina coralloides (OTU8)	■	■	■	
	Cantharellaceae	*Craterellus tubaeformis* (OTU19)	■			
		Craterellus tubaeformis (OTU18)	■	■		
	Hydnaceae	*Sistotrema citriforme* (OTU16)	■			
		Sistotrema FR1 (OTU44)	■		■	
Helotiales	Hyaloscyphaceae	*Hyaloscypha* (OTU2905)				■
		Hyaloscypha cf. *variabilis* (OTU4196)				■
	Mollisiaceae	*Phialocephala* cf. *fortinii* (OTU2797)				■
		Phialocephala cf. *fortinii* (OTU440)			■	
Mytilinidales	Gloniaceae	*Cenococcum geophilum* (OTU3500)				■
		Cenococcum geophilum (OTU1016)			■	
Russulales	Russulaceae	*Lactarius tabidus* (OTU37)				■
		Lactarius uvidus (OTU51)			■	
		Russula cf. *arvernensis* (OTU79)			■	
		Russula cf. *brevipes* (OTU29)				■
		Russula decolorans (OTU10)	■	■		
		Russula montana (OTU1)	■	■	■	■
		Russula paludosa (OTU75)			■	
		Russula turci (OTU327)				■
Thelephorales	Thelephoraceae	*Pseudotomentella nigra* (OTU20)				■
		Thelephora terrestris (OTU5)	■			
		Tomentella cf. *cinereoumbrina* (OTU366)				■
		Tomentellopsis (OTU88)		■		

3.1.9 Indicator operational taxnomic units

An indicator OTU analysis found that 33 OTUs were significantly associated with at least one tree species. Black spruce had 20 indicator OTUs with *Cortinarius caperatus* (OTU91) (p=0.0032), *Tylospora asterophora* (OTU28) (p=0.0041), and *Phialocephala* cf. *fortinii* (OTU312) (p=0.0098) as the three most significantly associated OTUs (Figure 3.9). White spruce had eight indicator OTUs with *Amphinema* sp. (OTU220) (p=0.0001), *Mycena purpureofusca* (OTU249) (p=0.0044), and *Lactarius picinus* (OTU1347) (p=0.0053) as the three most significantly associated OTUs (Figure 3.9). Five OTUs were indicators of BS and WS including *Apiotrichum porosum* (OTU202) (p=0.0087), Schizoporaceae (OTU147) (p=0.205), *Tomentella terrestris* (OTU87) (p=0.0292), *Mycena epipterygia* (OTU40) (p=0.0373), and Pucciniales sp. (OTU133) (p=0.0491). BF had no significantly associated indicator OTUs. Analysis was not run on WB due to low sample size.

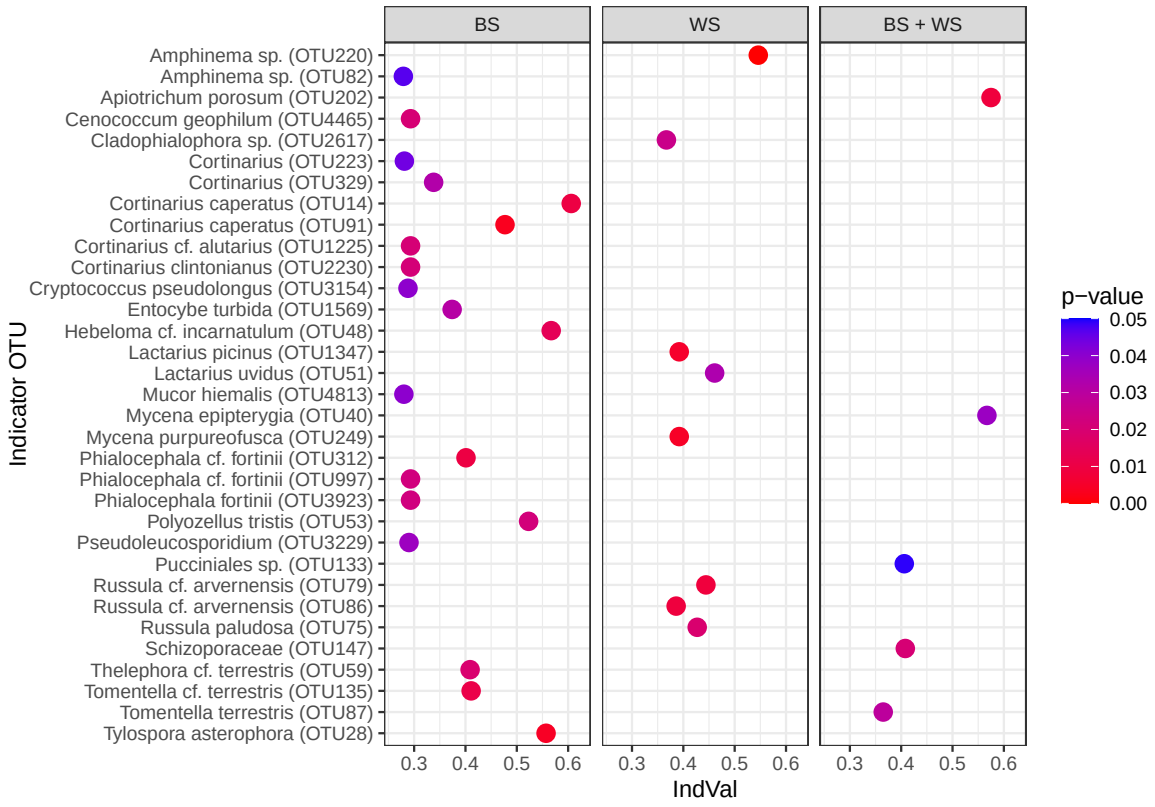

Figure 3.9: Indicator operational taxonomic units of black spruce and white spruce. BS represents black spruce and WS represents white spruce. IndVal, the indicator value index is a measured association between the fungal species and the tree species. Color of dot denotes p-value. Confidence of OTU identification can be found in Appendix Table A.4.

3.2 Seedlings and roots within and near seedling patches

3.2.1 Seedling samples

Of the 223 seedling samples collected, 42 were collected before outplanting, 90 in fall 2021, and 91 in spring 2022, and 50 were BF, 66 were black spruce BS and 112 were WS (Table 3.8). After clustering and removal of low abundance (<0.1%) and non-target reads, a total of 2,040,159 reads and 2432 OTUs were generated (Table 3.9). An average of 35.2 ±18.5 OTUs were generated per sample, with a minimum of one and a maximum of 82 OTUs per sample. Seedlings of BF generated 431,968 reads and 714 OTUs, seedlings of BS generated

595,995 reads and 1146 OTUs and seedlings of WS generated 1,012,196 reads and 1151 OTUs. Seedlings collected before outplanting generated 532,073 reads and 918 OTUs, seedlings collected in fall 2021 generated 1,047,450 and 1344 OTUs and seedlings collected in spring 2022 generated 460,636 reads and 713 OTUs. A species accumulation curve was generated and does not reach a horizontal asymptote (Figure 3.10).

Table 3.8: Count of seedling samples of each tree species collected in each season. BF represents balsam fir, BS represents black spruce, WS represents white spruce and WB represents white birch.

	Tree Species			
Season	**BF**	**BS**	**WS**	**Total**
Pre-planting	8	18	16	42
Fall 2021	18	24	48	90
Spring 2022	23	22	46	91
Total	50	66	112	223

Table 3.9: Read count of seedling samples after filtering, denoising, and chimera and non-target read removal in dada2 pipeline.

	Input Reads	**Filtered Reads**	**Denoised Reads**	**Non-Chimeric Reads**	**Target Reads**
Sum	13,543,808	3,612,840	3,517,713	2,073,598	2,040,159

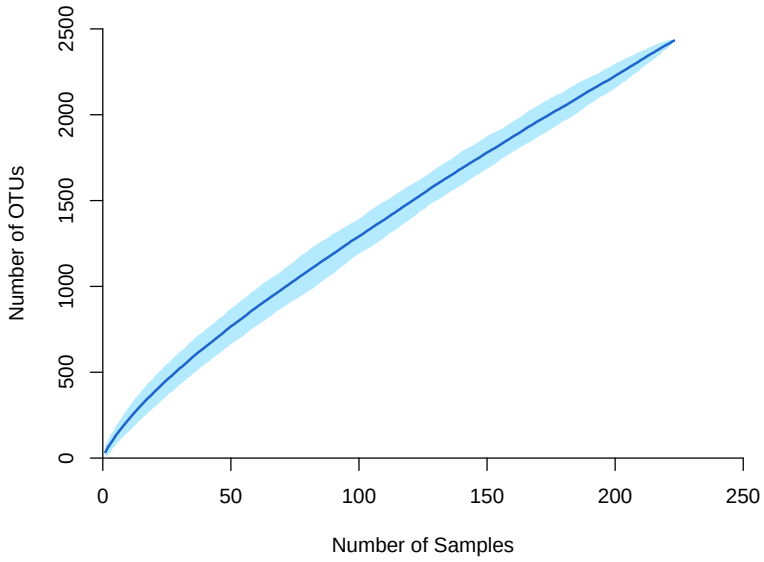

Figure 3.10: Species accumulation curve of seedling samples. Cumulative number of unique OTUs generated from each sample in a randomized order. Shaded area represents a 95% confidence interval.

3.2.2 Root samples within and near seedling patches

Of the 262 collected root samples from plots A, B and C, 181 were identified as BF, 41 as BS, 35 as WS and five as WB (Table 3.10). Five samples with a zero read count were excluded from analyses. After clustering and removal of low abundance (<0.1%) and non-target reads, a total of 1,953,370 reads and 2000 OTUs were generated (Table 3.11). An average of 30.6 ±18.3 OTUs were generated per sample, with a minimum of one and a maximum of 77 OTUs per sample. Samples collected in fall 2021 generated 1,484,721 reads and 1615 OTUs and samples collected in spring 2022 generated 468,649 reads and 703 OTUs, with 318 OTUs shared between seasons. Samples of BF generated 1,262,030 reads and 1422 OTUs, BS generated 313,631 reads and 595 OTUs, WS generated 337,467 reads and 551 OTUs and WB generated 40,242 reads and 124 OTUs. Of the 2000 total OTUs, 68 were shared between all tree species, 1015 were unique to BF, 282 were unique to BS, 259 were unique to WS, and 24 were unique to WB. A species accumulation curve was generated and does not reach a horizontal asymptote (Figure 3.11).

Table 3.10: Count of root samples of each tree species collected inside seedling patches in each season. BF represents balsam fir, BS represents black spruce, WS represents white spruce and WB represents white birch.

	Tree Species				
Season	**BF**	**BS**	**WS**	**WB**	**Total**
Fall 2021	85	24	23	3	135
Spring 2022	96	17	12	2	127
Total	181	41	35	5	262

Table 3.11: Read count of root samples inside seedling patches after filtering, denoising, and chimera and non-target read removal in dada2 pipeline.

	Input Reads	**Filtered Reads**	**Denoised Reads**	**Non-Chimeric Reads**	**Target Reads**
Sum	14,929,277	3,913,637	3,800,164	2,219,858	1,953,370

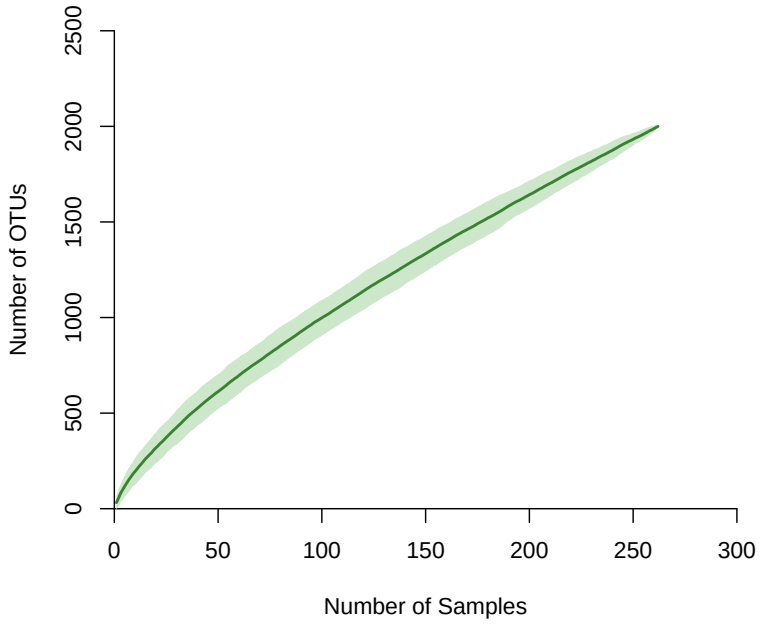

Figure 3.11: Species accumulation curve of root samples inside seedling patches. Cumulative number of unique OTUs generated from each sample in a randomized order. Shaded area represents a 95% confidence interval.

3.2.3 Phylum level comparisons

Relative abundance of Basidiomycota within pre-planted seedling samples ranged from 6.4% (BS) to 84.8% (BF), relative abundance of Ascomycota ranged from 14.2% (BF) to 93.3% (BS), relative abundance of Mucoromycota ranged from 0.0% (BF, BS) to 64.8% (BS) and relative abundance of other phyla ranged from 0.0% (BF, BS, WS) to 2.4% (BS) (Figure 3.12). Relative abundance of Basidiomycota within seedling samples collected in fall 2021 ranged from 13.1% (BF) to 87.1% (BF), relative abundance of Ascomycota ranged from 12.6% (BF) to 86.9% (BF), relative abundance of Mucoromycota ranged from 0.0% (BF, BS, WS) to 5.8% (WS) and relative abundance of other phyla ranged from 0.0% (BF, BS, WS) to 5.5% (WS) (Figure 3.12). Relative abundance of Basidiomycota within seedling samples collected in spring 2022 ranged from 17.9% (BS) to 100.0% (BF, BS, WS), relative abundance of Ascomycota ranged from 0.0% (BF, BS, WS) to 81.9% (BS), relative abundance of Mucoromycota ranged from 0.0% (BF, BS, WS) to 0.2% (BS, WS) and relative abundance of other phyla ranged from 0.0% (BF, BS, WS) to 6.1% (BF) (Figure 3.12).

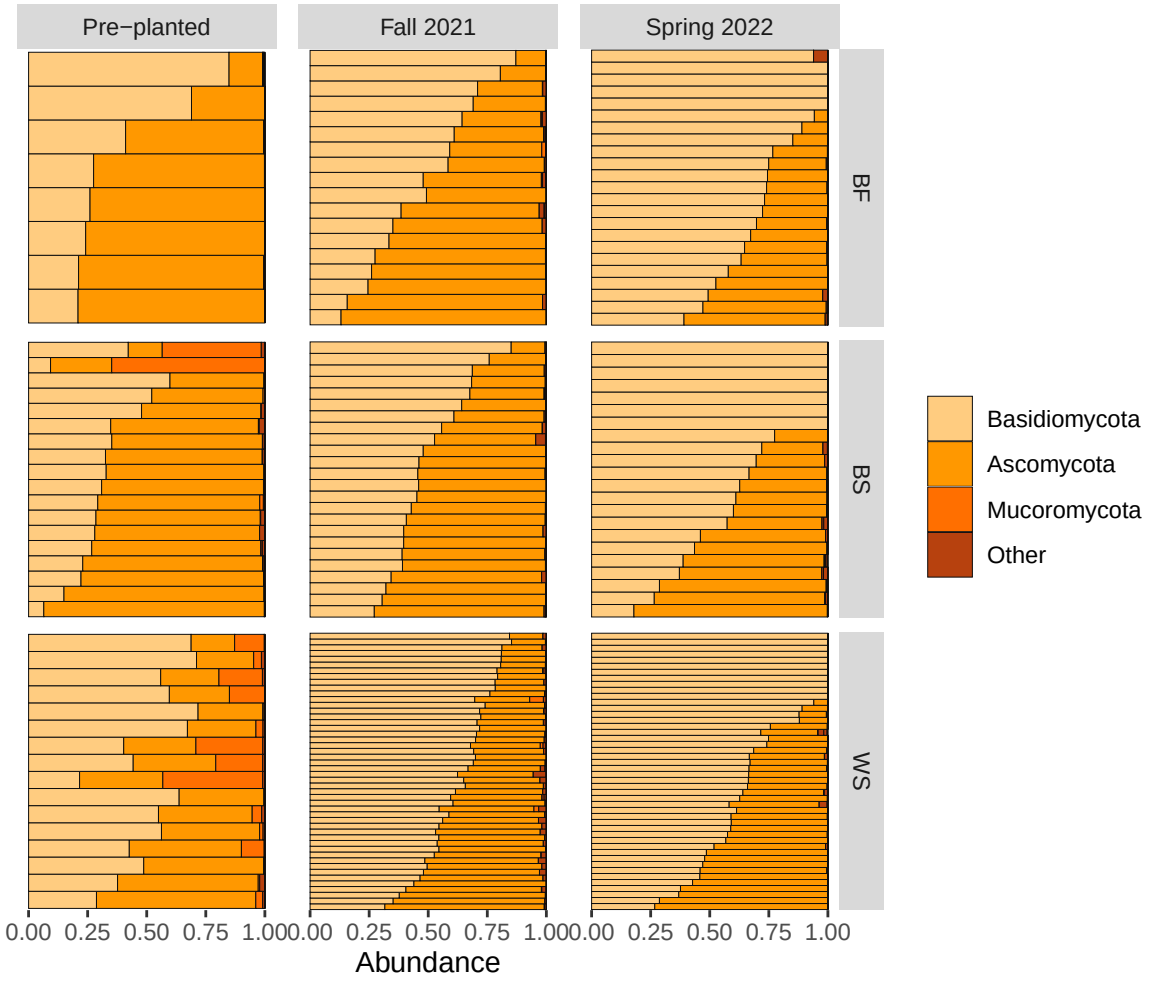

Figure 3.12: Relative abundances of operational taxonomic units in seedling samples separated by tree species and season identified to phylum level. Each row of each column represents one sample. BF represents balsam fir, BS represents black spruce, and WS represents white spruce. Relative abundance of Basidiomycota within seedling samples ranged from 6.4% (BS) to 100.0% (BF, BS, WS), relative abundance of Ascomycota ranged from 0.0% (BF, BS, WS) to 93.3% (BS), relative abundance of Mucoromycota ranged from 0.0% (BF, BS, WS) to 64.8% (BS) and relative abundance of other phyla ranged from 0.0% (BF, BS, WS) to 6.1% (BF).

3.2.4 Community composition of seedlings over time

The five most abundant OTUs on pre-planted BF seedlings were *Wilcoxina rehmii* (OTU17 and OTU21), *Mycena leptocephala* (OTU49 and OTU9), and *Thelephora terrestris* (OTU5) (Figure 3.13). The five most abundant OTUs on pre-planted BS seedlings were *Thelephora*

terrestris (OTU5), *Umbelopsis isabellina* (OTU31), *Amphinema* sp. (OTU2), *Russula montana* (OTU1) and *Mycena leptocephala* (OTU9) (Figure 3.14). The five most abundant OTUs on pre-planted WS seedlings were *Thelephora terrestris* (OTU5), *Amphinema* sp. (OTU2), *Amphinema byssoides* (OTU4), *Thelephora* cf. *terrestris* (OTU36), and *Umbelopsis isabellina* (OTU31) (Figure 3.15).

Balsam fir seedlings planted within the BF1 patch maintained a high abundance of *Wilcoxina rehmii* (OTU17 and OTU21) and *Mycena leptocephala* (OTU9) in fall 2021 and spring 2022 but showed a high abundance of *Mycena* cf. *cinerella* (OTU3) in spring 2022 (Figure 3.13). No OTUs were found on fall 2021 or spring 2022 seedlings in the BF1 patch that had not been found on pre-planted seedlings. BF seedlings planted within the BF2 patch maintained a high abundance of *Wilcoxina rehmii* (OTU17 and OTU21) and *Mycena leptocephala* (OTU9) in fall 2021 and spring 2022 (Figure 3.13). No OTUs were found on fall 2021 or spring 2022 seedlings in the BF2 patch that had not been found on pre-planted seedlings. BF seedlings planted within the BF3 patch maintained a high abundance of *Wilcoxina rehmii* (OTU17 and OTU21) and *Mycena leptocephala* (OTU9) in fall 2021 but increased in abundance of *Mycena* cf. *cinerella* (OTU3) and *Pseudotomentella nigra* (OTU20) in spring 2022 (Figure 3.13).

Black spruce seedlings planted within the BS1 patch maintained a high abundance of *Mycena leptocephala* (OTU9) but decreased in abundance of *Thelephora terrestris* (OTU5) in fall 2021 and spring 2022 (Figure 3.14). No OTUs were found on fall 2021 or spring 2022 seedlings in the BS1 patch that had not been found on pre-planted seedlings. BS seedlings planted within the BS2 patch maintained a high abundance of *Thelephora terrestris* (OTU5) and increased in abundance of *Amphinema byssoides* (OTU4) in fall 2021, but decreased in abundance of *Thelephora terrestris* (OTU5) and maintained high abundance of *Amphinema byssoides* (OTU4) in spring 2022 (Figure 3.14). No OTUs were found on fall 2021 or spring 2022 seedlings in the BS2 patch that had not been found on pre-planted seedlings. BS seedlings within the BS4 patch decreased in abundance of *Thelephora terrestris* (OTU5) and increased in abundance of *Piloderma sphaerosporum* (OTU7) in fall 2021 and spring 2022 (Figure 3.14).

White spruce seedlings within the WS1 patch maintained high abundance of *Amphinema* sp.

(OTU2) and *Amphinema byssoides* (OTU4), decreased in abundance of *Thelephora terrestris* (OTU5), *Thelephora* cf. *terrestris* (OTU36) and *Umbelopsis isabellina* (OTU31) in fall 2021 and spring 2022 and increased in abundance of *Mycena* cf. *cinerella* (OTU3) and *Tylospora fibrillosa* (OTU6) in spring 2022 (Figure 3.15). WS seedlings within the WS2 patch maintained high abundance of *Amphinema* sp. (OTU2) and *Amphinema byssoides* (OTU4), decreased in abundance of *Thelephora terrestris* (OTU5), *Thelephora* cf. *terrestris* (OTU36) and *Umbelopsis isabellina* (OTU31) in fall 2021 and spring 2022 and increased in abundance of *Mycena* cf. *cinerella* (OTU3), *Tylospora fibrillosa* (OTU6), *Russula decolorans* (OTU10), and *Piloderma sphaerosporum* (OTU7) in fall 2021 and spring 2022 (Figure 3.15). WS seedlings within the WS3 patch maintained high abundance of *Amphinema* (OTU2) and *Amphinema byssoides* (OTU4), decreased in abundance of *Thelephora terrestris* (OTU5), *Thelephora* cf. *terrestris* (OTU36), *Umbelopsis isabellina* (OTU31), and *Resinicium bicolor* (OTU69) in fall 2021 and spring 2022 and increased in abundance of *Mycena* cf. *cinerella* (OTU3), *Tylospora fibrillosa* (OTU6), and *Piloderma* sp. (OTU11) in fall 2021 and spring 2022 (Figure 3.15).

3.2.5 Community composition of roots over time

The five most abundant OTUs on BF roots collected from plots A, B and C in fall 2021 were *Russula montana* (OTU1), *Amphinema* sp. (OTU2), *Mycena* cf. *cinerella* (OTU3), *Tylospora fibrillosa* (OTU6), and *Clavulina coralloides* (OTU8). The five most abundant OTUs on BF roots collected from plots A, B and C in spring 2022 were *Russula montana* (OTU1), *Mycena* cf. *cinerella* (OTU3), *Tylospora fibrillosa* (OTU6), *Clavulina coralloides* (OTU8) and *Cortinarius caperatus* (OTU14) (Figure 3.13).

The five most abundant OTUs on BS roots collected from plots A, B and C in fall 2021 were *Piloderma sphaerosporum* (OTU7), *Cortinarius mammillatus* (OTU12), *Russula montana* (OTU1), *Clavulina coralloides* (OTU8) and *Tylospora fibrillosa* (OTU6). The five most abundant OTUs on BS roots collected from plots A, B and C in spring 2022 were *Russula montana* (OTU1), *Piloderma sphaerosporum* (OTU7), *Mycena* cf. *cinerella* (OTU3), *Russula decolorans* (OTU10) and *Clavulina coralloides* (OTU8) (Figure 3.14).

The five most abundant OTUs on WS roots collected from plots A, B and C in fall 2021 were *Tylospora fibrillosa* (OTU6), *Russula montana* (OTU1), *Mycena* cf. *cinerella* (OTU3), *Amphinema* sp. (OTU2), and *Piloderma sphaerosporum* (OTU7). The five most abundant OTUs on WS roots collected from plots A, B and C in spring 2022 were *Tylospora fibrillosa* (OTU6), *Amphinema* sp. (OTU2), *Russula montana* (OTU1), *Mycena* sp. (OTU81), and *Cortinarius roseomyceliosus* (OTU52) (Figure 3.15).

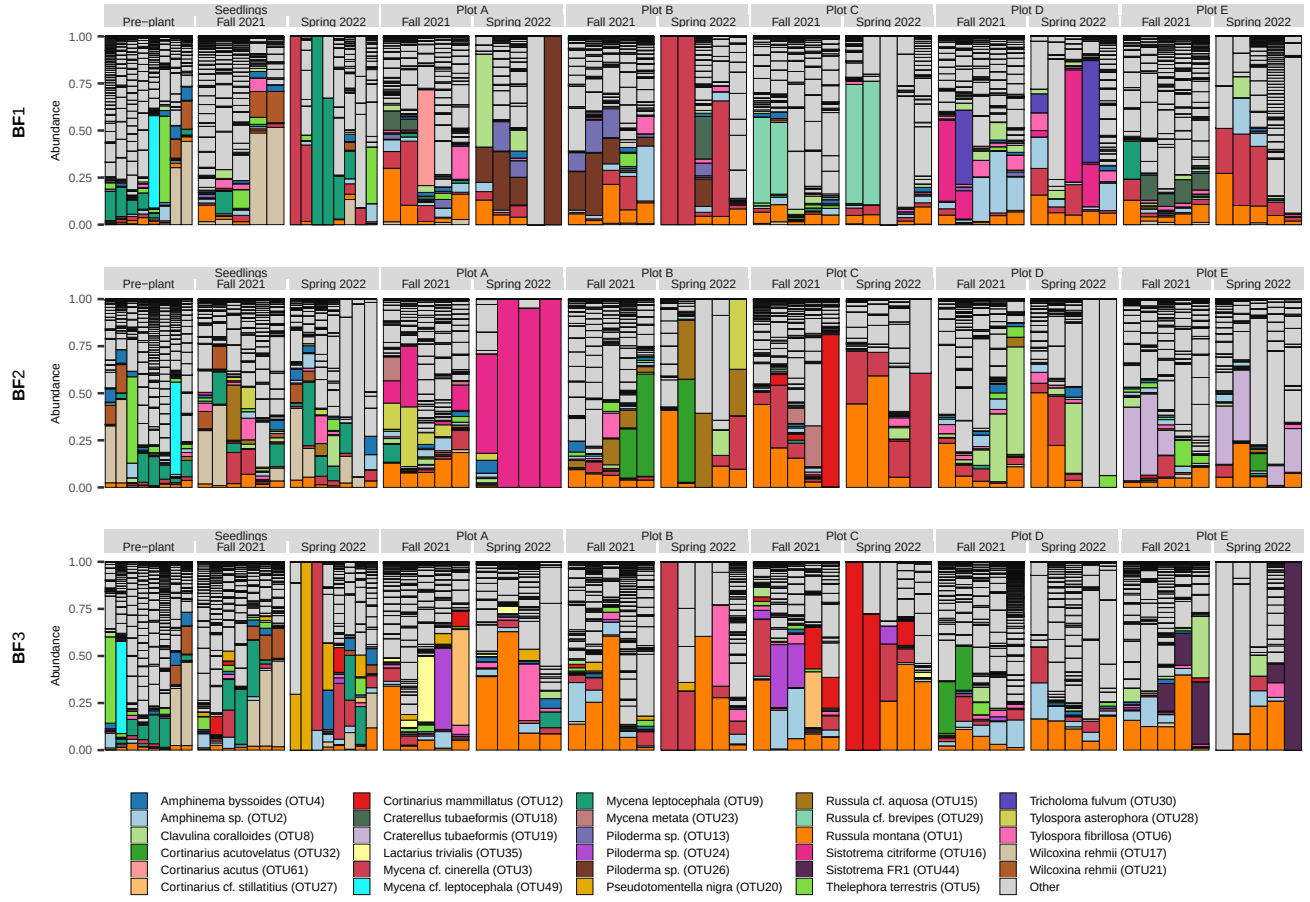

Figure 3.13: Relative abundances of operational taxonomic units in balsam fir samples by patch and season. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each column of each row represents one sample. Each column represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

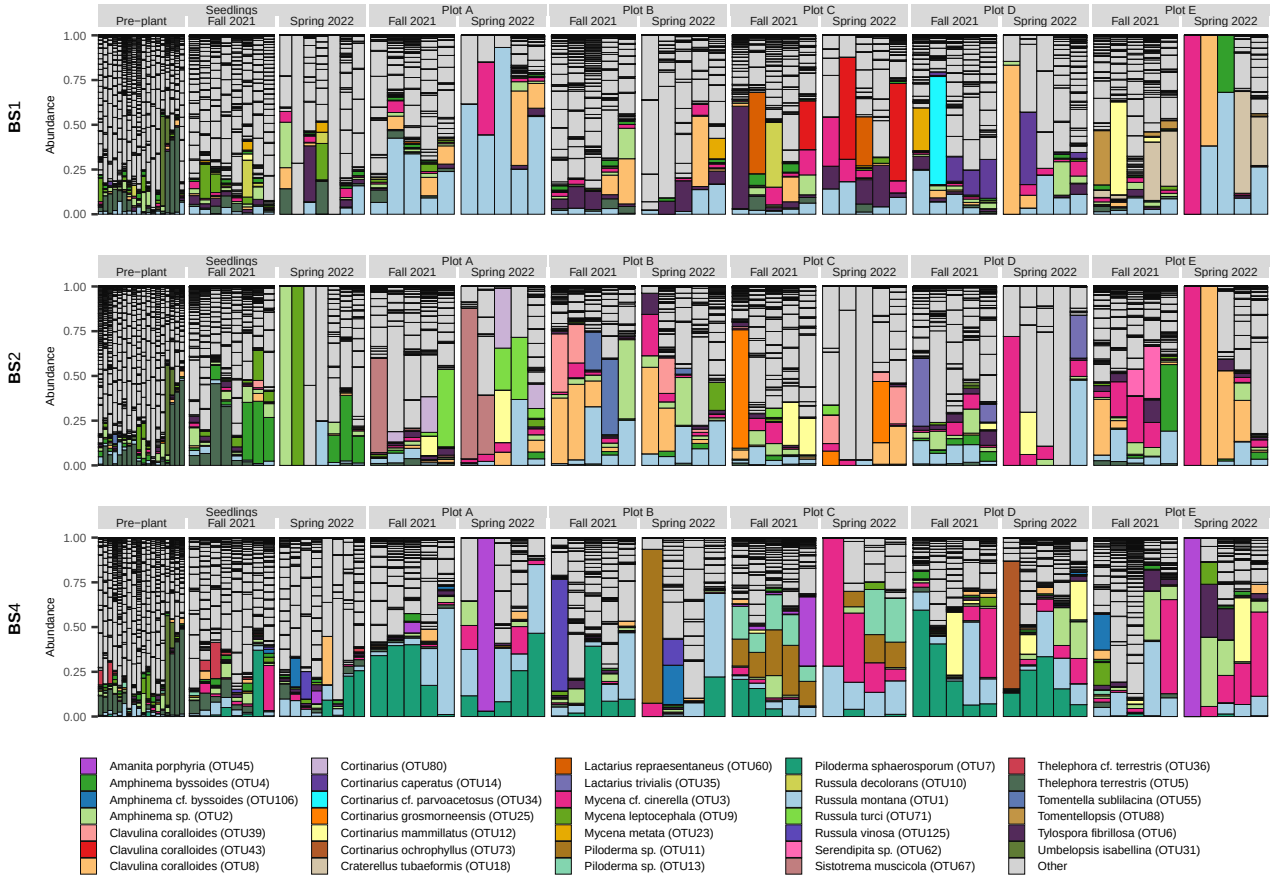

Figure 3.14: Relative abundances of operational taxonomic units in black spruce samples by patch and season. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each column of each row represents one sample. Each column represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

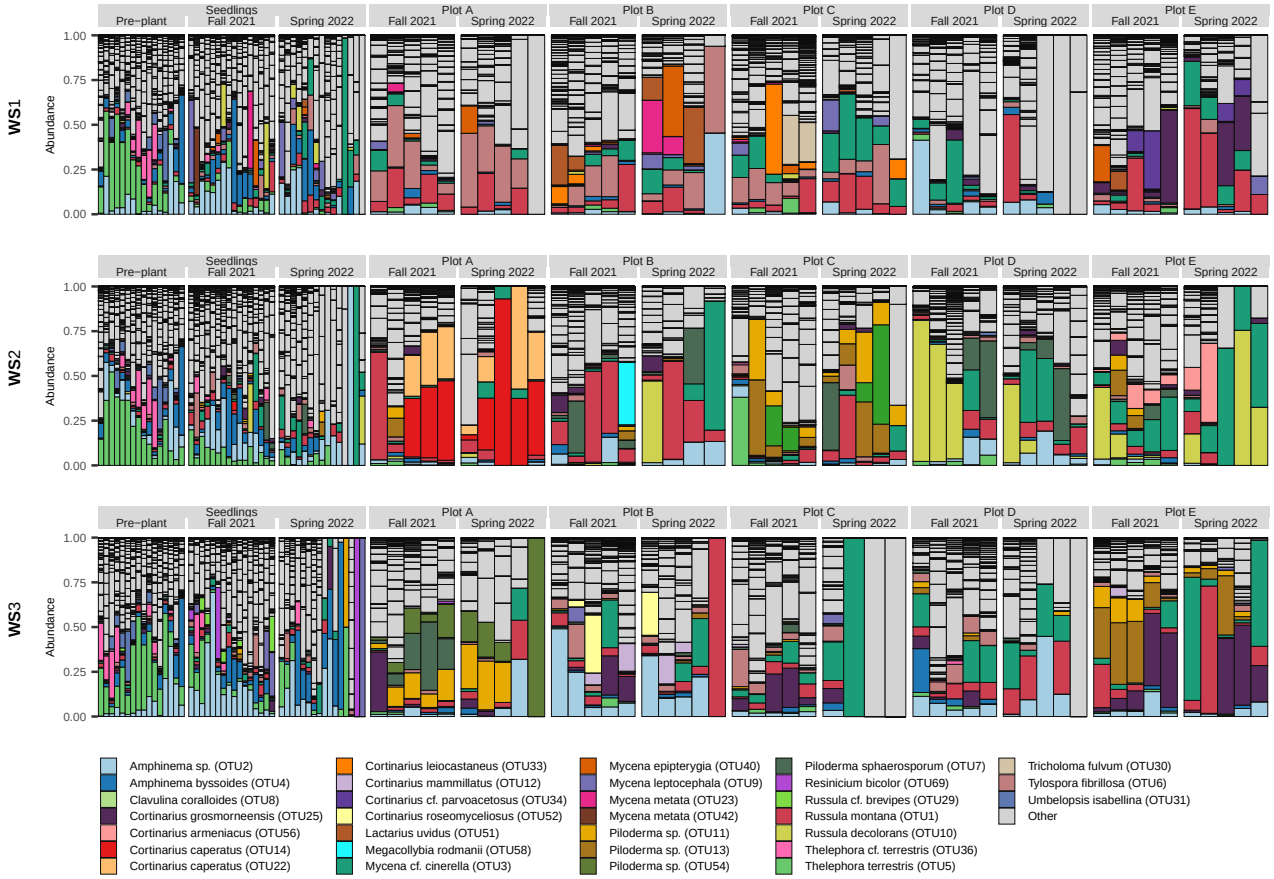

Figure 3.15: Relative abundances of operational taxonomic units in white spruce samples by patch and season. Most abundant 18 OTUs shown in colour; all other OTUs shown in grey, separated by black. Each column of each row represents one sample. Each column represents one sample. Confidence of OTU identification can be found in Appendix Table A.4.

3.2.6 Indicator operational taxonomic units of plots within seedling patches

An indicator OTU analysis of roots from plots A, B and C found that 48 OTUs were significantly associated with at least one tree species. Black spruce had 19 indicator OTUs with *Amanita porphyria* (OTU45) (p=0.002), *Clavulina coralloides* (OTU216) (p=0.0027) and *Cortinarius* cf. *aurae* (OTU99) (p=0.0056) as the three most significantly associated OTUs (Figure 3.16). White spruce had 25 indicator OTUs with Atheliaceae (OTU436) (p=0.0002), Schizoporaceae (OTU147) (p=0.0003), and *Mycena* cf. *sanguinolenta* (OTU1033) (p=0.0008) as the three most significantly associated OTUs (Figure 3.16). Four OTUs were indicators of BS and WS including Pucciniales sp. (OTU133) (p=0.0015), Sistotrema (OTU64) (p=0.0413), *Phialocephala fortinii* (OTU3923) (p=0.0467), and *Mycena leptocephala* (OTU74) (p=0.0499) (Figure 3.16). BF had no significantly associated indicator OTUs. Analysis was not run on WB due to low sample size.

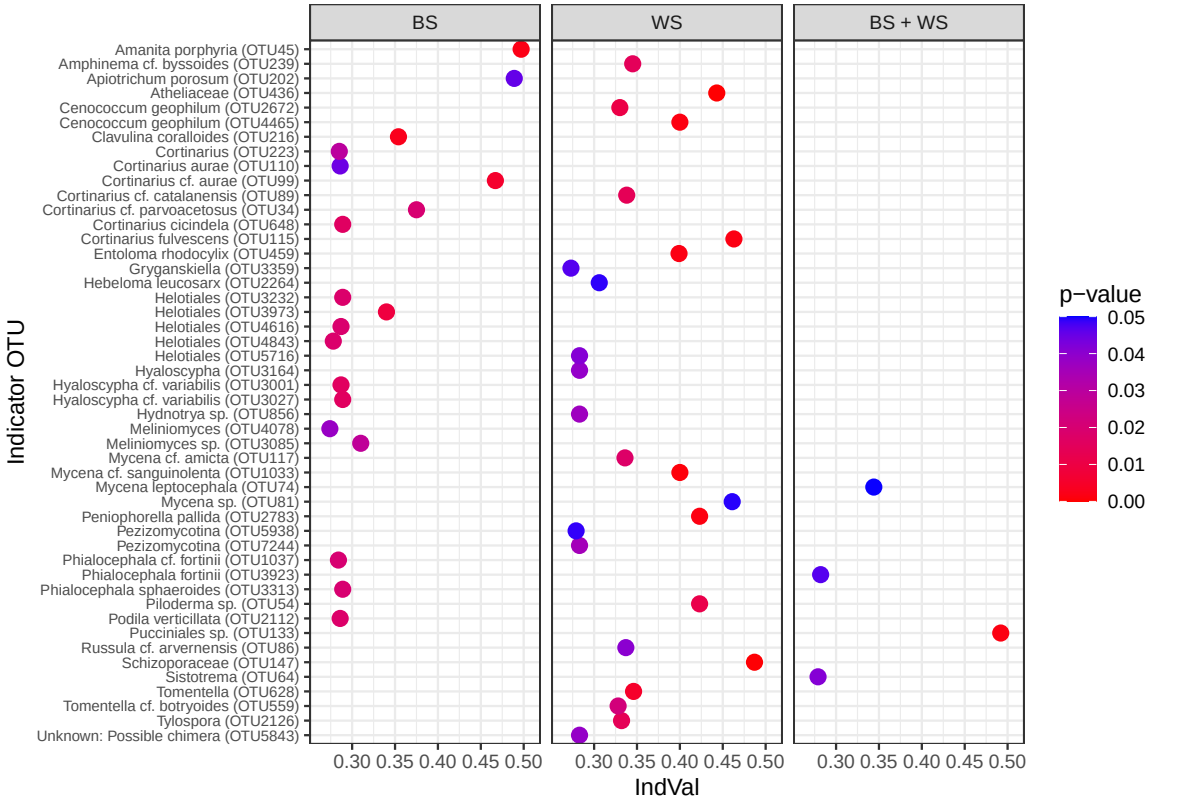

Figure 3.16: Indicator operational taxonomic units of black spruce and white spruce. BS represents black spruce and WS represents white spruce. IndVal, the indicator value index is a measured association between the fungal species and the tree species. Color of dot denotes p-value. Confidence of OTU identification can be found in Appendix Table A.4.

4 Discussion

4.1 Community composition of a spruce-fir forest in Newfoundland

The species accumulation curve did not reach a horizontal asymptote (Figure 3.2), indicating that sampling depth was inadequate to determine complete species richness. A horizontal asymptote would indicate that all possible OTUs have been sequenced, whereas the current graph indicates that additional sampling would have resulted in additional OTUs. Given the small quantity of root in each sample (0.1g), the high average number of OTUs per sample (31.3 ±18.9) is an indicator that clustering with a 99.5% threshold of similarity using vsearch in QIIME2 was not sufficient and the total OTU count is inflated. A lower threshold of similarity such as 99% may have resulted in a more realistic number of OTUs per sample and a species accumulation curve with a horizontal asymptote indicating adequate sampling depth.

There were no significant differences between the community composition of balsam fir (BF), black spruce (BS), and white spruce (WS) (Table 3.3); however, BF appears to host a higher diversity of ECM fungi, followed by BS then WS (Table 3.4). These results were in contrast to Kernaghan and Patriquin (2015) who found WS roots to support a more diverse community than BF roots in mixed spruce-fir forests in Nova Scotia and Quebec. The discrepancy may be due to sample size considering that Kernaghan and Patriquin (2015) sampled an equal number of root tips for each tree species, whereas BF was sampled in much higher proportions compared to BS and WS in this study (Table 3.1) thus generating more OTUs since the sampling depth was insufficient. This study emphasizes that a low diversity of host tree species can support a high diversity of ECM fungal species as only four host tree species were found to host

many OTUs. BF was the most generalist host, with only one unique abundant (>0.01%) OTU in comparison to BS (198 OTUs) and WS (46 OTUs) (See Section 3.1). The ECM community of the experimental site was colonized mainly by Basidiomycota and Ascomycota (Figure 3.3), a pattern which can be seen in other Canadian boreal forest studies (Kernaghan and Patriquin, 2015, Mah et al., 2001, Robertson et al., 2006).

There were significant differences between samples collected in fall and spring (Table 3.5). Seasonality has been shown to have an effect on ECM community composition of *Fagus sylvatica* in Germany (Pena et al., 2010), and of *Quercus petraea* and *Quercus robur* in France (Courty et al., 2008), with highest diversity found during the fall season in both studies. However, no effect of seasonality was found in studies of other host plants including *Quercus ilex* in France and *Pinus albicaulis* in California, USA (Richard et al., 2011, Glassman et al., 2017). The conflicting results of these studies may be due to the proportion of perennial mycelia, which grows year after year to annual mycelia, which grows and dies each year. However, in this study, a likely explanation for the significant differences is the poor quality of NGS runs in spring 2022. In comparison to fall 2021 samples (n=90), spring 2022 samples (n=90) had a lower average %Q30 (73.8% in spring 2022 vs 79.4% in fall 2021), many fewer reads generated per sample (1542 vs 12,537), less than half the total generated reads (372,378 vs 969,692), and approximately half of the OTUs (656 vs 1255).

Russula montana (OTU1), *Amphinema* sp. (OTU2), *Mycena* cf. *cinerella* (OTU3), and *Tylospora fibrillosa* (OTU6) were found on BF, BS, WS and white birch (WB) (Table 3.7), indicating low host specificity with hosts across different phyla. Species of *Russula*, *Amphinema*, *Mycena*, and *Tylospora* are often reported to be generalist fungi in the boreal region of Canada and have previously been found on BF (DeBellis et al., 2006, Kernaghan and Patriquin, 2015, Kernaghan et al., 2003), BS (Robertson et al., 2006), WB (DeBellis et al., 2006, Kernaghan and Patriquin, 2015, Kernaghan et al., 2003), WS (Kernaghan et al., 2003, Kernaghan and Patriquin, 2015), and tree species not included in this study such as *Populus tremuloides* (DeBellis et al., 2006, Kernaghan et al., 2003, Visser et al., 1998) and *Pinus banksiana* (Visser, 1995). *Tylospora fibrillosa* has previously been found on BS in Eastern Labrador (Reithmeier and Kernaghan, 2013) and *Russula montana* and *Mycena cinerella* have previously been reported

in fruiting body surveys of NL (Bazzicalupo et al., 2016, Burzynski et al., 2016). *Mycena* has historically been classified as a genus of wood-decomposing fungi rather than ectomycorrhizal fungi (Worrall et al., 1997, Tedersoo and Smith, 2013), but has recently been found on roots of BF, WS and WB in Nova Scotia and Quebec (Kernaghan and Patriquin, 2015). The presence of *Mycena* on roots in a previous study and *Mycena* cf. *cinerella* on roots in this study suggests that *Mycena* is not an exclusively wood-decomposing genus and may be ectomycorrhizal at times.

All abundant OTUs of *Cortinarius* (OTU63, OTU56, OTU14, OTU91, OTU89, OTU34, OTU12, and OTU73) and *Craterellus tubaeformis* (OTU18 and OTU19) were only found on BF and BS (Table 3.7). *Cortinarius* has previously been found on WS and WB in Canada (DeBellis et al., 2006, Kernaghan and Patriquin, 2015); the low sample size of WS (n=12) and WB (n=2) in this study was the likely reason for the lack of *Cortinarius* on WS and WB roots. *Craterellus tubaeformis* has not yet been reported on roots from a Canadian boreal forest, but has previously been reported in fruiting body surveys of NL (Burzynski et al., 2016). Outside of Canada, *Craterellus tubaeformis* has been found on roots of *Pinus densiflora* in Japan (Lian et al., 2006) and *Picea abies* in France (Buée et al., 2011).

Some OTUs appeared to show high host specificity, including *Cenococcum geophilum* (OTU1016 and OTU3500), *Lactarius tabidus* (OTU37) and *Lactarius uvidus* (OTU51), but other studies from Canadian boreal forests show that this apparent host specificity is likely exaggerated. *Cenococcum geophilum* (OTU1016 and OTU3500) was found only on WS and WB (Table 3.7), hosts which were also seen in DeBellis et al. (2006), but has been found on BF (DeBellis et al., 2006) and BS in midwestern USA (Hupperts and Lilleskov, 2022). *Lactarius* spp. were found only on WS and WB (Table 3.7), hosts which were also seen in DeBellis et al. (2006), Kernaghan et al. (2003), and Kernaghan and Patriquin (2015), but have been found on BF (DeBellis et al., 2006) and BS (Robertson et al., 2006). Since only the top 18 OTUs of each host tree species were highlighted in this study, it is likely that *Cenococcum geophilum* and species of *Lactarius* were associated with BF and BS but not included due to the high diversity seen in BF and BS in comparison to WS and WB (Table 3.7).

The overall community composition was found to be similar to that reported in boreal forest studies of roots in other parts of Canada, and fruiting body surveys and a small number of soil samples collected in NL. With the exception of seven OTUs, all abundant OTUs listed in Table 3.7 have previously been reported in other parts of Canada including studies of roots of BS in Labrador (Reithmeier and Kernaghan, 2013), BF, WS, WB and *Populus tremuloides* in Quebec and Nova Scotia (Kernaghan et al., 2003, Kernaghan and Patriquin, 2015, DeBellis et al., 2006), WS, WB, *Pinus banksiana* and *Pinus contorta* var. *latifolia* in Alberta (Danielson, 1984, Visser, 1995, Visser et al., 1998, Teste et al., 2012), BS in British Columbia (Robertson et al., 2006), two soil samples collected from NL as a part of a global study (Tedersoo et al., 2014), or fruiting body surveys of NL (Burzynski et al., 2016). *Cortinarius* cf. *parvoacetosus* (OTU34) and *Cortinarius mammillatus* (OTU12) have not been reported in root studies in Canada or fruiting body surveys of NL but have been reported as fruiting body observations in Quebec on MycoQuebec.org.

The five remaining OTUs that have not previously been reported on roots in Canada or observed in NL include *Cortinarius* cf. *catalanensis* (OTU89), *Mycena* cf. *amicta* (OTU117), *Mycena purpureofusca* (OTU249), *Russula* cf. *arvernensis* (OTU79), and *Tomentella* cf. *cinereoumbrina* (OTU366). *Cortinarius catalanensis* has been suggested as a synoynm of *Cortinarius* sp. IUMQ3813, a species that has been observed in Quebec (Landry et al., 2021). Fruiting bodies of *Mycena amicta* and *Mycena purpureofusca* have been reported in the Pacific Northwest (Redhead, 1997, Edmonds and Lebo, 1998), while *Russula arvernensis* and *Tomentella cinereoumbrina* have only been reported on roots and in soil in Europe (GenBank accession numbers OM964844.1, OM964845.1 and OM964846.1, Arraiano-Castilho et al., 2020). Due to constraints of previous techniques, many studies of root-associated ECM fungi in Canada have not been able to identify many OTUs to the species level. It is likely that some OTUs in this study that seemingly have not been previously found in Canada, have been found but were only identified to the genus level. Each genus of the five OTUs listed above has been reported in studies of roots from other parts of Canada (Kernaghan and Patriquin, 2015, Reithmeier and Kernaghan, 2013, Visser, 1995, Visser et al., 1998, Robertson et al., 2006), in the two soil samples from NL (Tedersoo et al., 2014), and in fruiting body surveys of NL (Burzynski et al.,

2016).

Fruiting bodies of *Cantharellus enelensis* were frequently observed in the experimental site (eight specimens collected, sequenced and deposited to GenBank; see Appendix A.3) but were not found on any root samples, even in low abundance likely as a result of the length of its ITS region (Schoch et al., 2012, Moncalvo et al., 2006). The chosen sequencing method of this study constrained the results as NGS preferentially captures short reads meaning that fungal genera including *Cantharellus* with a longer ITS2 region than the average 350-400 bases would have been excluded during sequencing, biasing the data towards fungi with shorter ITS2 regions.

Of the 20 indicator OTUs of BS, six were OTUs of *Cortinarius* and three were OTUs of *Phialocephala fortinii* or *Phialocephala* cf. *fortinii* (Figure 3.9). Of the eight indicator OTUs of WS, five were in the Russulaceae family including species of *Lactarius* and *Russula* (Figure 3.9). There are no commonalities between the five indicator OTUs of BS and WS. BF had no indicator species, likely as a result of the large number of BF samples in this study in comparison to BS and WS (Table 3.1). With an equal number of sampled BF, BS, and WS roots, more statistical support may have been found for indicator species of BF; however, since BF is a host of many generalist ECM fungi found on all four tree species in this study such as *Mycena*, *Amphinema* and *Russula* and is the most dominant tree at the experimental site (Appendix Figure A.1), it may truly have no significant unique ECM fungal associates.

4.2 Community composition of seedlings and roots near seedlings

The species accumulation curves for seedling samples and root samples near seedlings did not reach horizontal asymptotes (Figure 3.10, Figure 3.11), indicating that sampling depth was inadequate to determine complete species richness. Given the small quantity of root in each sample (0.1g), the high average numbers of OTUs per sample (35.2 ±18.5 for seedlings, 30.6 ±18.3 for roots) are indicators that clustering using vsearch in QIIME2 was not sufficient and the total OTU counts are inflated for the same reasons mentioned in Section 4.1.

4.2.1 Pre-planted seedlings

The community composition of pre-planted seedlings was similar to the community composition of nursery seedlings in other studies. Some OTUs found in high abundance, including *Wilcoxina rehmii* (OTU17 and OTU21), *Umbelopsis isabellina* (OTU31), and *Thelephora terrestris* (OTU5 and OTU36) (Figure 3.13, Figure 3.14, Figure 3.15), have previously been reported on seedlings of *Quercus garryana*, *Picea abies* and *Pinus sylvestris* (Trocha et al., 2006, Rudawska and Leski, 2021, Iwanski et al., 2006, Menkis et al., 2005, Stenström et al., 2014, Southworth et al., 2009, Colpaert et al., 1999). The remaining OTUs found in high abundance, *Mycena leptocephala* (OTU9), *Mycena* cf. *leptocephala* (OTU49), *Russula montana* (OTU1), *Amphinema* sp. (OTU2) and *Amphinema byssoides* (OTU4) (Figure 3.13, Figure 3.14, Figure 3.15) have not yet been reported on nursery seedlings, but other species or unidentified species within the genera *Russula* and *Amphinema* have been reported on seedlings of *Pinus sylvestris* and *Picea abies* (Menkis et al., 2005, Trocha et al., 2006, Rudawska and Leski, 2021, Iwanski et al., 2006, Aučina et al., 2014, Stenström et al., 2014, Southworth et al., 2009). No species of *Mycena* have been reported on nursery seedlings, but were likely overlooked in many studies as they are typically considered wood-decomposers as mentioned in section 4.1. *Cenoccocum geophilum* and *Laccaria* were not found in high abundance as commonly reported on nursery seedlings of *Picea abies* and *Pinus sylvestris* (Southworth et al., 2009, Battista et al., 2002, Menkis et al., 2005, Aučina et al., 2014, Trocha et al., 2006, Pietras et al., 2013); however, some studies have shown that nursery practices such as bare-root vs. container growing and fertilizer type and amount can influence ECM community composition on seedlings (Trocha et al., 2006, Rudawska et al., 2001).

Ascomycota had a higher abundance on nursery seedlings than Basidiomycota (Figure 3.12), a pattern commonly seen with nursery seedlings (Trocha et al., 2006, Aučina et al., 2014, Stenström et al., 2014, Menkis et al., 2005). Mucoromycota were found on nursery seedlings in proportions as high as 64.8% on a single sample (Figure 3.12) but have only been reported in two previous studies of nursery seedlings (Menkis et al., 2005, Stenström et al., 2014). The lack of reported Mucoromycota in other studies may be due to the commonly chosen method of

selection of morphotypes to identify ECM fungal species (Rudawska and Leski, 2021, Iwanski et al., 2006, Aučina et al., 2014, Pietras et al., 2013, Menkis et al., 2005, Battista et al., 2002, Southworth et al., 2009, Trocha et al., 2006) which would exclude Mucoromycota due to their inconspicuous morphotypes.

4.2.2 Changes in seedlings and roots after introduction of nursery seedlings

Seedlings of BF maintained a high abundance of *Wilcoxina rehmii* (OTU17 and OTU21) and *Mycena leptocephala* (OTU9) in all BF patches in fall 2021 and spring 2022 (Figure 3.13). *Thelephora terrestris* (OTU5) decreased in abundance on seedlings in BS1 and BS3 in fall 2021, but maintained high abundance in BS2 until spring 2022 (Figure 3.14). *Mycena leptocephala* (OTU9) was maintained in BS1 in fall 2021 and spring 2022 (Figure 3.14). Seedlings of WS maintained a high abundance of *Amphinema* sp. (OTU2) and *Amphinema byssoides* (OTU4) in all WS patches in fall 2021 and spring 2022 but decreased in abundance of *Thelephora terrestris* (OTU5), *Thelephora* cf. *terrestris* (OTU36) and *Umbelopsis isabellina* (OTU31) in all WS patches in fall 2021 and spring 2022 (Figure 3.15).

This study shows that fungi have varying persistence on seedlings once outplanted, as reported in studies of inoculated seedlings of BS (Gagné et al., 2006), WS (Gagné et al., 2006, Onwuchekwa et al., 2014) and other tree species (Hung and Trappe, 1987, Menkis et al., 2007, El Karkouri et al., 2006, Franco et al., 2014). Some OTUs including *Wilcoxina rehmii* (OTU17 and OTU21), *Mycena leptocephala* (OTU9), *Amphinema* sp. (OTU2) and *Amphinema byssoides* (OTU4) showed persistence of at least 20 months after outplanting in this study as previously reported in Franco et al. (2014), El Karkouri et al. (2006), Hung and Trappe (1987) and Pennanen et al. (2005) in which inoculant and non-inoculant ECM fungal species such as *Pisolithus*, *Suillis* spp., *Hebeloma crustuliniforme* and *Laccaria* spp. persisted for 17 months to five years. Other OTUs including *Thelephora terrestris* (OTU5), *Thelephora* cf. *terrestris* (OTU36) and *Umbelopsis isabellina* (OTU31) decreased in abundance in this study as similarly reported in Onwuchekwa et al. (2014), Gagné et al. (2006), and Menkis et al. (2007) in which inoculant and non-inoculant ECM fungal species including *Hebeloma crustuliniforme*,

Laccaria bicolor and *Suillus tomentosus* did not persist on seedlings past 15 months to five years. *Amphinema byssoides* (OTU4) maintained high abundance on WS in this study and was similarly found to persist on seedlings of WS six years after outplanting in Gagné et al. (2006).

In Dahlberg and Stenström (1991), native ECM fungi including *Cenococcum geophilum* and *Piloderma croceum* had colonized seedlings outplanted in forests after only one year and similarly, seven OTUs in this study appear to have transferred from roots within the seedling patches to seedlings. *Pseudotomentella nigra* (OTU20) appears to have transferred from roots within the BF3 patch to BF seedlings as it was found in high abundance on seedlings in spring 2022 and on roots in Plot A and B in fall 2021 and spring 2022, but not previously found on pre-planted seedlings (Figure 3.13). *Piloderma sphaerosporum* (OTU7) may have transferred from roots within the BS4 and WS2 patches to BS and WS seedlings as it was found in high abundance on seedlings in the BS4 and WS2 patches and high abundance on roots in plots A, B, C, and D of the BS4 patch and plots B, C, D and E of the WS2 patch, but very low abundance in pre-planted seedlings (Figure 3.14, Figure 3.15). *Mycena* cf. *cinerella* (OTU3) and *Tylospora fibrillosa* (OTU6) may have transferred from roots within all WS patches to WS seedlings as they were found in high abundance on seedlings in all WS patches and high abundance on roots in plots A, B, C, D and E of the WS patches, but very low abundance in pre-planted seedlings (Figure 3.15). *Russula decolorans* (OTU10) may have transferred from roots within the WS2 patch to WS seedlings as it was found in high abundance on seedlings in the WS2 patch and high abundance in plots B, C, D and E of the WS2 patch but very low abundance in pre-planted seedlings (Figure 3.15). *Piloderma* (OTU11) may have transferred from roots within the WS3 patch to WS seedlings as it was found in high abundance on seedlings in the WS3 patch and high abundance on roots in plots A, D and E of the WS3 patch but very low abundance in pre-planted seedlings (Figure 3.15).

Similar to *Mycena* as mentioned in section 4.1, *Resinicium* has also historically been classified as a genus of wood-decomposing fungi instead of ectomycorrhizal fungi (Ginns, 1986, Lindsey and Gilbertson, 1978). *Resinicium bicolor* was found in high abundance on seedlings of in the WS3 patch in fall 2021 and spring 2022 and on roots in plot A within the WS3 patch (Figure 3.15) and has previously been found on roots of *Betula pubescens* spp. *tortuosa* in Sweden

(Bödeker et al., 2014), suggesting that *Resinicium bicolor* is also not exclusively a wood-decomposer and may be ectomycorrhizal at times.

In contrast to the transfer of OTUs to seedlings, zero ECM fungi from seedlings seem to have transferred to roots within any of the seedling patches (Figure 3.13, Figure 3.14, Figure 3.15). Of the 48 indicator OTUs of roots near seedling patches (plots A, B and C), seven were also indicator OTUs of roots outside of seedling patches (plots D and E) (Figure 3.9, Figure 3.16). Six of the seven OTUs remained indicators of the same tree species or shared one common tree species with roots outside of seedling patches (Figure 3.9, Figure 3.16). *Cenococcum geophilum* (OTU4465) was an indicator OTU of BS outside of seedling patches and of WS near seedling patches, likely a result of the low sample size of BS and WS and insufficient sampling depth or clustering (Figure 3.9, Figure 3.16). Multiple species of *Cortinarius* were indicator OTUs of BS as also seen in roots outside of seedling patches (Figure 3.9, Figure 3.16).

This is the first study to investigate whether weedy ECM fungal species of nursery seedlings affect native ECM fungal species once outplanted and the results suggest that the common forestry practice of introducing weedy ECM fungal species as a byproduct of introducing nursery seedlings to forest ecosystems does not alter the composition of native ECM fungal communities, an important finding given that different fungi provide different benefits to forest ecosystems including differing P and N uptake and mobilization and C storage (Wallander, 2000, Baxter and Dighton, 2001,Clemmensen et al., 2015, Lindahl et al., 2021). Maintaining the composition of native ECM communities is critical in order to maintain the efficiency of forests; however, this study was only capable of showing short-term changes of roots and seedlings, up to a maximum of 20 months. Over time, more OTUs will likely transfer from roots to seedlings and OTUs still have the potential to transfer from seedlings to roots, as some species have previously been shown to have persistence on seedlings of at least six years (Gagné et al., 2006). This study was limited to showing that weedy ECM fungal species on nursery seedlings do not replace native ECM fungal species on roots within 20 months of outplanting.

Additionally, the experiments of this study were performed in a forested plot whereas nursery seedlings are typically outplanted in plots that have been clearcut. Previous studies show that after a clearcut, forests can exhibit a lower carbon input (Perry et al., 1984), a lower host plant diversity (Halpern and Spies, 1995), changes in soil conditions including moisture, temperature, and C, P and N storage (Perry et al., 1989, Johnson and Curtis, 2001, Hume et al., 2018), and disturbance to the forest floor if site preparation occurs (Harvey et al., 1976). These factors have been shown to alter ECM community composition (Parke et al., 1984, Jones et al., 2003, Barker et al., 2013); however, previous studies have found that the volume of ECM fungal inoculum, the number of active root tips, and the ECM fungal diversity in clearcut sites without site preparation do not begin to differ substantially from forested sites until one to two years after a clearcut (Harvey et al., 1980, Visser et al., 1998, Hagerman et al., 1999). Therefore, the results of this study are relevant to sites that are reforested with nursery seedlings within one to two years after clearcutting with no site preparation. Lastly, as previously mentioned in section 4.1, the chosen sequencing method, NGS, preferentially captures short reads, biasing the data towards fungi with shorter ITS2 regions.

4.3 Future considerations

For future studies, experiments should be performed long-term to monitor changes that occur more than 20 months after nursery seedlings are outplanted to confirm that the transfer of OTUs from seedlings to roots within the forest and potential loss of native ECM fungi does not occur past 20 months. In addition, future studies should be performed in clearcut sites to remove any biases that a non-disturbed forest site may introduce and extend the applicability of this study to sites that are reforested greater than one to two years after a clearcut.

The combination of ITS and an additional gene region, the 28S nuclear ribosomal large subunit (LSU), should be used in combination to remove the short read bias introduced by NGS. In fungi, LSU is considered to be less variable than the ITS region (Porras-Alfaro et al., 2014), exhibiting a more consistent length but it is generally inferior to ITS in species discrimination (Schoch et al., 2012). Sequencing both gene regions to determine community composition

would allow greater coverage of species diversity of the experimental site, in addition to increasing sampling depth or decreasing the similarity threshold in the clustering step.

Lastly, more studies should investigate the ECM community composition of forests in Newfoundland using molecular techniques to determine variability that may occur within the province. It is well known that many factors can affect community composition (Tedersoo et al., 2013, Tedersoo et al., 2012, Miyamoto et al., 2022, Bahram et al., 2011, Geml et al., 2012, Timling et al., 2012, Blaalid et al., 2014, (Toljander et al., 2006, Cox et al., 2010, Glassman et al., 2017, Tedersoo et al., 2014, Jarvis et al., 2013, Erlandson et al., 2016, Guo et al., 2020, Boeraeve et al., 2018, Twieg et al., 2007, Visser, 1995, Smith et al., 2002, Nara et al., 2003) and the factors influencing the community composition found at the site in this study are unknown. A cumulative list of all fungal species found in Newfoundland and Labrador since 2003 including non-ectomycorrhizal fungi has been recently updated to approximately 1700 species (Burzynski et al., 2023), whereas this study found approximately 1630 OTUs of only ectomycorrhizal fungi, suggesting that many ectomycorrhizal fungi in Newfoundland remain unlisted.

4.4 Conclusions

In conclusion, the ECM community composition of a mixed spruce-fir forest in Newfoundland was found to be similar to that of other boreal forest studies of BF, BS, WS, *Populus tremuloides* and *Pinus banksiana* in Quebec, Nova Scotia, Alberta, British Columbia and Labrador (DeBellis et al., 2006, Kernaghan and Patriquin, 2015, Kernaghan et al., 2003, Visser et al., 1998, Visser, 1995, Reithmeier and Kernaghan, 2013, Robertson et al., 2006, Danielson, 1984), with common abundant genera such as *Cortinarius*, *Mycena*, and *Russula*. The community composition of weedy ECM species developed on BF, BS and WS in the nursery was found to be similar to that of nursery seedlings of *Picea abies*, *Pinus sylvestris*, and *Quercus garryana*, sharing abundant taxa such as *Thelephora terrestris*, *Wilcoxina rehmii*, and *Umbelopsis isabellina* (Trocha et al., 2006, Rudawska and Leski, 2021, Iwanski et al., 2006, Menkis et al., 2005, Stenström et al., 2014, Southworth et al., 2009, Colpaert et al., 1999), but notably missing a high abundance of *Cenoccocum geophilum* and *Laccaria* seen in other studies (Southworth

et al., 2009, Battista et al., 2002, Menkis et al., 2005, Aučina et al., 2014, Trocha et al., 2006, Pietras et al., 2013).

Over time, some OTUs such as *Thelephora terrestris* and *Umbelopsis isabellina* decreased in abundance, while others such as *Wilcoxina rehmii* and *Amphinema byssoides* showed persistence throughout the entire study period. Seven OTUs appear to have transferred from roots to seedlings and no OTUs appear to have transferred from seedlings to roots within the 20 month study period, suggesting that outplanting nursery seedlings to reforest clearcut sites does not alter native ECM community composition for sites that are reforested within one to two years when no site preparation occurs. Since different fungi serve different functional roles in forest ecosystems (Wallander, 2000, Baxter and Dighton, 2001, Clemmensen et al., 2015, Lindahl et al., 2021), native ECM community composition must be conserved to maintain forest productivity, highlighting the importance of the finding that native ECM community composition is not altered. Long-term studies should be performed in clearcut sites in other areas of Newfoundland with a greater sampling depth and using a combination of ITS and LSU gene regions to capture complete species diversity in the province with less time-frame, site, sampling and sequencing biases.

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A Appendix

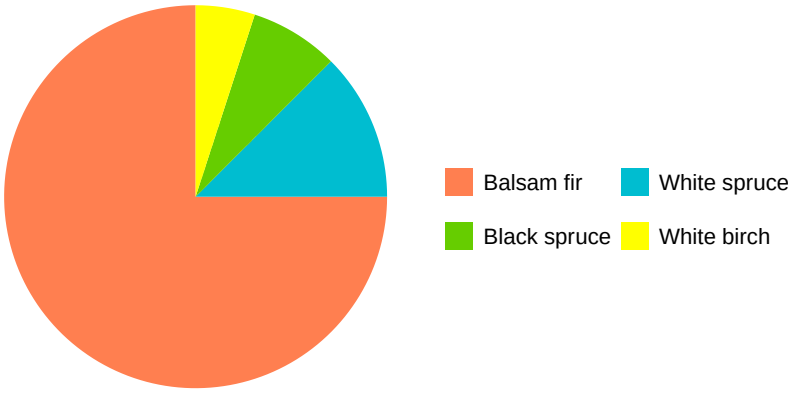

Figure A.1: Frequency of tree species in overstory of experimental site. Through point quarter sampling performed on 2021-09-24, it was determined that balsam fir represents 75% of the overstory, white spruce represents 12.5%, black spruce represents 7.5% and white birch represents 5%. 60 points (240 trees) were sampled on 6 transects of 10 m separated by 10 m.

Table A.1: Overstory tree metrics. Determined through point-quarter sampling performed on 2021-09-24. Equations used for calculations shown below table. 60 points (240 trees) were sampled on 6 transects of 10 m separated by 10 m.

	Overstory tree				
	Balsam fir	**Black spruce**	**White birch**	**White spruce**	**Stand**
Total distance (m)					401.03
Mean point-to-tree distance (m)	-	-	-	-	1.67
Trees/hectare	-	-	-	-	3581.53
Number of individuals (n_i)	214	7	5	14	240
Mean diameter at breast height (cm)	16.43	18.46	18.21	19.76	16.58
Relative density (%)	89.17	2.92	2.08	5.83	-
Density (stems/ha)	3193.53	104.46	74.62	208.92	3581.53
Basal area (m^2)	4.81	0.19	0.12	0.47	5.59
Average basal area (cm^2)	224.61	273.84	240.83	332.99	-
Total basal area (m^2/ha)	71.73	2.86	1.80	7.00	83.34
Relative basal area (%)	86.06	3.43	2.16	8.35	-
Frequency (%)	1.00	0.10	0.07	0.17	-
Relative frequency (%)	75	7.5	5	12.5	-
Importance value (IV)	0.83	0.05	0.03	0.09	-

Total distance (m) = Σ Distance from point − to − tree

Mean point − to − tree distance (m) = $\frac{\text{Total distance (m)}}{\text{Total number of trees}}$

Trees per hectare = $\frac{10000\ \text{ha}}{\text{Mean point-to-tree distance (m)}^2}$

Relative density (%)($\mathbf{p_i}$) = $\frac{n_i}{N}$

Density (stems per hectare) = $p_i \times$ Trees per hectare

Basal area (m^2) = Σ Basal area of each individual of a species

Average basal area (cm^2) = $\frac{\text{Basal area } (cm^2)}{n_i}$

Total basal area $\left(\frac{m^2}{ha}\right)$ = $\frac{\text{Density (stems per hectare)}}{\text{Average basal area } (m^2)}$

Relative basal area (%) = $\frac{\text{Total basal area } (\frac{m^2}{\text{hectare}})}{\text{Total basal area of all species } (\frac{m^2}{\text{hectare}})}$

Frequency (%) = $\frac{\text{Occurence of species among all sampling points}}{\text{Number of sampling points}}$

Relative frequency (%) = $\frac{\text{Frequency}}{\text{Total frequency}}$

Importance Value (IV) = $\frac{\frac{\text{Relative density}}{100} + \frac{\text{Relative basal area}}{100} + \frac{\text{Relative frequency}}{100}}{3}$

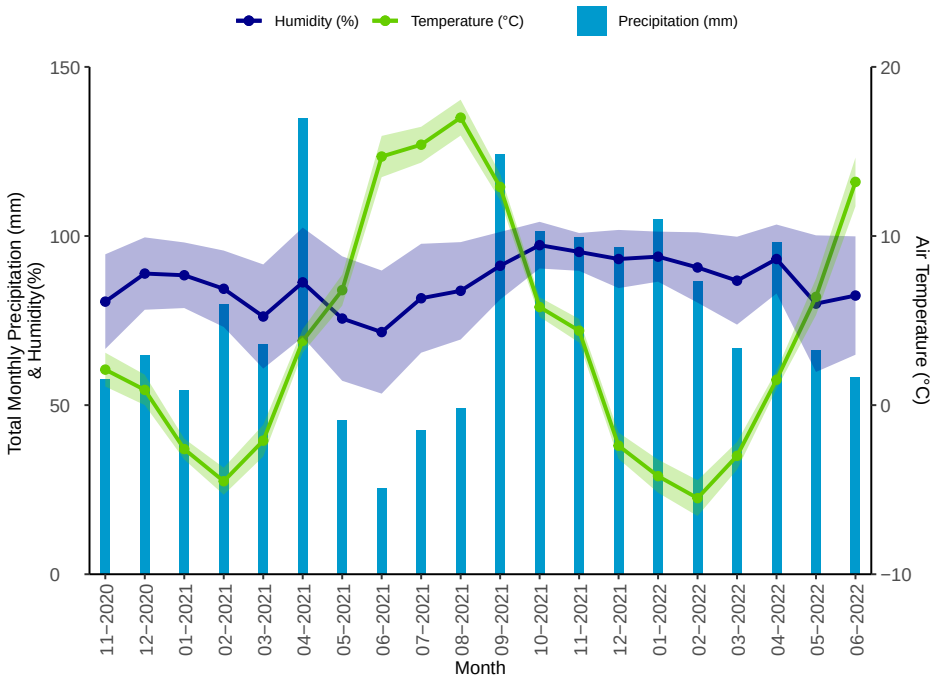

Figure A.2: Total monthly precipitation (mm), mean monthly air temperature (°C) and mean monthly humidity (%) for the duration of the study. Shaded areas represent a 95% confidence interval. Temperature and humidity data tracked using an Elitech Tlog B100H logger with the exception of data from 11-2020 to 05-2021, which was taken from Environment Canada Gander Airport CS Weather Station (48.95 N, -54.57 W). All precipitation data taken from Environment Canada Gander Airport CS Weather Station (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html).

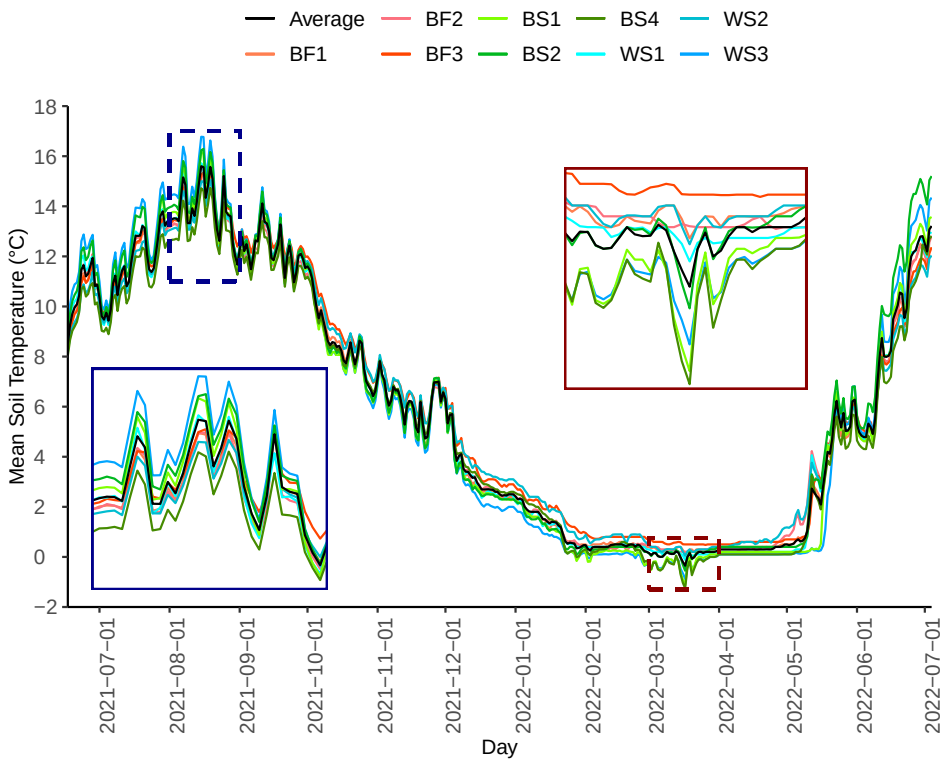

Figure A.3: Mean daily soil temperature at nine patch locations and average at experimental site. Average of all nine patches shown in black. Data from 2021-08-01 to 2021-09-01 (outlined in blue) and 2022-03-01 to 2022-04-01 (outlined in red) enlarged to show variation between patches. Data tracked using Elitech RC-51 loggers. Data points from 17:30-18:30 on 2021-10-17 were removed as data loggers were briefly removed from belowground to download data.

Table A.2: Understory vascular plants and bryophytes collected at experimental site. All specimens deposited at Dr. Laurie L. Consaul Herbarium at the University of Western Ontario (UWO No.). Names of vascular plants follow Canadensys (canadensys.net), names of bryophytes follow the Consortium of Bryophyte Herbaria (bryophyteportal.org), and names of botanical authors follow the form recommended in the International Plant Names Index (ipni.org).

Family	Name	Author	UWO No.
	Vascular Plants		
Araliaceae	*Aralia nudicaulis*	L.	55210
Asparagaceae	*Maianthemum canadense*	Desf.	55246
Betulaceae	*Alnus alnobetula*	(Ehrh). K. Koch	55243
	Alnus incana	(L.) Moench	55222
Caprifoliaceae	*Linnea borealis*	L.	55242
Cornaceae	*Cornus canadensis*	L.	55224
Cystopteridaceae	*Gymnocarpium dryopteris*	(L.) Newman	55216
Dryopteridaceae	*Dryopteris carthusiana*	(Vill.) H.P. Fuchs	55218
	Dryopteris intermedia	(Muhl. ex Willd.) A. Gray	55221
Ericaeceae	*Kalmia angustifolia*	L.	55226
	Moneses uniflora	(L.) A. Gray	55214
	Monotropa uniflora	L.	55208
	Orthilia secunda	(L.) House	55207
Grossulariaceae	*Ribes glandulosum*	Grauer	55220
Lycopodiaceae	*Dendrolycopodium obscurum*	(Michx.) A. Haines	55212
Orchidaceae	*Neottia cordata*	(L.) Rich.	55248
Osmundaceae	*Osmundastrum cinnamomeum*	(L.) C. Presl	55223
Primulaceae	*Lysimachia borealis*	(Raf.) U. Manns & Anderb.	55245
Rosaceae	*Amelanchier* sp.		55225
	Prunus pensylvanica	L. f.	55206
	Rubus pubescens	Raf.	55247
	Sorbus decora	(Sarg.) C.K. Schneid.	55211
Salicaceae	*Populus balsamifera*	L.	55205
	Populus tremuloides	Michx.	55241
Sapindaceae	*Acer spicatum*	Lam.	55209
Taxaceae	*Taxus canadensis*	Marshall	55244
Viburnaceae	*Sambucus racemosa*	L.	55215
	Viburnum cassinoides	L.	55219
	Viburnum edule	(Michx.) Raf.	55213
	Bryophytes & Lichens		
Dicranaceae	*Dicranum majus*	Turner	B5
Hylocomiaceae	*Hylocomium splendens*	(Hedw.) Schimp	B191
	Pleurozium schreberi	(Willd. ex Brid.) Mitt.	B190
Hypnaceae	*Calliergonella curvifolium*	(Hedw.) B.H. Allen	B1
Lepidoziaceae	*Bazzania trilobata*	(L.) Gray	L1
Peltigeraceae	*Peltigera neopolydactyla*	(Gyelnik) Gyelnik	2103
Pylaisiaeceae	*Ptilium crista-castrensis*	(Hedw.) De Not.	B3
Sphagnaceae	*Sphagnum capillifolium*	(Ehrh.) Hedw.	B192
	Sphagnum squarrosum	Crome	B189

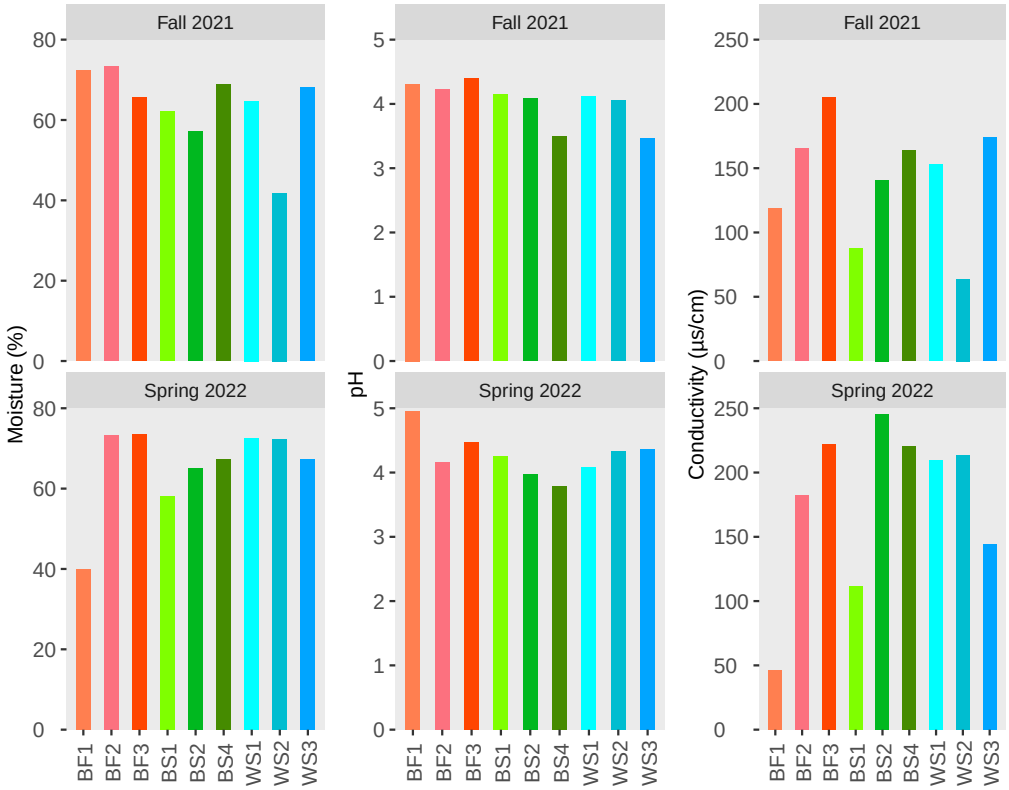

Figure A.4: Moisture (%), pH, and conductivity (µs/cm) at each of the nine patches at time of sampling. Soil was collected on 2021-10-17 and 2022-07-04. Moisture was measured by subtracting dry weight of soil sample from fresh weight of soil sample, divided by the fresh weight. pH and conductivity were measured with 10 g of soil in 100 mL distilled water using an Oakton WD-35634-35 PCTSTestr 50 Waterproof Pocket pH/Cond/TDS/Salinity Tester.

Table A.3: GenBank accession number, organism, sample identification, type of sample and date of collection of samples sequenced and submitted to GenBank.

Accession #	**Gene Region**	**Organism**	**Sample ID**	**Type**	**Date Collected**
OQ319473	rbcLa	*Picea mariana*	679	Root	2022-06-30
OQ319474	rbcLa	*Abies balsamea*	634	Root	2022-06-26
OQ319475	rbcLa	*Picea glauca*	210	Nursery seedling	2021-10-06
OQ319476	rbcLa	*Picea mariana*	30	Nursery seedling	2021-09-26
OQ319477	rbcLa	*Abies balsamea*	625	Nursery seedling	2022-06-26
OQ344479	trnH-psbA	*Abies balsamea*	625	Nursery seedling	2022-06-26
OQ344480	trnH-psbA	*Picea glauca*	565	Root	2022-06-23
OQ344481	trnH-psbA	*Betula papyifera*	444	Root	2022-06-19
OQ344482	trnH-psbA	*Picea glauca*	210	Nursery seedling	2021-10-06
OQ344483	trnH-psbA	*Picea mariana*	151	Root	2021-10-04
OQ344484	trnH-psbA	*Abies balsamea*	46	Root	2021-09-29
OQ344485	trnH-psbA	*Picea mariana*	30	Nursery seedling	2021-09-26
OQ351868	TEF-1	*Cantharellus enelensis*	362	Mushroom	2021-08-09
OQ351869	TEF-1	*Cantharellus enelensis*	363	Mushroom	2021-08-09
OQ351870	TEF-1	*Cantharellus enelensis*	364	Mushroom	2021-08-09
OQ351871	TEF-1	*Cantharellus enelensis*	366	Mushroom	2021-08-09
OQ351872	TEF-1	*Cantharellus enelensis*	367	Mushroom	2021-08-09
OQ351873	TEF-1	*Cantharellus enelensis*	368	Mushroom	2021-08-09
OQ351874	TEF-1	*Cantharellus enelensis*	369	Mushroom	2021-08-09
OQ351875	TEF-1	*Cantharellus enelensis*	365	Mushroom	2021-08-09

Table A.4: Certainty of identification and identity (%) of abundant operational taxonomic units numerically sorted. For OTU IDs that have been changed based on BLASTn results, the original ID from dada2 is to the right in square brackets.

	OTU	dada2	BLASTn (nr)		UNITE Blast	
No.	**ID**	**Certainty**	**Best BLAST Match**	**Identity (%)**	**UNITE SH #**	**Identity (%)**
1	*Russula montana*	0.954	MN992513	100.0	SH1182139.09FU	99.7
2	*Amphinema* sp.	0.997	OQ410914 (root)	100.0	SH0237947.09FU	99.6
3	*Mycena* cf. *cinerella* [*Mycena*]	0.995	HQ157912	100.0	SH0114381.09FU	99.3
4	*Amphinema byssoides* [*Amphinema*]	0.971	AY219839	100.0	SH0137097.09FU	100.0
5	*Thelephora terrestris*	1.000	MH861911	100.0	SH0189294.09FU	96.8
6	*Tylospora fibrillosa*	0.992	MF926576	100.0	SH0256804.09FU	100.0
7	*Piloderma sphaerosporum*	1.000	MK131527	100.0	SH0181969.09FU	100.0
8	*Clavulina coralloides* [*Clavulina cinerea*]	0.996	ON412804	100.0	SH1202236.09FU	99.3
9	*Mycena leptocephala*	0.963	MT644911	100.0	SH1062686.09FU	99.6
10	*Russula decolorans* [*Russula vinososordida*]	0.999	DQ367913	100.0	SH0107094.09FU	98.8
11	*Piloderma* sp.	0.995	MK131547	100.0	SH1188206.09FU	97.6
12	*Cortinarius mammillatus*	0.930	OQ321918	100.0	SH1152568.09FU	99.3
13	*Piloderma* sp.	0.993	MK131558	100.0	SH1188206.09FU	98.8
14	*Cortinarius caperatus*	0.815	OP749837	100.0	SH0248325.09FU	99.6
15	*Russula* cf. *aquosa* [*Russula aquosa*]	0.919	LC192783	98.5	SH1181301.09FU	98.3
16	*Sistotrema citriforme*	1.000	OQ410859 (root)	100.0	SH0250883.09FU	100.0
17	*Wilcoxina rehmii*	0.898	MF926519 (root)	99.7	SH0159837.09FU	96.5
18	*Craterellus tubaeformis*	0.999	OP749739	100.0	SH0886349.09FU	99.3
19	*Craterellus tubaeformis*	0.995	OM987312	99.1	SH0886357.09FU	98.6
20	*Pseudotomentella nigra*	1.000	KT800286	97.0	SH1099904.09FU	99.3
21	*Wilcoxina rehmii*	0.756	MF926519 (root)	99.7	SH0159837.09FU	96.8
22	*Cortinarius caperatus*	0.882	MN751061	99.7	SH0248325.09FU	99.6
23	*Mycena metata*	0.990	MH396636	100.0	SH1126130.09FU	98.5
24	*Piloderma* sp.	0.972	OQ410810 (root)	99.3	SH0919635.09FU	97.6
25	*Cortinarius grosmorneensis* [*Cortinarius*]	1.000	NR120094	100.0	SH1282662.09FU	100.0
26	*Piloderma* sp.	0.991	MK131558 (root)	99.3	SH1188206.09FU	99.2
27	*Cortinarius* cf. *stillatitius* [*Cortinarius vanduzerensis*]	0.993	MN992364	100.0	SH1132814.09FU	100.0
28	*Tylospora asterophora*	1.000	MG597438 (root)	100.0	SH0168510.09FU	100.0
29	*Russula* cf. *brevipes* [*Russula cremicolor*]	0.877	KY848511	100.0	SH0237758.09FU	99.7
30	*Tricholoma fulvum*	0.968	OP205427	100.0	SH1086505.09FU	100.0
31	*Umbelopsis isabellina*	0.896	MH863098	100.0	SH0181369.09FU	97.9
32	*Cortinarius acutovelatus*	0.987	AY083175	100.0	SH1303180.09FU	100.0
33	*Cortinarius leiocastaneus*	1.000	MN751335	100.0	SH1285094.09FU	100.0
34	*Cortinarius* cf. *parvoacetosus* [*Cortinarius obtusus*]	1.000	OP223488	99.7	SH1152060.09FU	99.6
35	*Lactarius trivialis*	1.000	KJ705209	100.0	SH1150224.09FU	99.7
36	*Thelephora* cf. *terrestris* [*Thelephora terrestris*]	1.000	LC376052	100.0	SH0189294.09FU	96.2
37	*Lactarius tabidus* [*Lactarius*]	1.000	KJ705214	100.0	SH0179331.09FU	99.4
39	*Clavulina coralloides* [*Clavulina cinerea*]	0.983	OM717006	99.4	SH1202236.09FU	98.6
40	*Mycena epipterygia*	1.000	MN992623	100.0	SH1126203.09FU	98.5
42	*Mycena metata*	0.992	ON963308 (root)	99.7	SH1126130.09FU	98.5
43	*Clavulina coralloides* [*Clavulina cinerea*]	0.995	ON412804	99.4	SH1202236.09FU	98.7
44	*Sistotrema* FR1 [*Hydnum*]	0.988	LC642051	100.0	SH1193668.09FU	100.0
45	*Amanita porphyria*	0.999	MN992299	100.0	SH1192464.09FU	100.0
48	*Hebeloma* cf. *incarnatulum* [*Hebeloma*]	1.000	NR175041	100.0	SH1162804.09FU	100.0
49	*Mycena* cf. *leptocephala* [*Mycena leptocephala*]	0.916	MN542079 (root)	99.7	SH1062686.09FU	99.6
50	*Piloderma* sp.	0.971	MT028116	99.7	SH0943092.09FU	97.2
51	*Lactarius uvidus*	0.993	KJ742416	100.0	SH1150151.09FU	100.0
52	*Cortinarius roseomyceliosus*	0.857	MN751744	100.0	SH1281854.09FU	100.0
53	*Polyozellus tristis* [*Polyozellus umbrinus*]	1.000	AJ889968	100.0	SH0159833.09FU	99.7
54	*Piloderma* sp.	0.988	MK131540 (root)	99.7	SH1188206.09FU	98.8
55	*Tomentella sublilacina*	0.963	KP753368	100.0	SH0189294.09FU	97.5
56	*Cortinarius armeniacus*	0.996	OQ322019	100.0	SH0255671.09FU	100.0
58	*Megacollybia rodmanii*	0.750	OP643071	100.0	SH1177498.09FU	100.0
59	*Thelephora* cf. *terrestris* [*Thelephora terrestris*]	1.000	JQ712012	99.4	SH0189294.09FU	96.5
60	*Lactarius repraesentaneus*	0.999	OQ987858	100.0	SH1150079.09FU	100.0
61	*Cortinarius acutus*	0.934	MN992324	100.0	SH1303777.09FU	98.5
62	*Serendipita* sp.	0.994	MZ017682 (root)	99.0	SH1218570.09FU	99.6
63	*Cortinarius acutus*	0.986	MN750851	100.0	SH0241458.09FU	99.7
64	*Sistotrema* [*Sistotrema muscicola*]	0.997	DQ365645 (root)	98.7	SH1210320.09FU	98.9
67	*Sistotrema muscicola*	1.000	JX561240 (root)	97.7	SH1210394.09FU	96.6
69	*Resinicium bicolor*	1.000	MF319079	100.0	SH1252020.09FU	100.0
71	*Russula turci*	1.000	MN992271	99.7	SH1180567.09FU	98.8
73	*Cortinarius ochrophyllus*	0.757	MN751405	100.0	SH1151612.09FU	99.6
74	*Mycena leptocephala*	0.940	MT294414	100.0	SH1062686.09FU	98.9
75	*Russula paludosa*	1.000	MN992516	100.0	SH0248000.09FU	98.3

	OTU	dada2	BLASTn (nr)		UNITE Blast	
No.	***ID***	**Certainty**	**Best BLAST Match**	**Identity (%)**	**UNITE SH #**	**Identity (%)**
79	*Russula* cf. *arvernensis* [*Russula arvernensis*]	0.838	NR184970	98.8	SH1181189.09FU	99.0
80	*Cortinarius* [*Cortinarius acutus*]	0.940	AF430290	98.7	SH0241458.09FU	97.7
81	*Mycena* sp.	0.843	ON113879 (root)	98.1	SH1034820.09FU	96.7
82	*Amphinema* sp.	0.778	KP814303	99.6	SH0137090.09FU	95.3
86	*Russula* cf. *arvernensis* [*Russula arvernensis*]	0.984	NR184970	98.8	SH1181160.09FU	98.4
87	*Tomentella terrestris*	0.925	AF272901	100.0	SH0255669.09FU	99.7
88	*Tomentellopsis* [*Tomentellopsis submollis*]	1.000	AJ410781 (root)	99.7	SH0247614.09FU	99.3
89	*Cortinarius* cf. *catalanensis* [*Cortinarius catalanensis*]	0.913	MN751631	98.6	SH0222489.09FU	98.8
91	*Cortinarius caperatus*	0.797	KU950452	99.7	SH0248325.09FU	99.6
99	*Cortinarius* cf. *aurae* [*Cortinarius aurae*]	0.999	MK131475 (root)	100.0	SH1303284.09FU	99.3
106	*Amphinema* cf. *byssoides* [*Amphinema byssoides*]	0.861	JQ711820 (root)	99.6	SH0137090.09FU	97.1
110	*Cortinarius aurae*	1.000	MN751592	100.0	SH1303284.09FU	99.6
115	*Cortinarius fulvescens* [*Cortinarius*]	1.000	MN751232	100.0	SH1809831.09FU	99.3
117	*Mycena* cf. *amicta* [*Mycena*]	0.901	OP035388	99.7	SH0182025.09FU	97.4
125	*Russula vinosa*	1.000	OQ322578	99.7	SH0195497.09FU	99.7
133	Pucciniales sp.	0.999	KR019816 (root)	100.0	SH1134289.09FU	99.2
135	*Tomentella* cf. *terrestris* [Thelephoraceae family]	1.000	MK868265 (soil)	100.0	SH0255669.09FU	95.9
147	Schizoporaceae [*Xylodon sambuci*]	0.809	MW238152 (soil)	967.0	SH1286425.09FU	97.0
202	*Apiotrichum porosum* [*Apiotrichum*]	1.000	MZ078477 (root)	99.6	SH2804501.09FU	99.6
216	*Clavulina coralloides* [*Clavulina cinerea*]	0.984	OP749688	99.4	SH1202236.09FU	98.6
220	*Amphinema* sp.	0.992	OQ410914 (root)	99.3	SH0237947.09FU	98.8
223	*Cortinarius*	0.997	OQ321998	98.0	SH1153130.09FU	98.9
239	*Amphinema* cf. *byssoides* [*Amphinema byssoides*]	0.810	MZ017868 (root)	99.6	SH0137090.09FU	97.1
249	*Mycena purpureofusca* [*Mycena*]	1.000	ON561528	99.7	SH1258690.09FU	98.9
312	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.993	MT738188	99.6	SH0255292.09FU	98.5
327	*Russula turci*	1.000	MN992271	99.7	SH1180567.09FU	98.1
329	*Cortinarius*	1.000	NR153058	97.5	SH0250893.09FU	95.1
366	*Tomentella* cf. *cinereoumbrina* [Thelephoraceae family]	1.000	MK956854 (root)	98.1	SH1140128.09FU	99.6
436	Atheliaceae [*Amphinema*]	0.795	OQ410914 (root)	925.0	SH0237947.09FU	91.4
440	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.994	MT738195 (root)	98.9	SH0255292.09FU	99.3
459	*Entoloma rhodocylix*	1.000	KJ001414	100.0	SH1260534.09FU	99.6
559	*Tomentella* cf. *botryoides* [*Tomentella botryoides*]	1.000	KY694394 (root)	99.7	SH0923638.09FU	99.3
628	*Tomentella* [Thelephoraceae family]	1.000	MF926573 (root)	99.0	SH0021174.09FU	98.9
648	*Cortinarius cicindela*	0.999	OP352899	100.0	SH1284275.09FU	100.0
856	*Hydnotrya* sp.	0.884	KU878593 (root)	99.7	SH1000819.09FU	99.6
997	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.991	MT276009	99.3	SH0255292.09FU	98.6
1016	*Cenococcum geophilum* [Gloniaceae family]	1.000	LC095122 (root)	99.3	SH1090012.09FU	98.2
1033	*Mycena* cf. *sanguinolenta* [*Mycena sanguinolenta*]	1.000	MK131672 (root)	100.0	SH1126067.09FU	94.6
1037	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.992	JQ711965 (root)	98.9	SH0255292.09FU	98.9
1225	*Cortinarius* cf. *alutarius* [*Cortinarius*]	1.000	MZ648197	97.5	SH0174585.09FU	95.5
1347	*Lactarius picinus* [*Lactarius*]	1.000	JQ446132	100.0	SH1176152.09FU	98.6
1569	*Entocybe turbida* [*Entocybe*]	1.000	MN992146	100.0	SH1149514.09FU	100.0
2112	*Podila verticillata* [*Podila humilis*]	1.000	MW268811	100.0	SH0246142.09FU	100.0
2126	*Tylospora* [*Tylospora fibrillosa*]	0.996	OQ410870 (root)	97.9	SH0256804.09FU	97.4
2230	*Cortinarius clintonianus*	0.956	MN751151	100.0	SH1251954.09FU	99.2
2264	*Hebeloma leucosarx* [*Hebeloma*]	1.000	OP749177	100.0	SH1162777.09FU	97.8
2617	*Cladophialophora* sp.	0.705	LC523843	98.5	SH1069521.09FU	98.3
2672	*Cenococcum geophilum* [Gloniaceae family]	1.000	LC095100 (soil)	99.6	SH1090012.09FU	98.2
2783	*Peniophorella pallida*	0.999	KP814371	99.7	SH1276903.09FU	98.9
2797	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.994	MT738188 (root)	99.0	SH0255292.09FU	98.9
2905	*Hyaloscypha*	0.966	OP135692	98.6	SH0238537.09FU	95.1
3001	*Hyaloscypha* cf. *variabilis* [*Hyaloscypha variabilis*]	0.961	MH844010 (root)	98.6	SH0236353.09FU	94.4
3027	*Hyaloscypha* cf. *variabilis* [*Hyaloscypha variabilis*]	0.967	MH843973 (root)	100.0	SH0236353.09FU	95.1
3085	*Meliniomyces* sp.	0.993	MK131622 (root)	100.0	SH0245054.09FU	96.4
3154	*Cryptococcus pseudolongus*	1.000	KY102944	100.0	SH1048924.09FU	100.0
3164	*Hyaloscypha*	0.756	MT276006	98.9	SH0238537.09FU	92.5
3229	*Pseudoleucosporidium* [*Pseudoleucosporidium fasciculatum*]	0.999	KJ778628	97.3	SH1326970.09FU	100.0
3232	Helotiales [*Phialea*]	0.725	LC131017 (root)	99.3	BLAST_NO_MATCH	
3313	*Phialocephala sphaeroides*	1.000	HQ157937	99.6	SH1241065.09FU	98.7
3359	*Gryganskiella* [*Mortierella turficola*]	0.749	JX270365 (soil)	100.0	SH1306549.09FU	98.3
3500	*Cenococcum geophilum* [*Glonium stellatum*]	0.705	MK069502 (root)	99.3	SH0243549.09FU	96.4
3923	*Phialocephala fortinii* [*Phialocephala helvetica*]	0.961	NR_103577	99.6	SH0255292.09FU	98.9
3973	Helotiales [Ascomycota phylum]	0.938	KU516628 (root)	97.5	BLAST_NO_MATCH	
4078	*Meliniomyces* [Helotiales order]	0.913	HQ157835 (root)	99.6	SH0238537.09FU	91.7
4196	*Hyaloscypha cf. variabilis* [*Hyaloscypha variabilis*]	0.959	MH844010 (root)	99.6	SH0236353.09FU	95.1
4465	*Cenococcum geophilum* [Gloniaceae family]	1.000	MK131421 (root)	100.0	SH0243549.09FU	97.1
4616	Helotiales [Helotiales order]	0.835	JQ272427 (root)	89.3	SH0153492.09FU	97.5
4813	*Mucor hiemalis*	0.981	MT514370	100.0	SH1270678.09FU	98.7
4843	Helotiales [Ascomycota phylum]	0.939	MT504585 (root)	98.4	SH0156630.09FU	85.1
5716	Helotiales [*Glutinomyces*]	0.728	KJ817304 (root)	98.6	SH0114406.09FU	93.2
5843	Unknown: Possible chimera [*Triscelophorus*]	0.991	OM987157 (root)	90.9	BLAST_NO_MATCH	
5938	Pezizomycotina [Dothideomycetes class]	0.983	MT678916 (root)	97.5	BLAST_NO_MATCH	
7244	Pezizomycotina [Acarosporales]	0.943	MT595798 (soil)	95.5	BLAST_NO_MATCH	

Table A.5: Certainty of identification and identity (%) of abundant operational taxonomic units alphabetically sorted. For OTU IDs that have been changed based on BLASTn results, the original ID from dada2 is to the right in square brackets.

OTU		dada2	BLASTn (nr)		UNITE Blast	
No.	**ID**	**Certainty**	**Best BLAST Match**	**Identity (%)**	**UNITE SH #**	**Identity (%)**
45	*Amanita porphyria*	0.999	MN992299	100.0	SH1192464.09FU	100.0
2	*Amphinema* sp.	0.997	OQ410914 (root)	100.0	SH0237947.09FU	99.6
82	*Amphinema* sp.	0.778	KP814303	99.6	SH0137090.09FU	95.3
220	*Amphinema* sp.	0.992	OQ410914 (root)	99.3	SH0237947.09FU	98.8
4	*Amphinema byssoides* [*Amphinema*]	0.971	AY219839	100.0	SH0137097.09FU	100.0
106	*Amphinema* cf. *byssoides* [*Amphinema byssoides*]	0.861	JQ711820 (root)	99.6	SH0137090.09FU	97.1
239	*Amphinema* cf. *byssoides* [*Amphinema byssoides*]	0.810	MZ017868 (root)	99.6	SH0137090.09FU	97.1
202	*Apiotrichum porosum* [*Apiotrichum*]	1.000	MZ078477 (root)	99.6	SH2804501.09FU	99.6
436	Atheliaceae [*Amphinema*]	0.795	OQ410914 (root)	925.0	SH0237947.09FU	91.4
1016	*Cenococcum geophilum* [Gloniaceae family]	1.000	LC095122 (root)	99.3	SH1090012.09FU	98.2
2672	*Cenococcum geophilum* [Gloniaceae family]	1.000	LC095100 (soil)	99.6	SH1090012.09FU	98.2
4465	*Cenococcum geophilum* [Gloniaceae family]	1.000	MK131421 (root)	100.0	SH0243549.09FU	97.1
3500	*Cenococcum geophilum* [*Glonium stellatum*]	0.705	MK069502 (root)	99.3	SH0243549.09FU	96.4
2617	*Cladophialophora* sp. [*Cladophialophora* sp.]	0.705	LC523843	98.5	SH1069521.09FU	98.3
8	*Clavulina coralloides* [*Clavulina cinerea*]	0.996	ON412804	100.0	SH1202236.09FU	99.3
39	*Clavulina coralloides* [*Clavulina cinerea*]	0.983	OM717006	99.4	SH1202236.09FU	98.6
43	*Clavulina coralloides* [*Clavulina cinerea*]	0.995	ON412804	99.4	SH1202236.09FU	98.7
216	*Clavulina coralloides* [*Clavulina cinerea*]	0.984	OP749688	99.4	SH1202236.09FU	98.6
223	*Cortinarius*	0.997	OQ321998	98.0	SH1153130.09FU	98.9
329	*Cortinarius*	1.000	NR153058	97.5	SH0250893.09FU	95.1
80	*Cortinarius* [*Cortinarius acutus*]	0.940	AF430290	98.7	SH0241458.09FU	97.7
32	*Cortinarius acutovelatus*	0.987	AY083175	100.0	SH1303180.09FU	100.0
61	*Cortinarius acutus*	0.934	MN992324	100.0	SH1303777.09FU	98.5
63	*Cortinarius acutus*	0.986	MN750851	100.0	SH0241458.09FU	99.7
56	*Cortinarius armeniacus*	0.996	OQ322019	100.0	SH0255671.09FU	100.0
110	*Cortinarius aurae*	1.000	MN751592	100.0	SH1303284.09FU	99.6
14	*Cortinarius caperatus*	0.815	OP749837	100.0	SH0248325.09FU	99.6
22	*Cortinarius caperatus*	0.882	MN751061	99.7	SH0248325.09FU	99.6
91	*Cortinarius caperatus*	0.797	KU950452	99.7	SH0248325.09FU	99.6
1225	*Cortinarius* cf. *alutarius* [*Cortinarius*]	1.000	MZ648197	97.5	SH0174585.09FU	95.5
99	*Cortinarius* cf. *aurae* [*Cortinarius aurae*]	0.999	MK131475 (root)	100.0	SH1303284.09FU	99.3
89	*Cortinarius* cf. *catalanensis* [*Cortinarius catalanensis*]	0.913	MN751631	98.6	SH0222489.09FU	98.8
34	*Cortinarius* cf. *parvoacetosus* [*Cortinarius obtusus*]	1.000	OP223488	99.7	SH1152060.09FU	99.6
27	*Cortinarius* cf. *stillatitius* [*Cortinarius vanduzerensis*]	0.993	MN992364	100.0	SH1132814.09FU	100.0
648	*Cortinarius cicindela*	0.999	OP352899	100.0	SH1284275.09FU	100.0
2230	*Cortinarius clintonianus*	0.956	MN751151	100.0	SH1251954.09FU	99.2
115	*Cortinarius fulvescens* [*Cortinarius*]	1.000	MN751232	100.0	SH1809831.09FU	99.3
25	*Cortinarius grosmorneensis* [*Cortinarius*]	1.000	NR120094	100.0	SH1282662.09FU	100.0
33	*Cortinarius leiocastaneus*	1.000	MN751335	100.0	SH1285094.09FU	100.0
12	*Cortinarius mammillatus*	0.930	OQ321918	100.0	SH1152568.09FU	99.3
73	*Cortinarius ochrophyllus*	0.757	MN751405	100.0	SH1151612.09FU	99.6
52	*Cortinarius roseomyceliosus*	0.857	MN751744	100.0	SH1281854.09FU	100.0
18	*Craterellus tubaeformis*	0.999	OP749739	100.0	SH0886349.09FU	99.3
19	*Craterellus tubaeformis*	0.995	OM987312	99.1	SH0886357.09FU	98.6
3154	*Cryptococcus pseudolongus*	1.000	KY102944	100.0	SH1048924.09FU	100.0
1569	*Entocybe turbida* [*Entocybe*]	1.000	MN992146	100.0	SH1149514.09FU	100.0
459	*Entoloma rhodocylix*	1.000	KJ001414	100.0	SH1260534.09FU	99.6
3359	*Gryganskiella* [*Mortierella turficola*]	0.749	JX270365 (soil)	100.0	SH1306549.09FU	98.3
48	*Hebeloma* cf. *incarnatulum* [*Hebeloma*]	1.000	NR175041	100.0	SH1162804.09FU	100.0
2264	*Hebeloma leucosarx* [*Hebeloma*]	1.000	OP749177	100.0	SH1162777.09FU	97.8
3973	Helotiales [Ascomycota phylum]	0.938	KU516628 (root)	97.5	BLAST_NO_MATCH	
4843	Helotiales [Ascomycota phylum]	0.939	MT504585 (root)	98.4	SH0156630.09FU	85.1
5716	Helotiales [*Glutinomyces*]	0.728	KJ817304 (root)	98.6	SH0114406.09FU	93.2
4616	Helotiales [Helotiales order]	0.835	JQ272427 (root)	89.3	SH0153492.09FU	97.5
3232	Helotiales [*Phialea*]	0.725	LC131017 (root)	99.3	BLAST_NO_MATCH	
2905	*Hyaloscypha*	0.966	OP135692	98.6	SH0238537.09FU	95.1
3164	*Hyaloscypha*	0.756	MT276006	98.9	SH0238537.09FU	92.5
3001	*Hyaloscypha* cf. *variabilis* [*Hyaloscypha variabilis*]	0.961	MH844010 (root)	98.6	SH0236353.09FU	94.4
3027	*Hyaloscypha* cf. *variabilis* [*Hyaloscypha variabilis*]	0.967	MH843973 (root)	100.0	SH0236353.09FU	95.1
4196	*Hyaloscypha* cf. *variabilis* [*Hyaloscypha variabilis*]	0.959	MH844010 (root)	99.6	SH0236353.09FU	95.1
856	*Hydnotrya* sp.	0.884	KU878593 (root)	99.7	SH1000819.09FU	99.6
1347	*Lactarius picinus* [*Lactarius*]	1.000	JQ446132	100.0	SH1176152.09FU	98.6
60	*Lactarius repraesentaneus*	0.999	OQ987858	100.0	SH1150079.09FU	100.0
37	*Lactarius tabidus* [*Lactarius*]	1.000	KJ705214	100.0	SH0179331.09FU	99.4
35	*Lactarius trivialis*	1.000	KJ705209	100.0	SH1150224.09FU	99.7

OTU		dada2	BLASTn (nr)		UNITE Blast	
No.	**ID**	**Certainty**	**Best BLAST Match**	**Identity (%)**	**UNITE SH #**	**Identity (%)**
51	*Lactarius uvidus*	0.993	KJ742416	100.0	SH1150151.09FU	100.0
58	*Megacollybia rodmanii*	0.750	OP643071	100.0	SH1177498.09FU	100.0
3085	*Meliniomyces* sp.	0.993	MK131622 (root)	100.0	SH0245054.09FU	96.4
4078	*Meliniomyces* sp. [Helotiales order]	0.913	HQ157835 (root)	99.6	SH0238537.09FU	91.7
4813	*Mucor hiemalis*	0.981	MT514370	100.0	SH1270678.09FU	98.7
81	*Mycena* sp.	0.843	ON113879 (root)	98.1	SH1034820.09FU	96.7
117	*Mycena* cf. *amicta* [*Mycena*]	0.901	OP035388	99.7	SH0182025.09FU	97.4
3	*Mycena* cf. *cinerella* [*Mycena*]	0.995	HQ157912	100.0	SH0114381.09FU	99.3
49	*Mycena* cf. *leptocephala* [*Mycena leptocephala*]	0.916	MN542079 (root)	99.7	SH1062686.09FU	99.6
1033	*Mycena* cf. *sanguinolenta* [*Mycena sanguinolenta*]	1.000	MK131672 (root)	100.0	SH1126067.09FU	94.6
40	*Mycena epipterygia*	1.000	MN992623	100.0	SH1126203.09FU	98.5
9	*Mycena leptocephala*	0.963	MT644911	100.0	SH1062686.09FU	99.6
74	*Mycena leptocephala*	0.940	MT294414	100.0	SH1062686.09FU	98.9
23	*Mycena metata*	0.990	MH396636	100.0	SH1126130.09FU	98.5
42	*Mycena metata*	0.992	ON963308 (root)	99.7	SH1126130.09FU	98.5
249	*Mycena purpureofusca* [*Mycena*]	1.000	ON561528	99.7	SH1258690.09FU	98.9
2783	*Peniophorella pallida*	0.999	KP814371	99.7	SH1276903.09FU	98.9
7244	Pezizomycotina [Acarosporales]	0.943	MT595798 (soil)	95.5	BLAST_NO_MATCH	
5938	Pezizomycotina [Dothideomycetes class]	0.983	MT678916 (root)	97.5	BLAST_NO_MATCH	
312	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.993	MT738188	99.6	SH0255292.09FU	98.5
440	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.994	MT738195 (root)	98.9	SH0255292.09FU	99.3
997	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.991	MT276009	99.3	SH0255292.09FU	98.6
1037	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.992	JQ711965 (root)	98.9	SH0255292.09FU	98.9
2797	*Phialocephala* cf. *fortinii* [*Phialocephala helvetica*]	0.994	MT738188 (root)	99.0	SH0255292.09FU	98.9
3923	*Phialocephala fortinii* [*Phialocephala helvetica*]	0.961	NR_103577	99.6	SH0255292.09FU	98.9
3313	*Phialocephala sphaeroides*	1.000	HQ157937	99.6	SH1241065.09FU	98.7
11	*Piloderma* sp.	0.995	MK131547	100.0	SH1188206.09FU	97.6
13	*Piloderma* sp.	0.993	MK131558	100.0	SH1188206.09FU	98.8
24	*Piloderma* sp.	0.972	OQ410810 (root)	99.3	SH0919635.09FU	97.6
26	*Piloderma* sp.	0.991	MK131558 (root)	99.3	SH1188206.09FU	99.2
50	*Piloderma* sp.	0.971	MT028116	99.7	SH0943092.09FU	97.2
54	*Piloderma* sp.	0.988	MK131540 (root)	99.7	SH1188206.09FU	98.8
7	*Piloderma sphaerosporum*	1.000	MK131527	100.0	SH0181969.09FU	100.0
2112	*Podila verticillata* [*Podila humilis*]	1.000	MW268811	100.0	SH0246142.09FU	100.0
53	*Polyozellus tristis* [*Polyozellus umbrinus*]	1.000	AJ889968	100.0	SH0159833.09FU	99.7
3229	*Pseudoleucosporidium* [*Pseudoleucosporidium fasciculatum*]	0.999	KJ778628	97.3	SH1326970.09FU	100.0
20	*Pseudotomentella nigra*	1.000	KT800286	97.0	SH1099904.09FU	99.3
133	Pucciniales sp.	0.999	KR019816 (root)	100.0	SH1134289.09FU	99.2
69	*Resinicium bicolor*	1.000	MF319079	100.0	SH1252020.09FU	100.0
15	*Russula* cf. *aquosa* [*Russula aquosa*]	0.919	LC192783	98.5	SH1181301.09FU	98.3
79	*Russula* cf. *arvernensis* [*Russula arvernensis*]	0.838	NR184970	98.8	SH1181189.09FU	99.0
86	*Russula* cf. *arvernensis* [*Russula arvernensis*]	0.984	NR184970	98.8	SH1181160.09FU	98.4
29	*Russula* cf. *brevipes* [*Russula cremicolor*]	0.877	KY848511	100.0	SH0237758.09FU	99.7
10	*Russula decolorans* [*Russula vinososordida*]	0.999	DQ367913	100.0	SH0107094.09FU	98.8
1	*Russula montana*	0.954	MN992513	100.0	SH1182139.09FU	99.7
75	*Russula paludosa*	1.000	MN992516	100.0	SH0248000.09FU	98.3
71	*Russula turci*	1.000	MN992271	99.7	SH1180567.09FU	98.8
327	*Russula turci*	1.000	MN992271	99.7	SH1180567.09FU	98.1
125	*Russula vinosa*	1.000	OQ322578	99.7	SH0195497.09FU	99.7
147	Schizoporaceae [*Xylodon sambuci*]	0.809	MW238152 (soil)	967.0	SH1286425.09FU	97.0
62	*Serendipita* sp.	0.994	MZ017682 (root)	99.0	SH1218570.09FU	99.6
64	*Sistotrema* [*Sistotrema muscicola*]	0.997	DQ365645 (root)	98.7	SH1210320.09FU	98.9
16	*Sistotrema citriforme*	1.000	OQ410859 (root)	100.0	SH0250883.09FU	100.0
44	*Sistotrema* FR1 [*Hydnum*]	0.988	LC642051	100.0	SH1193668.09FU	100.0
67	*Sistotrema muscicola*	1.000	JX561240 (root)	97.7	SH1210394.09FU	96.6
36	*Thelephora* cf. *terrestris* [*Thelephora terrestris*]	1.000	LC376052	100.0	SH0189294.09FU	96.2
59	*Thelephora* cf. *terrestris* [*Thelephora terrestris*]	1.000	JQ712012	99.4	SH0189294.09FU	96.5
5	*Thelephora terrestris*	1.000	MH861911	100.0	SH0189294.09FU	96.8
628	*Tomentella* [Thelephoraceae family]	1.000	MF926573 (root)	99.0	SH0021174.09FU	98.9
559	*Tomentella* cf. *botryoides* [*Tomentella botryoides*]	1.000	KY694394 (root)	99.7	SH0923638.09FU	99.3
366	*Tomentella* cf. *cinereoumbrina* [Thelephoraceae family]	1.000	MK956854 (root)	98.1	SH1140128.09FU	99.6
135	*Tomentella* cf. *terrestris* [Thelephoraceae family]	1.000	MK868265 (soil)	100.0	SH0255669.09FU	95.9
55	*Tomentella sublilacina*	0.963	KP753368	100.0	SH0189294.09FU	97.5
87	*Tomentella terrestris*	0.925	AF272901	100.0	SH0255669.09FU	99.7
88	*Tomentellopsis* [*Tomentellopsis submollis*]	1.000	AJ410781 (root)	99.7	SH0247614.09FU	99.3
30	*Tricholoma fulvum*	0.968	OP205427	100.0	SH1086505.09FU	100.0
2126	*Tylospora* [*Tylospora fibrillosa*]	0.996	OQ410870 (root)	97.9	SH0256804.09FU	97.4
28	*Tylospora asterophora*	1.000	MG597438 (root)	100.0	SH0168510.09FU	100.0
6	*Tylospora fibrillosa*	0.992	MF926576	100.0	SH0256804.09FU	100.0
31	*Umbelopsis isabellina*	0.896	MH863098	100.0	SH0181369.09FU	97.9
5843	Unknown: Possible chimera [*Triscelophorus*]	0.991	OM987157 (root)	90.9	BLAST_NO_MATCH	
17	*Wilcoxina rehmii*	0.898	MF926519 (root)	99.7	SH0159837.09FU	96.5
21	*Wilcoxina rehmii*	0.756	MF926519 (root)	99.7	SH0159837.09FU	96.8

Curriculum Vitae

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	University of Western Ontario Bachelor of Science, Honors Specialization of Biology 2016-2019
Honours and Awards:	Karen Auzins Scholarship (2022) Ruth Horner Arnold Award (2021)
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Publications:

1. Thorn, R.G., Banwell, A., Jee, I.K., Lebeuf, R. and Voitk, A. (2022) *Cantharellus betularum*, the "purple chanterelle" of NL. Omphalina 13(1): 17-20.

2. Thorn, R.G., Banwell, A., Pham, T.H., Vidal, N.P., Manful, C.F., Nadeem, M., Ivanov, A.G., Mroz, B.S., Bonneville, M.B., Huner, N.P.A., Piercey-Normore, M.D., and Thomas, R. (2021) Identification and analyses of the chemical composition of a naturally occurring albino mutant chanterelle. Scientific Reports 11: 20590.

3. Thorn, R.G., Banwell, A., Jee, I.K., Lebeuf, R. and Voitk, A. (2020) *Cantharellus betularum* Voitk and Thorn, sp. nov. Fungal Planet Description Sheet 1144. Persoonia 45 330-331.

Presentations:

1. Thorn, R.G. and Banwell, A. September 24 2022. An update on chanterelles: A new species, a new mutant and future research. Foray Newfoundland & Labrador. Bishop's Falls, NL, Canada. [Oral]

2. Thorn, R.G. and Banwell, A. June 1 2022. At the root of it: The introduction of nursery seedlings and their fungi to conifer forests. 3rd Annual CanFunNet Fungal Biology Conference. [Virtual; Oral]

3. Thorn, R.G. and Banwell, A. May 27 2021. A new albino mutant mushroom: Phylogenetic, genetic and chemical analyses separating white and golden chanterelles. The Joint CanFunNet and Great Lakes Mycology Conference. [Virtual; Oral]