Characterizing the ectomycorrhizal fungal community of whitebark pine (*Pinus albicaulis*) in Mount Revelstoke and Glacier National parks

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Abstract

Whitebark pine (WBP) (*Pinus albicaulis* Engelm.) is an endangered, high-elevation tree species and an obligate ectomycorrhizal mutualist. The ectomycorrhizal (ECM) fungal community with which it associates is central to its recovery. Previous efforts to describe this community have been focused in a single region of the species range (Greater Yellowstone Ecosystem) and relied on above ground sporocarp (mushroom) surveys. This project built upon that work by using NextGeneration sequencing of the ITS2 region to describe the WBP ECM fungal community in Mount Revelstoke and Glacier National parks of Canada. Samples were taken from root tips and adjacent soil of mature trees, naturally regenerated seedlings and planted seedlings. The major ECM lineages previously recorded with WBP were confirmed at our project site: generalist ascomycetes (*Cenococcum, Wilcoxina*), Agaricales (*Cortinarius, Inocybe, Tricholoma, Hygrophorus*), Atheliales (*Piloderma, Amphinema, Tylospora*), Suilloids (*Suillus, Rhizopogon*), Russuales (*Lactarius, Russula*) and Theleporales (*Pseudotomentella, Tomentella*). Twenty-one new species and two new genera were identified bringing the number of recorded WBP ECM associates to twenty-two genera and between fifty and fifty-five species. Compared with previous studies, a shift in the dominant ecological fungal guilds towards generalist associates was detected and attributed to the mixed conifer composition of the sites sampled. Distinct communities were detected on naturally regenerated and planted trees. Planted trees had lower colonization and diversity and lacked native ECM mycota. The ECM taxa identified here should be useful in the selection of planting sites, assessments of stand health and potential inoculation of planted seedlings. The assessment of planted seedlings reiterates the importance of selecting planting sites that support ECM development. This work makes a significant contribution to understanding the ECM fungal community and ecology of WBP.

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Chapter 1: Background and Introduction

1.1. Whitebark pine status and ecology

Pinus albicaulis Engelm. (whitebark pine; hereafter WBP) is a high-elevation stone pine, characterized by needles occurring in bundles of five, indehiscent cones requiring animal dispersal and a harsh alpine habitat. It is a keystone species providing the functions of regulating meltwater and stream flow, soil stabilization, facilitating the establishment of secondary plant communities and producing food for wildlife (ECCC, 2017). Its distribution spans the alpine and sub-alpine regions of the Pacific Northwest mountain ranges from California to northwestern British Columbia. Throughout this range, *P. albicaulis* forest are in decline; this decline is attributed to the cumulative effects of white pine blister rust (*Cronartium ribicola* J. C. Fisch.), mountain pine beetle (*Dendroctonus ponderosae* Hopkins), fire suppression and climate change (ECCC, 2017). WBP is listed as endangered in Canada under the Species at Risk Act (2012) and is a candidate for listing in the USA. The decline of WBP stimulated a range-wide recovery effort (Keane et al., 2012) that includes numerous government and not-for-profit agencies in the US and Canada. Parks Canada is one of the agencies leading work to conserve and restore WBP across its Canadian range. The work for this thesis was undertaken in Mount Revelstoke and Glacier National parks, located in southwestern British Columbia.

The status and recovery work of *P. albicaulis* have brought focus to the ectomycorrhizal fungal community with which it associates. Pines, including WBPs, are obligate mutualists (Mohatt, Cripps, & Lavin, 2008) meaning they require ectomycorrhizal fungal associations for normal growth and survival. Within the ectomycorrhizal fungal associations of pines, there exists some level of fungal host specificity. Certain species or clades of fungi are restricted to the genus *Pinus* or the smaller clade of five-needled pines (Mohatt et al., 2008). This suggests a co-evolutionary history between pines and a subset of their ectomycorrhizal fungi (Wu, Mueller, Lutzoni, Huang, & Guo, 2000; Mohatt et al., 2008).

Together these characteristics demonstrate that ectomycorrhizal fungi, including some that are species- or clade-specific, are necessary for the fitness of *P. albicaulis.* The description of the ectomycorrhizal fungal community of *P. albicaulis* in Mount Revelstoke and Glacier National parks of Canada is the focus of this thesis.

1.2. What are ectomycorrhizae?

Mycorrhiza refers to a usually mutualistic symbiosis between the roots of a plant and the mycelia (fungal tissue) of a fungal partner. This symbiosis is present in nearly all land plants; some 80% of land plants engage in mycorrhizae of some form (van der Heijden, Martin, Selosse, & Sanders, 2015). Ectomycorrhizae are a form of mycorrhizae defined by the presence of (1) a layer of fungal tissue wrapped around the outside of a plant root (sheath or mantle), (2) fungal cells (hyphae) that penetrate in between the cortical cells of the plant root and (3) an external mycelium that extends into the surrounding substrate (Smith & Read, 2008) (Figure 1). The primary function of the mycorrhiza is to act as an interface for the exchange of water and nutrients (from fungus to plant) and photosynthates (from plant to fungus); this exchange is essential to plant fitness and can be the dominant source of nutrient uptake (van der Heijden et al., 2015).

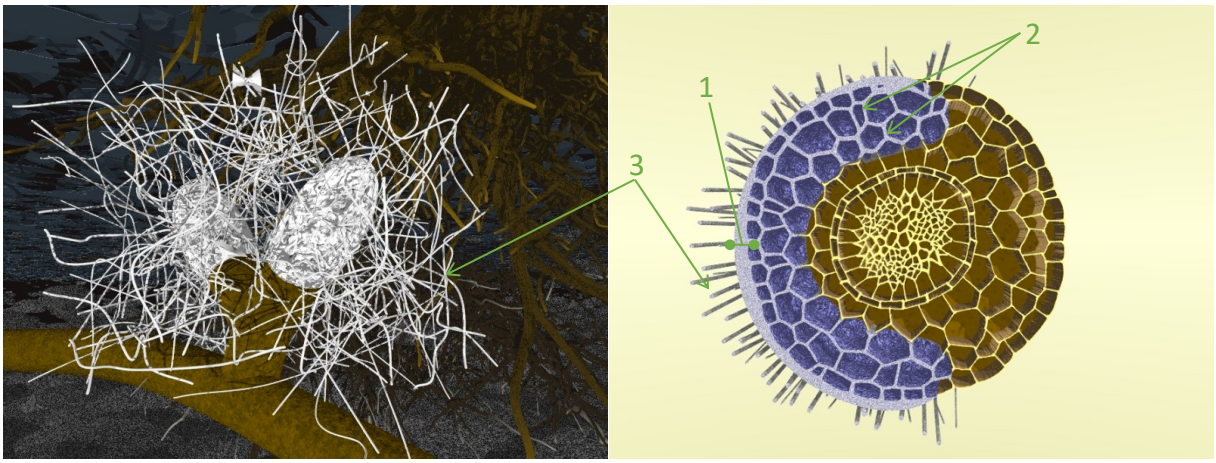

Figure 1. Schematic of ectomycorrhizae. Their appearance *in situ* (left) and in cross section (right). Numbers show the mantle (1), enlarged cell walls of Hartig net (2) and external hyphae (3).

Ectomycorrhizae are distinctive and critical to the functioning of temperate forest ecosystems. Relative to other types of mycorrhizal fungi, ectomycorrhizal fungi associate with relatively small subset of plants (~6000 species), most of which are coniferous trees (Pinaceae) and woody angiosperm species (van der Heijden et al., 2015). In these systems, ectomycorrhizae are critical to nutrient dynamics. It is estimated that 80% of plant nitrogen (N) and phosphorus (P) can come from ectomycorrhizal fungal partners, and some ECM fungi display saprophytic abilities that give them access to organic nutrient pools (van der Heijden et al., 2015). In addition, ectomycorrhizal fungi can form complex mycorrhizal networks: plant-to-plant connections formed by the fungal mycelium that can facilitate the exchange of water, carbon and nutrients (Simard et al., 2012). Mycorrhizal networks have major implications for numerous forest processes including seedling establishment, competition, diversity and community dynamics (Simard & Durall, 2004). Plant species vary in their dependence on mycorrhizal functions. WBP is (as introduced above) an obligate mutualist, depending on these functions for normal growth and survival.

1.3. Literature review: what we know about the whitebark pine ectomycorrhizal fungal community

Existing knowledge of the *P. albicaulis* ectomycorrhizal fungal community consists of three kinds of studies. These include those aimed explicitly at the description of the fungal community (Cripps & Antibus, 2011; Johnson, Kendall, & Coen, 1994; Mohatt et al., 2008; Mohatt, 2006), studies analyzing the benefits and recovery applications of the fungal community (Antibus, Hobbie, & Cripps, 2018; Cripps, Alger, & Sissons, 2018; Cripps & Grimme, 2011; Jenkins, Cripps, & Gains-Germain, 2018; Jenkins, 2017; Lonergan, Cripps, & Smith, 2013; Lonergan, 2012; Trusty & Cripps, 2011; Trusty, 2009) and scattered reports of ectomycorrhizal fungi occurring in *P. albicaulis* forests (covered in Mohatt, 2006). Mohatt, Cripps, & Lavin (2008) and Cripps & Antibus (2011) form the most significant work aimed explicitly at species discovery. These studies were focused in the Greater Yellowstone Ecosystem and used

sporocarps collected in pure WBP stands combined with morphological and DNA analysis of seedling root tips to identify the ectomycorrhizal fungal community. Together they report 32 ectomycorrhizal fungi species that associate with WBP. These species can be organized into three broad groups: "(1) generalists that associate with many trees such as pines, spruce, and fir (*Amphinema, Cenococcum, Piloderma*, and thelephoroid fungi), (2) associates of high-elevation western conifers (*Cortinarius, Russula, Lactarius, Tricholoma, Hygrophorus*, and fungi associated with snowbanks); and (3) specialists specific for pines such as five-needled pines or stone pines (*Suillus, Rhizopogon,* and *Chroogomphus*)" (Keane et al., 2012, pg. 15).

Among the 32 fungal species associated with WBP, the greatest diversity exists within the Boletales and Cortinariales orders; the most abundant species observed was the generalist, asexual ascomycete, *Cenoccocum geophilum* Fr.. The pine-specific group (number 3 above) is commonly known as the Suilloid group and is perhaps the most important. The Suilloid group is specific at least at the genus level in *Pinus* and often shows host specificity at an even finer level. These ectomycorrhizal fungi have the potential to decline along with the five-needled pine forests. At the same time, however, they may provide *P. albicaulis* a competitive advantage over secondary successional tree species (spruce and fir) (Cripps & Antibus, 2011). Notably, *Suillus subalpinus* M. M. Moser is known to associate only with WBP and has been proposed for listing on the IUCN Red List (Osmundson, 2016) .

The working species profile created by Mohatt et al. (2008) and Cripps & Antibus (2011) provided the basis for applied studies and recovery work with the WBP ectomycorrhizal fungal community. Cripps & Grimme (2011) showed that successful inoculation of seedlings can occur under greenhouse conditions; the degree of success varied with species or strain of fungus, and the parameters of inoculum type, soil substrate, pH and fertilizer regime. Lonergan, Cripps, & Smith (2013) and Cripps, Alger, & Sissons (2018), used the best candidate inoculant strain identified by Cripps and

Grimme (2011), *Suillus sibiricus* (Singer) Singer, in a large-scale out-planting experiment. Their results showed that the benefit of inoculation on seedling survival was dependent on site conditions in early years, but after seven years, there was no significant benefit of inoculation across all treatments (Cripps et al., 2018). The current understanding is that inoculation is a useful and necessary component of recovery in sites devoid of native soil mycota, such as severe burns or plots isolated from other WBP stands. Given that WBP seedling survival decreases the most in the second and third years after establishment, an inoculation benefit persisting only through the early years is nonetheless useful (Cripps et al., 2018).

Considerable work has been done investigating the effect of fire on the WBP ectomycorrhizal fungal community. Trusty & Cripps (2011) and Trusty (2009) compared the ectomycorrhizal fungi of naturally regenerated and planted seedlings on burned and unburned sites. They found that burning accounted for the majority (>60%) of the shift in the fungal community between seedlings; seedlings regenerating in burns largely lost the native pine-specific Suilloid group and instead were dominated by non-host specific species typical of burned environments. Seedling origin (natural/planted) had only a minor effect on the observed community. Planted seedlings hosted thelephoroid and *Suillus* species that were lacking on naturally regenerated seedlings, and naturally regenerated seedlings hosted *Phialocephala* and *Coltricia* species that were lacking on planted seedlings; however, these differences were insufficient to separate the communities in principal coordinate analysis (Trusty & Cripps, 2011). Jenkins, Cripps, & Gains-Germain (2018) investigated the ectomycorrhizal-fire relationship in a greenhouse experiment by comparing *Suillus*-colonized seedlings to non-colonized seedlings planted in burned soils. They found that *Suillus* colonization increased seedling biomass and total N content and was associated with N-isotope partitioning.

The latest research has examined use of nitrogen by the WBP ectomycorrhizal fungal community. Antibus, Hobbie, & Cripps (2018) examined nitrogen use within the Suilloid group and demonstrated strong intra- and interspecific variation; this niche partitioning supports the maintenance of species diversity and complete resource use by the Suilloid group.

1.4. Research questions and objectives

The existing body of research on the description of the *P. albicaulis* ectomycorrhizal fungal community is extensive. However, there is a need for more discovery research examining the native ectomycorrhizal fungal community, the effects of environmental variables on this community and applied questions in recovery.

The existing species profile of the native ectomycorrhizal fungal community is incomplete for three reasons: (1) it is heavily reliant on surveys of sporocarps (mushrooms) considered to be putative associates; (2) DNA confirmation has been restricted to the root tips of seedlings; and (3) the work has been concentrated in a single region of the WBP range, the Greater Yellowstone Ecosystem (Cripps & Antibus, 2011; Mohatt et al., 2008; Mohatt, 2006). There is inherent uncertainty in using sporocarp collection. Fungal fruiting is stimulated by different variables, fruiting bodies of some species are inconspicuous (hypogenous or resupinate) and some species don't fruit at all (Mohatt, 2006). In addition, it is well demonstrated that the aboveground (sporocarp) and belowground fungal communities are decoupled; that is, what we see as mushrooms does not correspond to what occurs belowground as mycorrhizae (Gardes & Bruns, 1996). Sporocarp collection will miss species that are not fruiting. Associates determined by sporocarp collection can only be understood as *putative* associates (Mohatt, 2006). Mycorrhizal communities are successional, though traditional, forest-based, linear models of succession are insufficient to describe the complexity of ectomycorrhizal fungal community succession (Twieg, Durall, & Simard, 2007). This applies to WBP communities and thus DNA sampling

from multiple age classes is necessary to capture the entire community. There are some indications that environmental variables, in addition to host identity, can influence the ectomycorrhizal fungal community (Miyamoto, Sakai, Hattori, & Nara, 2015; Toljander, Eberhardt, Toljander, Paul, & Taylor, 2006). Sampling sites in other parts of the WBP range, especially those differing significantly in forest type, soil and climate is also required to capture the full extent of the community.

In addition to gaps in the description of the mycorrhizal fungal community, little work has been done to assess the extent to which planted seedlings are colonized by the native ectomycorrhizal soil mycota. Trusty & Cripps (2011) and Trusty (2009) are the only works that have specifically addressed this question. Robust descriptions of the ectomycorrhizal communities on out-planted seedlings has important implications in management and recovery.

This thesis is an effort to contribute to the discovery and description of the native ectomycorrhizal fungal community and to focus on its applied implications by assessing differences among planted and naturally occurring trees. It asks three primary questions:

1. What are the ectomycorrhizal fungal communities on *P. albicaulis* (tree community) and in the soils of *P. albicaulis* stands (soil community) in the treeline forests of Mount Revelstoke and Glacier National parks of Canada?
2. How do the tree and soil ectomycorrhizal fungal communities compare between tree types (mature trees, naturally regenerated seedlings and planted seedlings)?
3. How do the tree and soil ectomycorrhizal fungal communities compare to the community described previously by Mohatt, Cripps, & Lavin (2008) and Cripps & Antibus (2011) in the Greater Yellowstone Ecosystem?

These questions can be reiterated into two objectives:

1. To describe the tree and soil ectomycorrhizal fungal communities of *P. albicaulis* in Mount Revelstoke and Glacier National parks.
2. To compare the tree and soil ectomycorrhizal fungal communities of *P. albicaulis* among tree types (mature trees, naturally regenerated seedlings and planted seedlings) and with previously described communities.

The research formed by these questions contributes to four major applications:

1. **Practical use in recovery efforts of *P. albicaulis*.** This refers to the assessment of ectomycorrhizal fungal colonization on planted seedlings, the identification of the best candidate ectomycorrhizal fungi species for the inoculation of planted seedlings and the providing baseline knowledge to incorporate ectomycorrhizal fungi into planting site selection and assessments of WBP stand health (among other applications).
2. **Conservation of host specific fungi (Suilloid group).** Fungi specific to *P. albicaulis* or a larger clade including *P. albicaulis* are threatened by its decline (Cripps & Antibus, 2011; Osmundson, 2016). Their identification is a prerequisite to their conservation.
3. **Improved understanding of *P. albicaulis* mycorrhizal fungal patterns and dynamics.** Greater understanding of the variability in mycorrhizal fungal associates across the range of WBP, and variation with the age and origin of the trees, can contribute to development of practical recovery efforts (1).
4. **Species discovery.** The Kingdom Fungi is marked by a lack of knowledge. Five percent of the some 2.2 to 3.8 million fungi species have been named (WIllis, 2018). Discovery and identification of species or species associations is the first step toward understanding the status and functioning of these species in ecosystems and their role in ecosystem recovery.

Chapter 2: Methods

2.1. Study area

This work was carried out in Mount Revelstoke and Glacier National parks of Canada (research permit#: GLA-2019-32862) (Figure 2). Recovery work and research surrounding WBP is well established in the parks. The region differs significantly in climate and dominant forest type from other locations where sampling of the *P. albicaulis* ectomycorrhizal fungal community has occurred (Greater Yellowstone Ecosystem; Rocky Mountains) (Cripps & Antibus, 2011; Mohatt et al., 2008; Mohatt, 2006). Mount Revelstoke and Glacier National parks are located in the Columbia Mountains region, which is characterized as an interior rainforest. The lower elevation forests are in the Interior Cedar-Hemlock biogeoclimatic zone, and these transition to the Engelmann Spruce-Subalpine Fir biogeoclimatic zone in the subalpine. *P. albicaulis* generally occurs in mixture with sub-alpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and Engelmann spruce (*Picea engelmanni* L. H. Karst.). This contrasts the Greater Yellowstone Ecosystem, where the climate is much drier. The lower elevations are treeless and dominated by grassland-shrub vegetation; continuous coniferous stands are present at mid and sub-alpine elevations and contain Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), lodgepole pine (*Pinus contorta* Douglas ex Loudon), sub-alpine fir and Engelmann spruce. WBP occurs at high elevations in pure or mixed stands and co-occurs at some sites with limber pine (*Pinus flexilis* E. James).

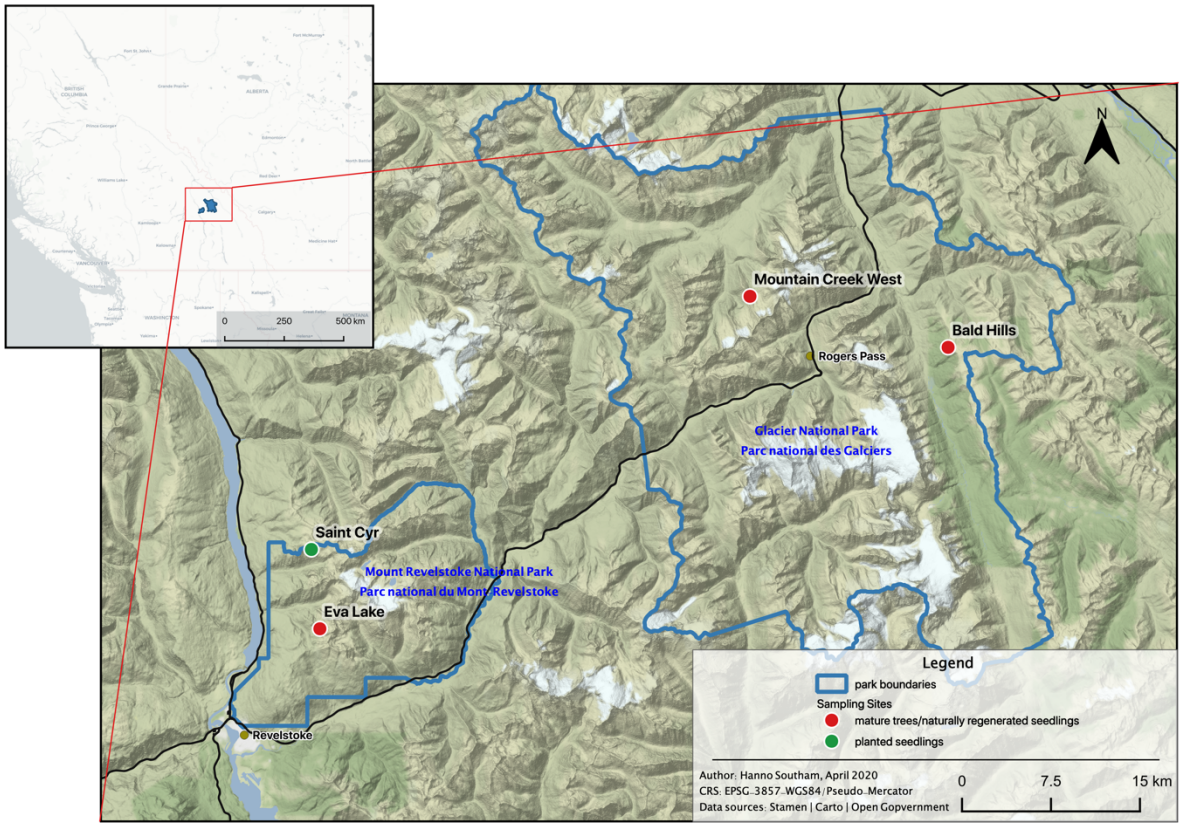

Figure 2. Map of study area and locations of sampling sites. Created in QGIS version 3.10.4 (QGIS Development Team, 2020).

2.1.1. Mature tree and natural seedling sampling sites

Sampling of mature trees and naturally regenerated seedlings occurred at three sites that are associated with a long-term monitoring project tracking the incidence of blister rust in the Canadian Rocky and Columbia mountains (Shepherd et al. 2018; Smith et al., 2008; Smith, Shepherd, Gillies, & Stuart-Smith, 2013) (Figure 2). The sites were nearly uniform in their site characteristics (Table 1). Elevation centred around 2000m and aspects were typically southwest. Mean annual temperature (MAT) and mean annual precipitation (MAP) ranged from (-0.6)-0°C and 1899-2084mm respectively. The dominant soil types were Orthic and Eluviated Drystic Brunisols and Orthic Humo-Ferric Podzols. The characteristic vegetation types were subalpine fir-mountain hemlock open forests (ELC Vegetation Type O20) and subalpine fir-whitebark pine open forests (O22) (Achuff, Holland, Coen, & Tighem, 1984). The

understory vegetation was typified by ericaceous shrubs (*Vaccinium* spp.*, Cassiope mertensiana* (Bong.) G.Don and *Phyllodoce empetriformis* (Sm.) D. Don).

2.1.2. Planted seedling sampling site

Sampling of planted seedlings occurred at one site, Saint Cyr, that is part of a trial project by the BC Ministry of Forest, Lands and Natural Resource Operations and Parks Canada seeking to identify rust-resistant strains of *P. albicaulis* (Figure 2). As part of that trial, 2559 putatively blister rust-resistant seedlings (known as plus-seedlings) were planted at four different sites in Mount Revelstoke and Glacier National parks between 2014 and 2018. Two age classes are present at the Saint Cyr site: one planted in September 2017 and a second planted in September 2018. Seedlings were sampled from both age classes.

The Saint Cyr site differs from the mature tree/naturally regenerated seedling sites in two major ways. The site was burned by a low-mid severity wildfire in the summer of 2003 (K. Macauley, Parks Canada, personal communication, 18 March 2020) and naturally occurring WBP is rare. Burned sites are understood to be ideal planting locations and so the site is representative of active out-planting recovery efforts (Keane et al., 2012); however, it is not comparable to sites where WBP stands naturally occur elsewhere in the Park. The Saint Cyr site also differs in soil and vegetation types (Table 1), some of which is attributable to its history of fire. The dominant soil types are slightly more developed Orthic Sombric Brunisols and Orthic Sombric Humo-Ferric Podzols (note: no attempt was made to describe the post-fire soil). The dominant vegetation types include subalpine fir-mountain hemlock open forest (ELC Vegetation Type O20), heath tundra (L5) and herb meadow (H16) (Achuff et al., 1984). The vegetative community observed during sampling is captured by these three vegetation types but was also marked by a post-fire community of herbaceous species (e.g. *Castilleja*, *Erythronium grandiflorum* Pursh.). The elevation is ~2100m and the aspect south-southeast. MAT ranged from (-0.3)-(-0.1)°C and MAP ranged from 1966-2011mm (Table 1).

Table 1. Site characteristics of the sampling sites. Ecosite, soil types and vegetation types are from the Ecological Land Classification (ELC) of Mount Revelstoke and Glacier National parks (Achuff et al., 1984). MAT and MAP are averages from 1981-2010 estimated from ClimateWNA (Wang, Hamann, Spittlehouse, & Carroll, 2016). (*) indicates estimated from GoogleEarth.

Site Name	ELC Ecosite	Soil Types	Vegetation Types	Elevation	Aspect	MAT	MAP
			Mature Tree/Natural Seedling Sites				
Mountain Creek West	AK6	Orthic & Eluviated Dystric Brunisols, Orthic Humo-Ferric Podzol	subalpine fir-mountain hemlock open forest (O20), subalpine fir-whitebark pine open forest (O22)	2145-2161m	210-273°	(-0.6)-(-0.5) °C	2073-2084mm
Bald Hills	AK6	Orthic & Eluviated Dystric Brunisols, Orthic Humo-Ferric Podzol	subalpine fir-mountain hemlock open forest (O20), subalpine fir-whitebark pine open forest (O22)	2068-2087m	234-270°	(-0.1)-0°C	1899-1915mm
Eva Lake	AK6	Orthic & Eluviated Dystric Brunisols, Orthic Humo-Ferric Podzol	subalpine fir-mountain hemlock open forest (O20), subalpine fir-whitebark pine open forest (O22)	2080-2103m	190-250°*	(-0.2)-0.4°C	1895-1991mm
			Planted Seedling Site				
Saint Cyr Planting Site	JD4	Orthic Sombric Brunisol, Orthic Sombric Humo-Ferric Podzols	subalpine fir-mountain hemlock open forest (O20), heath tundra (L5), herb meadow (H16)	2093-2129m	140-169°	(-0.3)-(-0.1)°C	1966-2011mm

2.2. Field sampling and sample processing

2.2.1. Mature tree sampling

Thirty mature trees were sampled across the three sites. Trees were randomly selected from the long-term health monitoring transects established at the sites (Shepherd et al., 2018; Smith et al., 2008; Smith et al., 2013) to test how the ectomycorrhizal fungal community changed with tree health and incidence of blister rust (unpublished data not reported on here). The sampling protocol at each mature tree followed Birch (2019), developed for bristlecone pine (*Pinus longaeval* D.K. Bailey) and limber pine *(Pinus flexilis* E. James). These species occupy similar habitats to WBP and sometimes co-occur.

At each tree, three fine root samples were collected. Roots were traced outward from the bole and a trowel was used to dig down and find fine roots. Only fine roots with a visible connection to the parent root were taken. Samples were placed in Ziploc bag or Falcon tube and pooled by tree. Three soil samples were collected adjacent to where fine roots were sampled. Litter on the soil surface was scraped away and a trowel was used to collect soil to a depth of 15cm (this included the F and H horizons of the organic layer). Soil samples were pooled by tree and stored in a Ziploc bag.

Upon return from the field, soil samples were placed in the freezer until processing. Fine root samples were refrigerated until processing. Fine root samples were processed within 48 hours of collection and soil samples within 45 days of collection.

2.2.2. Seedling sampling

Twelve naturally regenerated seedlings and twelve planted seedlings were sampled. Naturally regenerated seedlings were sampled randomly from the same sites as the mature trees; these seedlings were taken from outside the established health monitoring transects so as not to affect long term data collection. Planted seedlings were randomly sampled across the two age classes present at the Saint Cyr planting site, with six seedlings collected from the 2017 planting and six seedlings from the 2018 planting.

Some of the naturally regenerated and planted seedlings were sampled from clusters of seedlings that reflects the natural spatial distribution of WBP. WBP possesses a unique coevolutionary dispersal strategy with the Clarks Nutcracker (*Nucifraga columbiana*), where seeds are harvested from WBP cones and stored in soil seed caches (Tomback, 1982). This strategy often results in WBP germinating in clusters that can fuse at the stem. Out-planting of WBP for recovery tries to emulate these seedling clusters by planting three individual seedlings in close proximity. Planted seedling clusters were sufficiently spaced to allow sampling of individual stems without causing significant damage to neighboring seedlings in the cluster. For naturally regenerated seedlings, we treated clusters as single seedlings because it was impossible to sample individual stems. After processing, this resulted in 15 individual stems in the sample.

To collect the seedlings, a trowel was used to dig around the root mass of the seedlings. The whole seedling, root mass and adhering soil were collected and placed in a Ziploc bag. A soil sample was also collected adjacent to the seedling following the same methods as for mature trees. Seedlings were

refrigerated and soil samples were frozen until processing. Seedlings were processed within 48 hours and soil samples within 45 days.

2.2.3. Sample processing

Fine root samples were gently washed free of soil, debris and roots of other species with water. The root mass of seedlings was intact, providing an opportunity to compare seedling root structure between naturally regenerated and planted seedlings. Cleaned seedling roots were scanned with a WinRHIZO root scanning system (unpublished data not reported on here) and fine root samples were inspected for colonized root tips. Colonized root tips were identified by conspicuous morphological characteristics (e.g. tuberculate mycorrhizae or an enlarged mantle), the absence of root hairs and, if necessary, the presence of a Hartig net in cross section. Fifty colonized root tips, or all colonized tips if less than fifty were present, were taken per sample and placed in a microtube. Microtubes were frozen at -30°C until they were sent for sequencing.

Soil samples were taken out of the freezer twenty-four hours before processing. Soil was sieved through progressively finer sieves (12.0, 6.0, 4.0, 2.0mm) to separate soil from root fragments and prepare them for DNA analysis. Notably, 12 soil samples associated with mature trees were sieved to 1.0mm unintentionally; this represents a plausible source of error in the results. Approximately 7.5ml of sieved soil was placed in 15ml Falcon tubes and frozen again at -30°C until being sent for sequencing. Frozen soil and fine root samples were shipped together on dry ice to the Integrated Microbiome Resource (IMR) (Dalhousie University, Halifax, NS).

A subset of the samples collected in the field were used for a morphotyping analysis and not processed (unpublished data not reported on here). The total number of samples sent for sequencing was 52 fine root samples (30 mature trees, 13 naturally regenerated seedlings and 9 planted seedlings) and 55 soil samples (30 mature trees, 13 naturally regenerated seedlings and 12 planted seedlings).

2.2.4. Precautions against sample contamination

Precautions were taken to prevent contamination throughout field sampling and sample processing. Between sampling trees in the field, all implements were sprayed with 10% bleach solution and wiped clean. During fine root processing in the lab, all trays, dishes and implements contacting samples were rinsed between samples. Sieves and trays used for processing soil samples were submerged in 10% bleach solution for 5 minutes, rinsed clean and wiped dry between samples.

2.3 Genetics

2.3.1. DNA extraction and sequencing

DNA extraction and Next-Generation sequencing of the ITS2 region was performed by the IMR using their standard protocols (Comeau, Douglas, & Langille, 2017). The ITS2 region is understood as one of the "metabarcode" regions of fungi (Blaalid et al., 2013). DNA was extracted using the QIAGEN PowerSoil DNA Kit and amplified by polymerase chain reaction (PCR) with the ITS86(F) and ITS4(R) fungal specific primers. Next-generation sequencing was carried out with an Illumina MiSeq. 1.8 Cassava demultiplexed paired-end fastq sequences were returned as the final product.

2.3.2. Bioinformatics

With the exception of Trimmomatic, all bioinformatic processing was performed in QIIME 2 using a modified pipeline (Bolyen et al., 2019). Sequences were run through Trimmomatic to trim low-quality fragments (threshold: sliding window = 5 bp, quality score = 20) (Bolger, Lohse, & Usadel, 2014) . The sequences were then imported into QIIME 2. CutAdapt (Martin, 2011) was used to remove adapters and ITSxpress (Rivers, Weber, Gardner, Liu, & Armstrong, 2018) was used to extract the ITS2 region. Denoising, dereplication and removal of chimeras was done with DADA2 (Callahan et al., 2016) to produce an abundance table and representative sequences of the resulting amplicon sequence variants (ASVs). ASVs are a replacement for operational taxonomic units (OTUs) that use exact sequence variants

as oppose to clustering sequences based on a similarity threshold (historically 97% similarity) (Callahan, McMurdie, & Holmes, 2017).

ASVs were assigned taxonomy using a fungal specific classifier of the entire ITS region created by the IMR (date created: 2 February 2019) (Comeau et al., 2017). Taxonomic classification was based on 99% similarity with reference sequences in the UNITE database (Kõljalg et al., 2013). ASVs were then filtered in progressive steps. ASVs occurring at frequencies lower than 0.1% of the mean sample depth were removed; this threshold corresponds to the percent abundance of contaminant ASVs from sequential sequencing runs with Illumina MiSeq (Comeau et al., 2017). ASVs passing this threshold were filtered to only those classified to the level of genus or lower. These taxa were compared with the FUNGuild database to determine mycorrhizal status (Nguyen et al., 2016); taxa not described in FUNGuild were assessed by referencing the literature. Only ASVs belonging to ectomycorrhizal taxa were retained. This produced the working dataset of ectomycorrhizal fungal taxa. This database was exported out of QIIME 2 for downstream analyses.

2.4. Ectomycorrhizal fungal community description and analysis

All analyses were performed in R (version 3.6.2) (R Core Team, 2019) mostly using the phyloseq package (McMurdie & Holmes, 2013); phyloseq is a package for microbiome analysis that incorporates numerous other packages commonly used for community analysis (e.g. vegan, ade4, ape, picante) and the ggplot package (for graphics). Two datasets were used in the analyses – a rarefied dataset and an unrarefied dataset. The unrarefied dataset retained low abundance samples and was used to create the master list of taxa occurring in the tree and soil communities (Table 5 & 9). The rarefied dataset was used in all diversity analyses and was created by rarefying the raw dataset exported from QIIME 2 to a sequencing depth of 2000 reads; this threshold was determined from rarefaction curves generated in QIIME 2. The term *sample type* is used frequently below; it refers to a fine root sample or soil sample taken from one of the three tree types (mature tree, naturally regenerated seedling or planted

seedling). Fine root samples from mature trees is a sample type. All statistical tests were considered significant at α=0.05.

2.4.1. Community Composition and Alpha Diversity

Community composition was measured at the species, genera and family level using relative abundance (RA) and sample counts as metrics. Relative abundance was estimated as the mean RA (proportion of reads) of each taxon across all the samples in a given sample type from the rarefied dataset. Sample counts were defined as the number (or percent) of samples a taxon occurred in and were retrieved from the unrarefied dataset.

Alpha diversity was estimated with observed richness (count of # of taxa in a sample) and Shannon's diversity metrics at the ASV and species levels. Diversity estimates and plots were done in phyloseq. ANOVA (package: car) (Fox & Weisberg, 2018) and pairwise t-tests with a Bonferroni adjustment (package: emmeans) (Lenth, 2020) were used to test for differences in alpha diversity between tree types after assumptions had been checked. The Shapiro-Wilk test was used to test the normality assumption and the Bartlett test was used to test for equal variance.

2.4.2. Beta Diversity

Beta diversity was estimated with the Bray Curtis dissimilarity metric (Bray & Curtis, 1957). The datasets used for beta diversity calculations were subsets of the larger rarefied dataset filtered to include only the most abundant genera whose mean relative abundance summed to >90% of the total abundance in a given sample type. This filtering was done to better resolve differences between sample types. No transformations were applied to the Bray Curtis distance matrix. Ordination was performed with Principal Component Analysis (PCoA) and the two axes explaining the greatest proportion of variation were graphed. Estimates of beta diversity and ordinations were done in phyloseq.

The statistical tests used to assess compositional differences between sample types were betadisper, Adonis and ANOSIM (package: vegan) (Oksanen et al., 2019). betadisper was used to check

the assumption of homogeneity of beta diversity variances between sample types. Adonis and ANOSIM were used to test for significance of compositional differences between sample types.

Chapter 3: Results

3.1. Qualitative observations

Tree types (mature trees, naturally regenerated seedlings and planted seedlings) differed qualitatively in dominant ectomycorrhizal fungi, number of colonized root tips and root structure. Fifty colonized root tips were consistently collected from all mature trees (Table 2). Some naturally regenerated seedlings failed to meet the sampling goal of 50 root tips (Table 2), but the ectomycorrhizal fungi observed among naturally regenerated seedlings were similar to those on mature trees. Planted seedlings, however, were distinct from mature trees and naturally regenerated seedlings. All planted seedlings had fewer than 50 root tips and some samples had none or only a few (2-5) colonized root tips (Table 2). The planted seedlings were dominated by a single morphotype (Figure 3) and their root structure was drastically different from that of naturally regenerated seedlings. Planted seedlings had more root mass, finer roots, more root tips and often retained the shape of a planting container (Figure 4).

Table 2. Summary statistics of colonized root tips (CRT) collected from each tree type. The maximum number of CRT collected per tree was 50.

Tree Type	Mean CRT	Median CRT	Min CRT	Max CRT
Mature Tree (n=30)	50	50	50	50
Natural Seedling (n=13)	40	50	7	50
Planted Seedling (n=10)	5	4	0	13

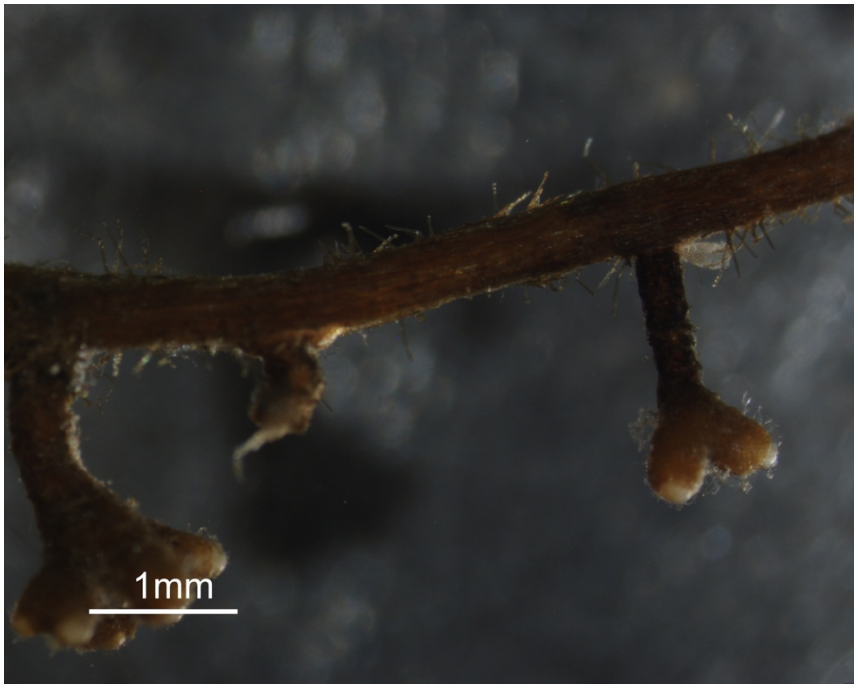

Figure 3. Dominant ectomycorrhizae morphotype observed on the planted seedlings.

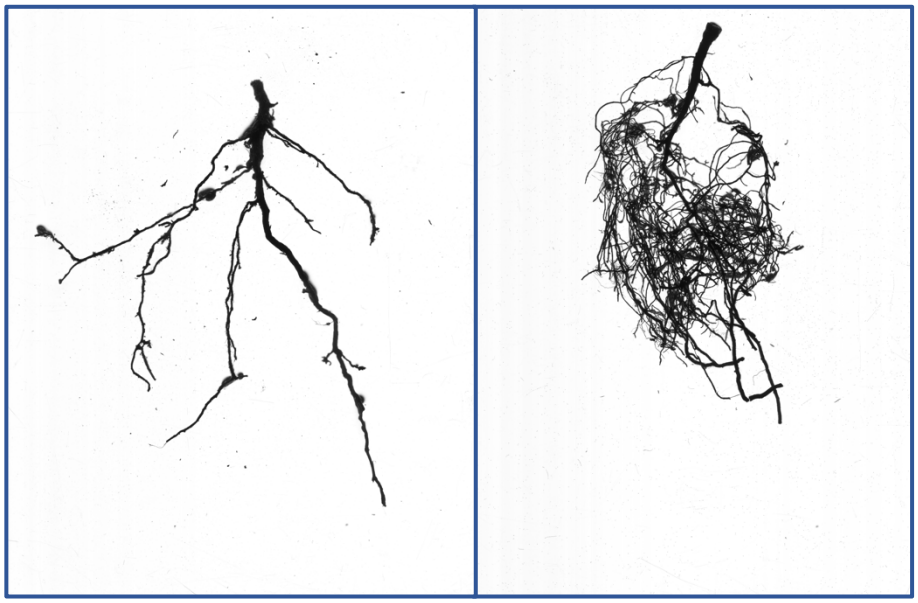

Figure 4. Representative samples of seedling root structure; naturally regenerated seedlings (left) and planted seedlings (right).

3.2. Bioinformatics

Sequencing returned 6 270 821 reads from 109 samples with a mean per sample depth of 57 530. After processing (trimming to isolate the ITS2 region, denoising, dereplication and removal of chimeras) this data grouped into 5 409 ASVs across 107 samples with a mean per sample frequency of

45 760 reads. The requirement for ASVs to appear at an abundance of >0.1% of the mean sequencing depth reduced the dataset to include 2 665 ASVs across 106 samples (mean per sample frequency of 45 748 reads). Taxonomic classification of the ASVs and retention of only those assigned to the level of genus left 1 340 ASVs in 106 samples with a mean per sample frequency of 35 250 reads. Selecting only ectomycorrhizal taxa produced the dataset used for species identification: 451 ASVs across 105 samples (mean per sample frequency of 24 267 reads) that resolve into 105 taxa at the genus or species level based on 99% similarity with taxa in the UNITE database. Rarefying to a standard sequencing depth (2000 reads) produced the second dataset used for diversity analyses; rarefying removed an average of 11 samples and 30-70 ASVs.

3.3. Ectomycorrhizal fungal communities and diversity

The complete set of taxa identified on root tips (tree community) and in the adjacent soil (soil community) are reported in Tables 5 & 9 respectively.

3.3.1. Tree communities

3.3.1.a. Alpha diversity

A total of 283 ASVs grouped into 59 species and 31 genera in 23 families appeared in the tree community. After rarefying, the community was reduced to 55 species and 31 genera in 23 families. There were no significant differences in alpha diversity between tree types at the ASV or species level using observed richness or Shannon's diversity metrics (Tables 3 and 4; Figure 5). There was a consistent pattern in alpha diversity among tree types; diversity tended to decrease from mature trees, to naturally regenerated seedlings, to planted seedlings (Table 3; Figure 5).

Table 3. Mean per sample observed richness by tree type in tree community. Two levels included: # of ASVs and # of species.

	Tree Community		
Tree Type	**ASV**	**Species**	**Sample Size (n)**
Mature Tree	16.345	9.483	29
Natural Seedling	13.769	7.923	13
Planted Seedling	13.286	6.857	7

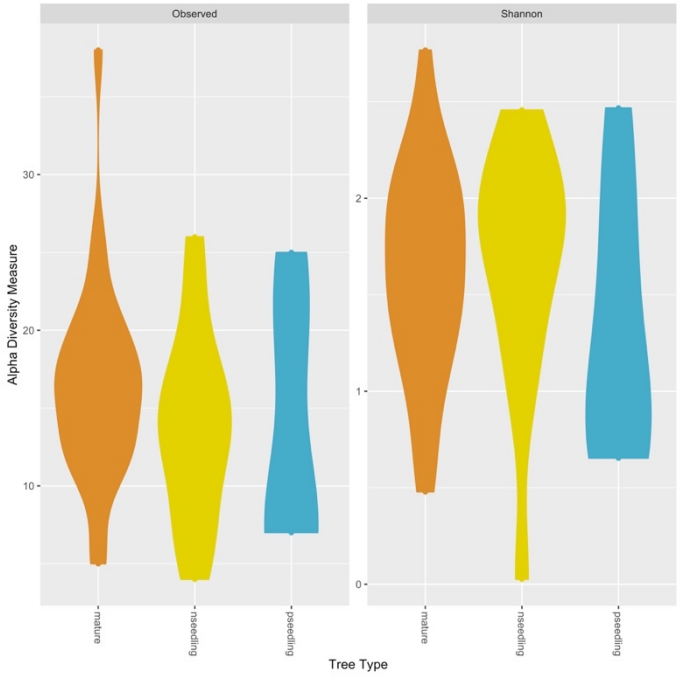

Figure 5. Comparison of observed richness (left) and Shannon's diversity index (right) among tree types in tree community. Colours: mature trees (mature; orange), naturally regenerated seedlings (nseedling; yellow-green) and planted seedlings (pseedling; blue). Violin plots show spread of data; the width of the object is proportional to the frequency of observations at that richness value.

Table 4. Type III ANOVA table of test for differences in α-diversity between tree types in tree community. Test used a simple linear model with a single factor (Tree Type). Separate tests were performed at ASV and species levels for observed richness and Shannon's diversity index.

Factor/Metric	Sum Sq	Df	F value	Pr(>F)
ASV Level				
Observed richness				
Tree Type	87.228	2	1.024	0.367
Residuals	1958.446	46	NA	NA
Shannon's diversity index				
Tree Type	0.598	2	0.836	0.440
Residuals	16.464	46	NA	NA
Species Level				
Observed richness				
Tree Type	47.844	2	2.361	0.106
Residuals	465.992	46	NA	NA
Shannon's diversity index				
Tree Type	0.727	2	1.760	0.183
Residuals	9.494	46	NA	NA

3.3.1.b. Community composition

The mature tree community contained 49 species and 29 genera in 23 families. Rarefying removed 3 species, but did not remove any genera or families. Mean per sample richness was 16 ASVs and 9 species (Table 3). Seven families contained the genera making up 90% of the total abundance: Atheliaceae (RA=0.383; dominant genera: *Piloderma* (RA=0.266), *Amphinema* (RA=0.068), *Tylospora* (RA=0.049)), Helotiaceae (RA=0.243; dominant genera: *Meliniomyces* (RA=0.236)), Gloniaceae (RA=0.129; dominant genera: *Cenococcum* (RA=0.129)), Cortinariaceae (RA=0.072; dominant genera: *Cortinarius* (RA=0.072)), Suillaceae (RA=0.051; dominant genera: *Suillus* (RA=0.051)), Albratellaceae (RA=0.031; dominant genera: *Leucophelps* (RA=0.028)) and Rhizopogonaceae (RA=0.021; dominant genera: *Rhizopogon* (RA=0.021)) (Table 6; Figure 6).

The naturally regenerated seedling community included 26 species and 18 genera in 14 families. Rarefying reduced the community to 21 species and 16 genera in 12 families. The mean per sample richness was 14 ASVs and 8 species (Table 3). The genera making up 90% of the total abundance were grouped into six families: Helotiaceae (RA=0.331; dominant genera: *Meliniomyces* (RA=0.330)), Atheliaceae (RA=0.229; dominant genera: *Piloderma* (RA=0.182), *Amphinema* (RA=0.046)), Cortinariaceae (RA=0.125; dominant genera: *Cortinarius* (RA=0.125)), Suillaceae (RA=0.080; dominant genera: *Suillus* (RA=0.080)), Rhizopogonaceae (RA=0.077; dominant genera: *Rhizopogon* (RA=0.077)) and Gloniaceae (RA=0.066; dominant genera: *Cenococcum* (RA=0.066)) (Table 6; Figure 6).

The planted seedling community consisted of 18 species and 17 genera in 14 families. Rarefying removed 1 species and did not affect the number of observed genera or families. Mean per sample richness was 13 ASVs and 7 species (Table 3). Here, the genera making up 90% of the total abundance grouped into only five families: Helotiaceae (RA=0.435; dominant genera: *Meliniomyces* (RA=0.433)), Leotiaceae (RA=0.195; dominant genera: *Pezoloma* (RA=0.195)), Suillaceae (RA=0.138; dominant genera:

Suillus (RA=0.138)), Russulaceae (RA=0.199; dominant genera: *Russula* (RA=0.119)) and Endogonaceae (RA=0.24; dominant genera: *Endogone* (RA=0.024)) (Table 4; Figure 3).

Table 5. Ectomycorrhizal fungal taxa (genera or species) of tree community. Taxa identified from root tip samples of mature trees (MT), naturally regenerated seedlings (NS) and planted seedlings (PS) of whitebark pine. Counts are the number of samples a taxon was identified in. Sample size (N): MT=30, NS=13, PS=9. Bolding indicates high confidence in assigning ectomycorrhizal association. (+) indicates taxa previously identified with WBP. (*) indicates taxa previously identified as putative WBP associates.

Taxa	# of Samples			
	Total	MT	NS	PS
Ascomycota				
Dothideomycetes				
Mytilinidales				
Gloniaceae				
***Cenococcum*+**				
Cenococcum_unidentified	**33**	21	8	4
Leotiomycetes				
Helotiales				
Helotiaceae				
Hymenoscyphus				
Hymenoscyphus_tetracladius	**4**	3	0	1
Meliniomyces				
Meliniomyces_bicolor	**10**	6	3	1
Meliniomyces_variabilis	**45**	27	11	7
Meliniomyces_unidentified	**50**	30	13	7
Mycosymbioces				
Mycosymbioces_mycenaphila	**3**	2	1	0
Mycosymbioces_unidentified	**12**	9	1	2
Helotiales_fam_Incertae_sedis				
Acephala				
Acephala_unidentified	**1**	0	0	1
Hyaloscyphaceae				
Pezizella				
Pezizella_unidentified	**2**	2	0	0
Leotiaceae				
Pezoloma				
Pezoloma_ciliifera	**6**	0	1	5
Pezoloma_ericae	**33**	18	12	3
Discinaceae				
Hydnotrya				
Hydnotrya_unidentified	**5**	5	0	0
Pezizomycetes				
Pezizales				
Pyronemataceae				
***Wilcoxina*+**				
Wilcoxina_unidentified	**5**	3	1	1
***Wilcoxina_rehmii*+**	**1**	1	0	0
Basidiomycota				
Agaricomycetes				
Agaricales				
Cortinariaceae				
***Cortinarius*+**				
Cortinarius_unidentified	**16**	9	7	0
Cortinarius_alboamarescens	**2**	1	1	0
Cortinarius_barlowensis	**1**	1	0	0
Cortinarius_borgsjoeensis	**1**	0	1	0
Cortinarius_boulderensis	**1**	1	0	0
Cortinarius_brunneus	**1**	1	0	0
Cortinarius_caesiobrunneus	**1**	0	1	0
Cortinarius_caperatus	**6**	3	2	1
Cortinarius_carabus	**1**	0	1	0

Taxa	# of Samples			
	Total	MT	NS	PS
Cortinarius_cinnamomeus	**2**	0	2	0
Cortinarius_grosmorneensis	**1**	1	0	0
Cortinarius_lux-nymphae	**1**	1	0	0
Cortinarius_neofurvolaesus	**2**	2	0	0
Cortinarius_ochrophyllus	**1**	1	0	0
Cortinarius_privignus	**1**	1	0	0
Cortinarius_sarcoflammeus	**1**	1	0	0
Cortinarius_semisanguineus	**5**	5	0	0
Cortinarius_testaceofolius	**1**	1	0	0
Entolomataceae				
Entoloma				
Entoloma_cetratum	**3**	1	2	0
Hygrophoraceae				
Hygrophorus [+]				
Hygrophorus_purpurascens	**2**	1	0	1
Inocybaceae				
Inocybe [+]				
Inocybe_soluta	**1**	1	0	0
Lyophyllaceae				
Lyophyllum				
Lyophyllum_shimeji	**2**	2	0	0
Tricholomataceae				
Tricholoma [+]				
Tricholoma_equestre [+]	**1**	1	0	0
Atheliales				
Atheliaceae				
Amphinema [+]				
Amphinema_byssoides [+]	**2**	2	0	0
Amphinema_unidentified	**20**	15	3	2
Piloderma [+]				
Piloderma_bicolor	**25**	19	5	1
Piloderma_byssinum [+]	**5**	3	1	1
Piloderma_sphaerosporum	**22**	14	8	0
Piloderma_unidentified	**4**	3	1	0
Tylospora				
Tylospora_fibrillosa	**13**	11	2	0
Boletales				
Gomphidiaceae				
Gomphidius				
Gomphidius_glutinosus	**1**	1	0	0
Rhizopogonaceae				
Rhizopogon [+]				
Rhizopogon_unidentified	**11**	10	0	1
Rhizopogon_bacillisporus	**5**	3	1	1
Rhizopogon_brunneiniger	**6**	3	2	1
Rhizopogon_salebrosus	**2**	2	0	0
Suillaceae				
Suillus [+]				
Suillus_acidus	**2**	2	0	0
Suillus_brevipes	**4**	1	0	3
Suillus_punctatipes	**4**	1	2	1
Suillus_unidentified	**9**	6	3	0
Cantharellales				
Cantharellales_fam_Incertae_sedis				
Sistotrema				
Sistotrema_unidentified	**5**	3	1	1
Russulales				
Albatrellaceae				
Albatrellus				
Albatrellus_flettii	**1**	0	1	0
Leucogaster				
Leucogaster_unidentified	**1**	1	0	0
Leucogaster_nudus	**1**	1	0	0
Leucophelps				

Taxa	# of Samples			
	Total	MT	NS	PS
Leucophleps_spinispora	**1**	1	0	0
Russulaceae				
***Lactarius*⁺**				
Lactarius_caespitosus*	**4**	0	4	0
Lactarius_rufus*	**10**	9	1	0
Russula				
Russula_adusta	**1**	0	1	0
Russula_fragilis	**1**	1	0	0
Russula_griseascens	**6**	6	0	0
Russula_paludosa	**1**	0	1	0
Russula_puellaris	**2**	2	0	0
Russula_turci	**4**	1	2	1
Russula_vesca	**3**	0	0	3
Russula_xerampelina	**2**	1	0	1
Thelephorales				
Bankeraceae				
Phellodon				
Phellodon_melaleucus	**1**	1	0	0
Thelephoraceae				
***Pseudotomentella*⁺**				
Pseudotomentella_unidentified	**5**	3	2	0
Pseudotomentella_mucidula	**2**	2	0	0
***Pseudotomentella_nigra*⁺**	**1**	1	0	0
***Tomentella*⁺**				
Tomentella_sublilacina	**3**	2	0	1
Mucoromycota				
Endogonomycetes				
Endogonales				
Endogonaceae				
Endogone				
Endogone_unidentified	**1**	0	0	1
Endogone_lactiflua	**4**	1	1	2

Table 6. Dominant ectomycorrhizal genera in tree community by tree type. Taxa make up 90% of the total abundance in a given tree type. Relative abundance is the mean relative abundance of each genera across samples. # samples and % samples are presence/absence data. Sample size (n) applies only to presence absence data (unrarefied dataset).

	Abundance Metric		
Tree Community/Taxa	**RA**	**# samples**	**% samples**
Mature Tree (n=30)			
Piloderma	0.266	24	80.0
Meliniomyces	0.236	30	100.0
Cenococcum	0.129	21	70.0
Cortinarius	0.072	19	63.3
Amphinema	0.068	15	50.0
Suillus	0.051	8	26.7
Tylospora	0.049	11	36.7
Leucophleps	0.028	1	3.3
Rhizopogon	0.021	15	50.0
Natural Seedlings (n=13)			
Meliniomyces	0.330	13	100.0
Piloderma	0.182	9	69.2
Cortinarius	0.125	9	69.2
Suillus	0.080	5	38.5
Rhizopogon	0.077	2	15.4
Cenococcum	0.066	8	61.5
Amphinema	0.046	3	23.1
Planted Seedlings (n=9)			
Meliniomyces	0.433	9	100.0
Pezoloma	0.195	7	77.8
Suillus	0.138	3	33.3
Russula	0.119	4	44.4
Endogone	0.024	2	22.2

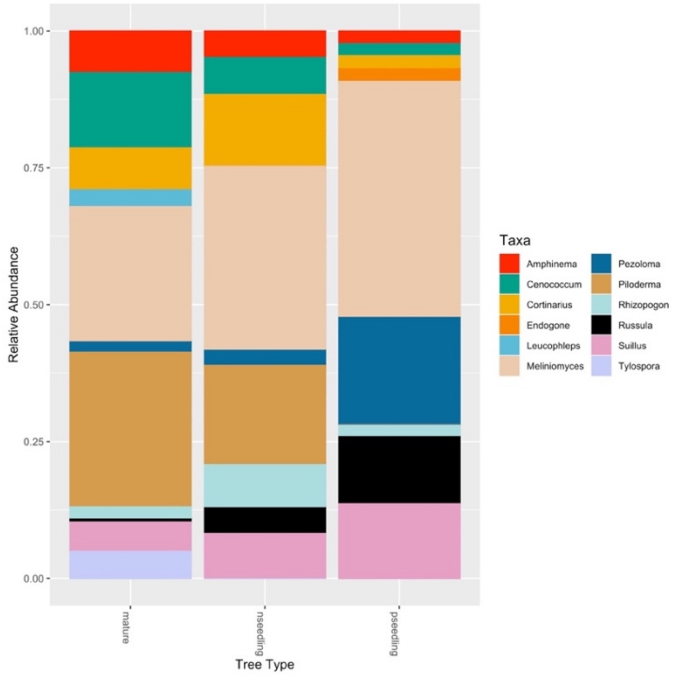

Figure 6. Relative abundance of the dominant genera (Table 6) in the tree community separated by tree type. Relative abundance measured as proportion reads in a given tree type. Position: mature trees (mature; left), natural seedlings (nseedling; middle) and planted seedlings (pseedling; right).

3.3.1.c. Beta diversity

Beta diversity was estimated with the Bray-Curtis dissimilarity metric on a subset of the untransformed rarefied community abundance data containing only ASVs in the dominant genera (Table 6). The first two axes of Principal Component Analysis (PCoA) explained 43.9% of the variation in this distance matrix; communities belonging to the three tree types showed some visual separation along these axes (Figure 7). Statistical testing to confirm this interpretation returned contrasting results. Multivariate dispersion was not statistically different among tree types allowing for tests of compositional difference (F=0.179, p=0.837) (betadisper in Table 7). Tree type was significant in the Adonis test for community dissimilarity indicating distinct communities on the natural (mature tree and naturally regenerated seedlings) and planted tree types (F=1.878, R^2=0.076, p=0.02) (Table 7). The ANOSIM test of similarity between communities gave the opposite result; tree type was not significant indicating rank similarity within groups was not significantly different than rank similarity between groups (R=0.059, p=0.219) (Table 7).

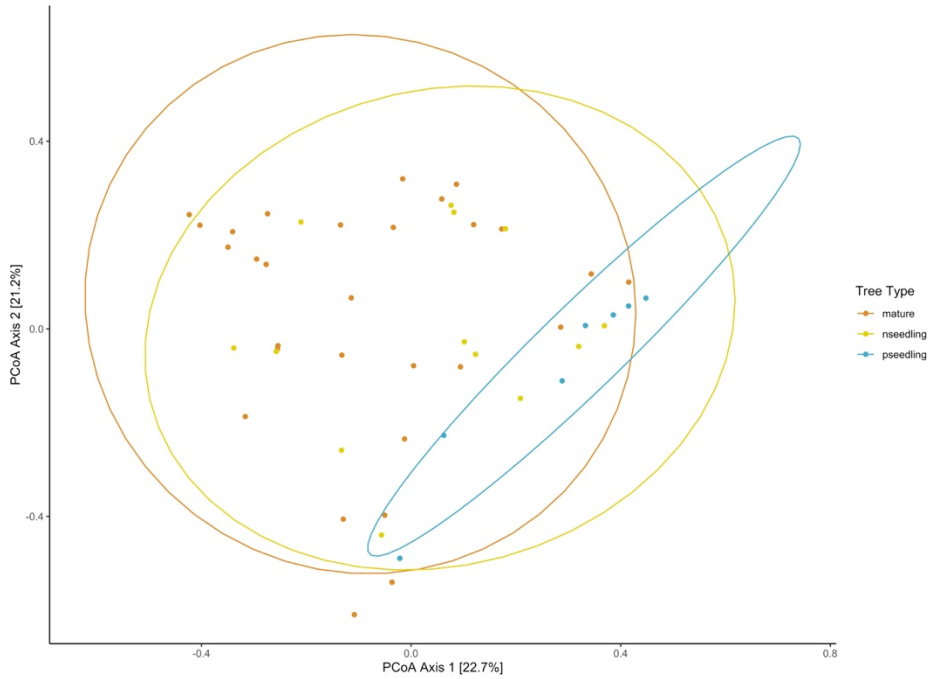

Figure 7. Principal coordinate analysis (PCoA) comparing communities of the three tree types in the tree community. Colour: mature tree (mature; orange), naturally regenerated seedling (nseedling; yellow), planted seedling (pseedling; blue). Uses untransformed rarefied community abundance data filtered to the dominant

genera (Table 6) and a Bray-Curtis distance metric. Percentages are the proportion of variation in community composition explained by each axis.

Table 7. Results from betadisper, Adonis and ANOSIM tests comparing communities of the three tree types in the tree community. betadisper tests assumption that multivariate variance (dispersion) is equal amongst groups. Adonis and ANOSIM are methods for testing significance of differences in community composition. Tests performed on a Bray-Curtis distance matrix created from untransformed rarefied community abundance data filtered to the dominant genera (Table 6).

Betadisper						
	df	**Sums of Squares**	**Mean Squares**	**F value**	**Pr(>F)**	
tree_type	2	0.008	0.004	0.179	0.837	
Residuals	46	1.067	0.023	-	-	
Adonis						
	df	**Sums of Squares**	**Mean Squares**	**F value**	**R2**	**Pr(>F)**
tree_type	2	0.961	0.4804	1.878	0.076	**0.02**
Residuals	46	11.766	0.2558	-	0.925	-
Total	48	12.727	-	-	1.000	-
ANOSIM						
	R Statistic	**Significance**				
Results	0.059	0.219				

3.3.2. Soil communities

3.3.2.a. Alpha diversity

The soil community contained 366 ASVs falling into 87 species and 39 genera in 28 families. After rarefying, 86 species and 38 genera in 27 families remained. Alpha diversity at ASV and species levels expressed as observed richness and Shannon's diversity index differed between tree types (Table 8 and 9; Figure 8). The soil communities of mature trees and naturally regenerated seedlings did not differ from each other, but both had significantly greater diversity than the planted seedling soil community (Table 10).

Table 8. Mean per sample observed richness by tree type (# of ASVs or # of species) in soil community. Two levels included: # of ASVs or # of species.

Soil Community			
Tree Type	**ASV**	**Species**	**Sample Size (n)**
Mature Tree	27.357	15.714	28
Natural Seedling	26.500	14.917	12
Planted Seedling	7.600	4.800	5

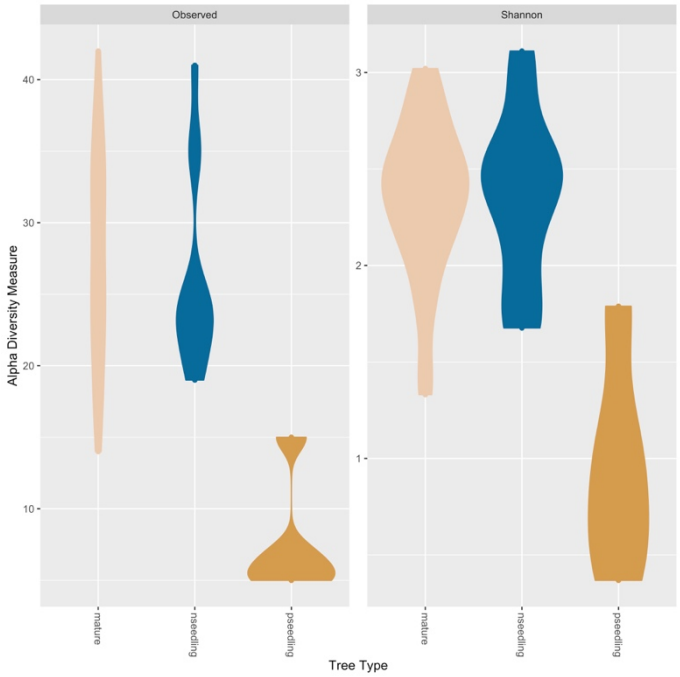

Figure 8. Comparison of observed richness (left) and Shannon's diversity index (right) among tree type in soil community. Colours: mature trees (mature; beige), naturally regenerated seedlings (nseedling; blue) and planted seedlings (pseedling; brown). Violin plots show spread of data; the width of the object is proportional to the frequency of observations at that richness value.

Table 9. Type III ANOVA table of test for differences in α-diversity between tree types in the soil community. Test used a simple linear model with a single factor (Tree Type). Separate tests were performed at ASV and species levels for observed richness and Shannon's diversity index.

Factor/Metric	Sum Sq	Df	F value	Pr(>F)
ASV Level				
Observed richness				
Tree Type	1696.171	2	16.844	**4.25E-06**
Residuals	2114.629	42	NA	NA
Shannon's diversity index				
Tree Type	8.728	2	23.241	**1.60E-07**
Residuals	7.886	42	NA	NA
Species Level				
Observed richness				
Tree Type	511.813	2	12.306	**6.22E-05**
Residuals	873.431	42	NA	NA
Shannon's diversity index				
Tree Type	5.270	2	15.338	**9.98E-06**
Residuals	7.215	42	NA	NA

Table 10. Pairwise t-tests for differences in alpha diversity between tree types in the soil community. Test uses a simple linear model with a single factor (Tree Type). Tree types: mature tree (MT), natural seedling (NS) and planted seedling (PS). p-values are adjusted with the Bonferroni correction to account for number of pairs.

Contrast	Estimate	SE	df	t-ratio	p-value
ASV Level					
MT-NS	-0.006	0.150	42	-0.042	1.000
MT-PS	1.399	0.210	42	6.652	**<.0001**
NS-PS	1.406	0.231	42	0.231	**<.0001**
Species Level					
MT-NS	0.103	0.143	42	0.720	1.000
MT-PS	1.111	0.201	42	5.519	**<.0001**
NS-PS	1.008	0.221	42	4.567	**0.0001**

3.3.2.b. Community Composition

The mature tree soil community contained 72 species and 34 genera in 24 families. Rarefying reduced the community to 71 species and 33 genera in 23 families. The mean per sample richness was 27 ASVs and 16 species (Table 8). The dominant families were: Helotiaceae (RA=0.292; dominant genera: *Meliniomyces* (RA=0.289)), Atheliaceae (RA=0.211; dominant genera: *Piloderma* (RA=0.156), *Amphinema* (RA=0.024), *Tylospora* (RA=0.031)), Leotiaceae (RA=0.102; dominant genera: *Pezoloma* (RA=0.102)), Russulaceae (RA=0.091; dominant genera: *Russula* (RA=0.031), *Lactarius* (RA=0.060)), Cortinariaceae (RA=0.088; dominant genera: *Cortinarius* (RA=0.088)), Gloniaceae (RA=0.060; dominant genera: *Cenococcum* (RA=0.060)), Lyophyllaceae (RA=0.031; dominant genera: *Lyophyllum* (RA=0.031)) and Rhizopogonaceae (RA=0.030; dominant genera: *Rhizopogon* (RA=0.30)) (Table 11; Figure 9).

The naturally regenerated seedling soil community held 42 species and 25 genera in 19 families. Rarefying did not remove any taxa. The mean per sample richness was 27 ASVs and 15 species (Table 8). The most abundant genera grouped into nine families: Leotiaceae (RA=0.222; dominant genera: *Pezoloma* (RA=0.222)), Helotiaceae (RA=0.183; dominant genera: *Meliniomyces* (RA=0.176)), Cortinariaceae (RA=0.151; dominant genera: *Cortinarius* (RA=0.151)), Atheliaceae (RA=0.211; dominant genera: *Piloderma* (RA=0.096)), Gloniaceae (RA=0.081; dominant genera: *Cenococcum* (RA=0.081)), Suillaceae (RA=0.080; dominant genera: *Suillus* (RA=0.080)), Russulaceae (RA=0.074; dominant genera:

Russula (RA=0.055)), Hydnaceae (RA=0.031; dominant genera: *Hydnum* (RA=0.031) and Inocybaceae (RA=0.021; dominant genera: *Inocybe* (RA=0.021)) (Table 11; Figure 9).

The planted seedling soil community contained 9 species and 14 genera in 11 families. After rarefying, the community consisted of 7 species, 11 genera and 8 families. The mean per sample richness was 8 ASVs and 5 species (Table 8). The major families were: Helotiaceae (RA=0.587; dominant genera: *Calyptrozyma* (RA=0.448), *Rhizocyphus* (RA=0.124)), Leotiaceae (RA=0.318; dominant genera: *Pezoloma* (RA=0.318)) and Atheliaceae (RA=0.051; dominant genera: *Amphinema* (RA=0.0511)) (Table 11; Figure 9).

Table 9. Ectomycorrhizal fungal taxa (genera or species) of soil community. Taxa identified to from soil samples taken adjacent to mature trees (MT), naturally regenerated seedlings (NS) and planted seedlings (PS) of whitebark pine. Counts are the number of samples a taxa was identified in. Sample size (N): MT=29, NS=12, PS=12.

Taxa	# of Samples			
	Total	MT	NS	PS
Ascomycota				
Dothideomycetes				
Mytilinidales				
Gloniaceae				
Cenococcum_unidentified	**38**	27	10	1
Eurotiomycetes				
Eurotiales				
Elaphomycetaceae				
Elaphomyces_asperulus	**3**	2	1	0
Leotiomycetes				
Helotiales				
Helotiaceae				
Calyptrozyma_unidentified	**17**	2	6	9
Hymenoscyphus_tetracladius	**3**	3	0	0
Meliniomyces_bicolor	**14**	9	5	0
Meliniomyces_variabilis	**38**	27	11	0
Meliniomyces_unidentified	**45**	28	11	6
Mycosymbioces_mycenaphila	**2**	2	0	0
Mycosymbioces_unidentified	**17**	12	3	2
Rhizoscyphus_monotropae	**2**	0	0	2
Helotiales_fam_Incertae_sedis				
Acephala_unidentified	**2**	0	0	2
Leotiaceae				
Pezoloma_ciliifera	**5**	0	1	4
Pezoloma_ericae	**50**	28	12	10
Pezizomycetes				
Pezizales				
Discinaceae				
Hydnotrya_unidentified	**15**	11	4	0
Hydnotrya_michaelis	**2**	2	0	0
Pezizaceae				
Chromelosporium_carneum	**1**	0	0	1
Pyronemataceae				
Wilcoxina_unidentified	**14**	8	6	0
Wilcoxina_rehmii	**3**	3	0	0
Basidiomycota				
Agaricomycetes				

Taxa	# of Samples			
	Total	MT	NS	PS
Agaricales				
Cortinariaceae				
Cortinarius_unidentified	**22**	16	5	1
Cortinarius_acutus	**2**	2	0	0
Cortinarius_alboamarescens	**2**	0	2	0
Cortinarius_aureovelatus	**2**	1	1	0
Cortinarius_barlowensis	**1**	1	0	0
Cortinarius_biformis	**1**	1	0	0
Cortinarius_boulderensis	**1**	1	0	0
Cortinarius_brunneus	**4**	2	2	0
Cortinarius_caesiobrunneus	**5**	5	0	0
Cortinarius_caperatus	**8**	3	5	0
Cortinarius_causticus	**1**	0	1	0
Cortinarius_cinnamomeus	**1**	1	0	0
Cortinarius_clarobrunneus	**1**	0	1	0
Cortinarius_colus	**3**	2	1	0
Cortinarius_colymbadinus	**4**	3	1	0
Cortinarius_diasemospermus	**3**	1	2	0
Cortinarius_dolabratus	**3**	3	0	0
Cortinarius_grosmorneensis	**2**	2	0	0
Cortinarius_lux-nymphae	**1**	1	0	0
Cortinarius_mattiae	**1**	0	1	0
Cortinarius_multiformis	**1**	1	0	0
Cortinarius_nauseosouraceus	**1**	1	0	0
Cortinarius_ochrophyllus	**2**	2	0	0
Cortinarius_pluvius	**1**	0	1	0
Cortinarius_privignatus	**2**	1	1	0
Cortinarius_privignus	**3**	3	0	0
Cortinarius_renidens	**1**	0	1	0
Cortinarius_scaurus	**1**	1	0	0
Cortinarius_semisanguineus	**8**	8	0	0
Cortinarius_semivestitus	**1**	0	0	1
Cortinarius_testaceofolius	**1**	1	0	0
Cortinarius_uraceus	**2**	2	0	0
Entolomataceae				
Entoloma_cetratum	**2**	0	0	2
Hydnangiaceae				
Laccaria_unidentified	**4**	3	1	0
Hygrophoraceae				
Hygrophorus_camarophyllus	**1**	1	0	0
Hygrophorus_purpurascens	**4**	3	1	0
Inocybaceae				
Inocybe_impexa	**1**	0	1	0
Inocybe_soluta	**5**	4	1	0
Inocybe_teraturgus	**2**	1	1	0
Lyophyllaceae				
Lyophyllum_shimeji	**2**	2	0	0
Tricholomataceae				
Tricholoma_equestre	**1**	1	0	0
Tricholoma_saponaceum	**3**	3	0	0
Tricholoma_vaccinum	**1**	1	0	0
Atheliales				
Atheliaceae				
Amphinema_byssoides	**1**	1	0	0
Amphinema_unidentified	**25**	17	7	1
Piloderma_bicolor	**27**	21	6	0
Piloderma_byssinum	**3**	2	1	0
Piloderma_sphaerosporum	**25**	18	7	0
Piloderma_unidentified	**6**	5	1	0
Tylospora_fibrillosa	**17**	14	3	0
Boletales				
Boletaceae				
Boletus_edulis	**1**	1	0	0
Gomphidiaceae				

Taxa	# of Samples			
	Total	MT	NS	PS
Chroogomphus_filiformis	**11**	6	5	0
Rhizopogonaceae				
Rhizopogon_unidentified	**22**	17	5	0
Rhizopogon_bacillisporus	**12**	7	4	1
Rhizopogon_brunneiniger	**4**	3	1	0
Rhizopogon_salebrosus	**6**	6	0	0
Suillaceae				
Suillus_acidus	**2**	2	0	0
Suillus_brevipes	**2**	2	0	0
Suillus_punctatipes	**6**	3	3	0
Suillus_unidentified	**15**	9	6	0
Cantharellales				
Cantharellales_fam_Incertae_sedis				
Sistotrema_adnatum	**2**	2	0	0
Sistotrema_autumnale	**3**	3	0	0
Sistotrema_sernanderi	**1**	0	0	1
Sistotrema_unidentified	**7**	5	2	0
Clavulinaceae				
Clavulina_cinerea	**3**	1	2	0
Hydnaceae				
Hydnum_unidentified	**2**	0	2	0
Gomphales				
Gautieriaceae				
Gautieria_unidentified	**2**	2	0	0
Ramaria_unidentified	**2**	2	0	0
Russulales				
Albatrellaceae				
Albatrellus_flettii	**2**	2	0	0
Leucogaster_nudus	**1**	1	0	0
Leucophleps_spinispora	**2**	2	0	0
Russulaceae				
Lactarius_caespitosus	**4**	1	3	0
Lactarius_rufus	**19**	14	5	0
Russula_adusta	**3**	0	3	0
Russula_aeruginea	**1**	1	0	0
Russula_fragilis	**2**	2	0	0
Russula_griseascens	**13**	10	3	0
Russula_paludosa	**3**	3	0	0
Russula_turci	**4**	2	2	0
Russula_vesca	**3**	3	0	0
Russula_xerampelina	**5**	3	1	1
Thelephorales				
Thelephoraceae				
Pseudotomentella_unidentified	**8**	6	2	0
Pseudotomentella_mucidula	**15**	12	3	0
Pseudotomentella_nigra	**6**	5	1	0
Tomentella_badia	**1**	0	1	0
Tomentella_sublilacina	**10**	6	4	0
Mucoromycota				
Endogonomycetes				
Endogonales				
Endogonaceae				
Endogone_lactiflua	**1**	1	0	0

Table 11. Dominant ectomycorrhizal genera in soil community by tree type. Taxa make up 90% of the total abundance in a given tree type. Relative abundance is the mean relative abundance of each genera across samples. # samples and % samples are presence/absence data. Sample size (n) applies only to presence absence data (unrarefied dataset).

	Abundance Metric		
Soil Community/Taxa	**RA**	**# samples**	**% samples**
Mature Tree Soil (n=29)			
Meliniomyces	0.289	28	96.6
Piloderma	0.156	24	82.8
Pezoloma	0.102	28	96.6
Cortinarius	0.088	23	79.3
Lactarius	0.060	14	48.3
Cenococcum	0.060	27	93.1
Tylospora	0.031	14	48.3
Russula	0.031	19	65.5
Lyophyllum	0.031	2	6.9
Rhizopogon	0.030	25	86.2
Amphinema	0.024	17	58.6
Natural Seedling Soil (n=12)			
Pezoloma	0.222	12	100.0
Meliniomyces	0.176	12	100.0
Cortinarius	0.151	9	75.0
Piloderma	0.096	9	75.0
Cenococcum	0.081	10	83.3
Suillus	0.080	7	58.3
Russula	0.055	6	50.0
Hydnum	0.031	2	16.7
Inocybe	0.021	2	16.7
Planted Seedling Soil (n=12)			
Calyptrozyma	0.448	9	75.0
Pezoloma	0.318	11	91.7
Rhizoscyphus	0.124	2	16.7
Amphinema	0.051	1	8.3

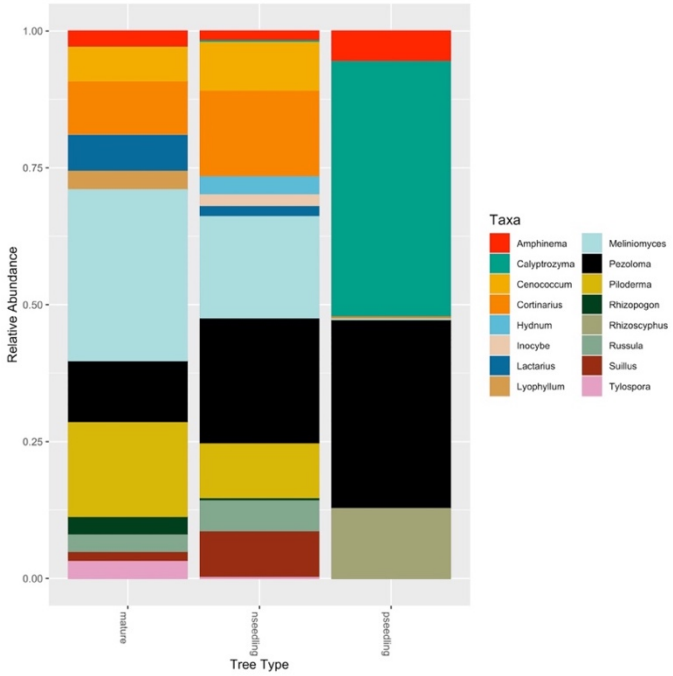

Figure 9. Relative abundance of the dominant genera (Table 11) in the soil community separated by tree type. Relative abundance measured as proportion reads in a given tree type. Position: mature trees (mature; left), natural seedlings (nseedling; middle) and planted seedlings (pseedling; right).

3.3.2.c. Beta diversity

Similar to the soil community, beta diversity was estimated with Bray-Curtis dissimilarity on untransformed rarified community abundance data filtered to ASVs in the most abundant genera (Table 11). PCoA explained 43.9% of the variation in this distance matrix in the first two axes and showed clear visual separation of the planted seedling community from the communities associated with mature trees and naturally regenerated seedlings (Figure 10). Statistical testing confirmed this result. Multivariate dispersion was not significantly different between groups (F=1.433, p=0.250) (betadisper in Table 11). Both Adonis and ANOSIM tests for compositional differences were significant (Adonis: F=5.743, R^2=0.215, p=0.001; ANOSIM: R=0.4311, p=0.001) (Table 11).

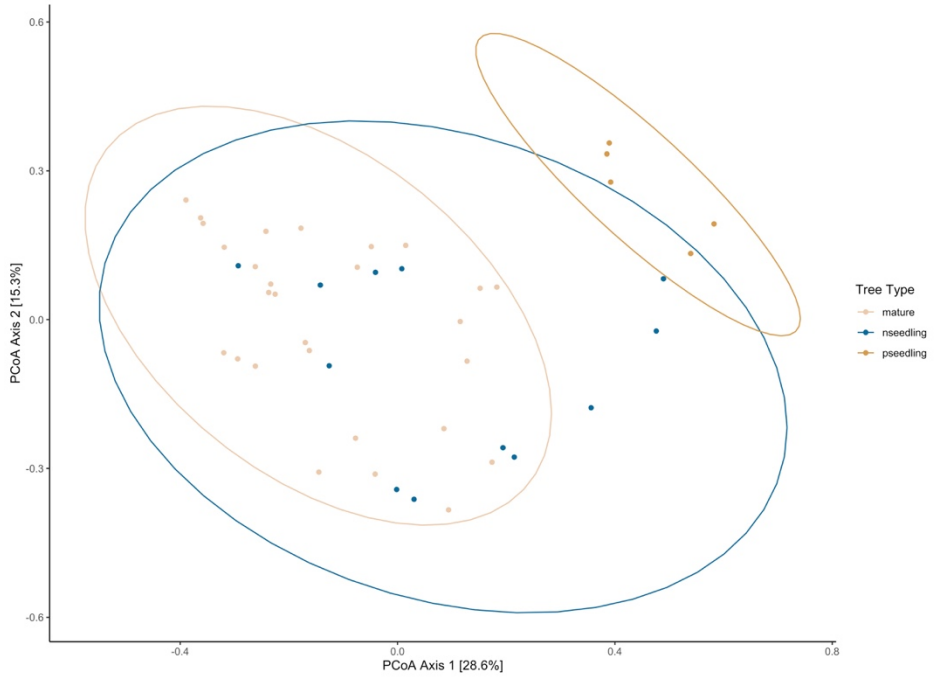

Figure 10. Principal coordinate analysis (PCoA) comparing communities of the three tree types in the soil community. Colour: mature trees (mature; beige), naturally regenerated seedlings (nseedling; blue) and planted seedlings (pseedling; brown). Uses untransformed rarefied community abundance data filtered to the dominant genera (Table 11) and a Bray-Curtis distance metric. Percentages are the proportion of variation in community composition explained by each axis.

Table 12. Results from betadisper, Adonis and ANOSIM tests comparing communities of the three tree types in the soil community. betadisper tests assumption that multivariate variance (dispersion) is equal amongst groups. Adonis and ANOSIM are methods for testing significance of differences in community composition. Tests performed on a Bray-Curtis distance matrix created from untransformed rarefied community abundance data filtered to the dominant genera (Table 11).

Betadisper						
	df	**Sums of Squares**	**Mean Squares**	**F value**	**Pr(>F)**	
tree_type	2	0.034	0.017	1.433	0.250	
Residuals	42	0.494	0.012	-	-	
Adonis						
	df	**Sums of Squares**	**Mean Squares**	**F value**	**R2**	**Pr(>F)**
tree_type	2	2.406	1.203	5.743	0.215	**0.001**
Residuals	42	8.798	0.2095	-	0.785	-
Total	44	11.204	-	-	1.000	-
Anosim						
	R Statistic	**Significance**				
Results	0.4311	**0.001**				

Chapter 4: Discussion

4.1. The ectomycorrhizal fungi of whitebark pine

The ectomycorrhizal fungi associating with WBP are diverse. A total of fifty-nine species and thirty-one genera were identified at some abundance in the tree community. Of these taxa, only a subset warrant confident designation as confirmed associates. Some taxa are identified in only a few samples and some cannot be sufficiently resolved to the level of species using the ITS2 region alone (Table 5; Supplementary Table 1). Taxonomically the taxa in the tree community fall into three phyla – the Ascomycota, Basidiomycota and Mucoromycota. These phyla are discussed below.

4.1.1. Ascomycota

The Ascomycota can be broken into three distinct groupings. *Cenococcum* is a widespread generalist species that is well known with WBP (Cripps & Antibus, 2011; Mohatt et al., 2008); the single *Cenococcum* ASV identified here almost certainly is *C. geophilum* Fr.. *Wilcoxina* species are generalist E-strain fungi that have similarly been identified with WBP (Trusty & Cripps, 2011). The novel taxa identified here are those in the Helotiales. These ascomycetous associates display complicated root associations that are not limited to ectomycorrhizal habits (see below) (Tedersoo et al., 2009). These taxa were abundant in the samples, but the debate over the nature of the root associations they form makes it impossible to define them as ectomycorrhizal.

4.1.2. Basidiomycota

The Basidiomycota, unsurprisingly, contained the majority of the taxa identified in the tree community. Five major orders contained taxa that are confidently described as ectomycorrhizal associates or those that warrant discussion: Agaricales, Atheliales, Boletales, Russuales and Theleporales. In the Agaricales, *Cortinarius* was dominant. Most of the ASVs in *Cortinarius* classified to the level of species were found in only a few (1-3) samples; only *C. caperatus* (6 samples) and *C.*

semisanguineus (5 samples) appeared repeatedly (Table 5). *Cortinarius* species often require both the ITS and RPB2 regions to be resolved (C. Cripps, personal communication, 30 July 2019). Taxa identified in only a few samples should be taken cautiously. The genus has been identified repeatedly with WBP (Cripps & Antibus, 2011; Mohatt et al., 2008) but none of the previously identified species were recovered here. *Cortinarius* is undoubtedly an important associate to the WBP communities at our study sites but confirmation of species is difficult. *Inocybe*, *Tricholoma* and *Hygrophorus* are all genera identified at low abundances that have been confirmed with WBP previously (Cripps & Antibus, 2011; Mohatt et al., 2008). Previous identification supports describing them as true associates. *Hygrophorus,* however, warrants further investigation. The nutritional modes of this genus are varied and do not always align with an ectomycorrhizal habit (Seitzman, Ouimette, Mixon, Hobbie, & Hibbett, 2011). Notably missing from the Agaricales taxa that have been identified with WBP previously are *Amanita, Dermocybe,* and *Leucopaxillus* (Cripps & Antibus, 2011; Mohatt et al., 2008) .

The Atheliales included three important genera: *Amphinema, Piloderma* and *Tylospora*. *Amphinema* and *Piloderma* both included taxa recorded with WBP previously (*A. byssoides* (Pers.) J. Erikss. and *P. byssinum* (P. Karst.) Jülich) (Cripps & Antibus, 2011; C. Cripps, personal communication, 30 July 2019). Two new species in *Piloderma* were identified as associates – *P. bicolor* (Peck.) Jülich and *P. sphaerosporum* Jülich. *Tylospora* was represented by a single species (*T. fibrillose* (Burt) Donk) and has not been reported previously on WBP.

The Boletales contains the pine or WBP specific Suilloid group. Here this group was dominated by *Rhizopogon* (*R. bacillisporus* A.H. Sm., *R. brunneiniger* A.H. Sm. and *R. salebrosus* A.H. Sm.) and *Suillus* (*S. acidus* (Peck) Singer, *S. brevipes* (Peck) Kuntze and *S. punctatipes* (Snell & E.A. Dick) Singer). None of the species identified here have been identified with WBP previously (Cripps & Antibus, 2011; Mohatt et al., 2008), but the pattern of the Suilloid group making up an important component of the WBP community holds. None of the other Suilloid genera previously identified with WBP (*Boletus edulis* Bull.

and *Chroogomphus* sp.) were identified in the tree community here (though both were found in the soil community; Table 9).

Russuales was made up of taxa in the *Lactarius* and *Russula* genera*;* both genera have been identified with WBP (Cripps & Antibus, 2011; Mohatt et al., 2008). Neither of the *Lactarius* species identified here (*L. caespitosus* Hesler & A.H. Sm. and *L. rufus* (Scop.) Fr.) had been confirmed as WBP associates previously but both species were collected in WBP stands and identified as putative associates in Barge & Cripps (2016). *Russula* presented as a similar situation to *Cortinarius;* most species appeared in only a few samples, limiting the number that can be confirmed as associates. *R. griseascens* (Bon & Gaugué) Marti and *R. turci* Bres. were the only species appearing in more than three samples and are considered tentative confirmations.

Theleporales is the last significant order in Basidiomycota and contained two genera: *Pseudotomentella* and *Tomentella*. *P. nigra* (Höhn & Litsch.) Svrček. appeared in only one sample but has been identified with WBP before (Cripps & Antibus, 2011; Mohatt et al., 2008). *P. mucidula* (P. Karst.) Svrček is a new associate but only appeared in two samples. *Tomentella* has only been identified with WBP to the level of genus previously; here, the genus resolved consistently as *T. sublilacina* (Ellis & Holw.) Wakef..

4.1.3. Mucoromycota

A single genus (*Endogone*) was identified in the phylum Mucoromycota. No taxa from this phylum have been identified with WBP before. The genus appeared in a reasonable number of samples and has been shown to associate with pines before (e.g. Fassi, Fontana, & Trappe, 1969) indicating it is likely a true associate.

4.1.4. Putting it together: taxonomically and ecologically

The taxonomic discussion above can be organized into a simpler description of the fungal community based on abundance. The Atheiales is a dominant order typified by *Piloderma, Amphinema*

and *Tylospora*. The Helotiales is equally important and is made up largely of two genera – *Meliniomyces* and *Pezoloma*. Suilloids (*Suillus* and *Rhizopogon*) and the Agaricales (mostly *Cortinarius*) occur regularly at medium abundances. *Cenococcum* accounts for the majority of the non-Helotian ascomycetous community and also occurs at intermediate levels. The remaining taxa occur rarely or mostly on planted seedlings.

This community can also be organized ecologically. Cripps & Antibus (2011) developed ecological divisions for the WBP community that included: (1) generalists, (2) high elevation conifer associates and (3) pine or five-needled pine specialists. Given the overlap of taxa identified here with the community they described, the same divisions can be used. The taxa falling into the generalist category include: *Cenococcum, Wilcoxina, Amphinema, Piloderma, Pseudotomentalla* and *Tomentella.* High elevation conifer associates include: *Cortinarius, Russula, Lactarius, Tricholoma, Hygrophorus* and *Tylospora*. The pine specialist group is restricted to *Suillus* and *Rhizopogon*.

These ecological groupings do however miss an important clade identified here – the Helotiales. This group warrants the creation of a fourth ecological division as "secondary ascomycete associates". The ecologies of these fungi are poorly understood. The group contains saprophytic, root endophytic, ericoid mycorrhizal and ectomycorrhizal taxa that often co-colonize root tips already colonized by true ectomycorrhizal taxa (Tedersoo et al., 2009). Their abundance here demonstrates they are a consistent component of the WBP rhizosphere and likely have significant ecological roles. Importantly, the Helotiales have been linked to pine-beetle effected stands, signaling they may be indicators of forests in poor health (see section 4.4.2; Karst et al., 2015).

4.1.5. The soil ectomycorrhizal community of whitebark pine stands

A total of eighty-seven species and thirty-nine genera of ectomycorrhizal fungi were identified at some abundance in the soil community. These figures should be regarded as overestimates; like in the tree community, numerous taxa appeared in only a few samples and were not sufficiently identified by

the ITS2 region alone. The soil community matches the tree community almost exactly. Only seven taxa that were present in tree community were not present in the soil community and most (4) of these taxa were *Cortinarius* species that were low in abundance and lack confident classification at the species level. This congruence between the root tips and soil community is notable but should also be expected because soil samples were taken directly adjacent to the fine root samples. The soil community should not be understood as a representative sample of the soil community in the sub-alpine forests of the study region, but rather the community in the soils directly adjacent to WBP trees; this is the soil community experienced by WBP fine roots.

The majority of the taxa in the soil community that were not present in the tree community occurred at low abundance and were scattered throughout the orders or genera present in the tree community (e.g. *Cortinarius* species). Three taxa stand out as needing attention: *Tricholoma vaccinium* (Schaeff.) P. Kumm.*, Boletus edulis* Bull. and *Chroogomphus*. These taxa have been shown to associate with WBP previously (Cripps & Antibus, 2011; Mohatt et al., 2008; C. Cripps personal communication, 30 July 2019) and are likely to do so in this system as well.

4.2. Weighing up: comparison with previously described communities and other high-elevation North American pines

Thirty-four species or genera were identified as ectomycorrhizal WBP associates with high confidence (Supplentary Table 1; bold in Table 5). This figure excludes the speciose Helotian group whose ectomycorrhizal status is debated. It also excludes taxa that were low in abundance or poorly resolved at the species level; these taxa may be contaminants, misidentified or true associates (e.g. *Cortinarius* and *Russula* species). These exclusions mean the actual figure is likely higher. This level of richness compares well with previous descriptions of the WBP ectomycorrhizal fungal community (Cripps & Antibus, 2011; Mohatt et al., 2008; Trusty & Cripps, 2011; unpublished records) that have collectively identified thirty-five to forty species or genera. Relative to other high elevation pines, this

richness is high. Bidartondo, Baar & Bruns (2001) documented only ten species with bristlecone pine (*P. longaeva*). Cripps & Antibus (2011) recorded twenty-six species as putative associates of limber pine (*P. flexilis*). A recent study comparing the ability of *P. longaeva* and *P. flexilis* to shift their range upslope in response to climate change recorded only fifteen ectomycorrhizal species in bioassay (Shemesh, Boaz, Millar, & Bruns, 2019). Of the thirty-four taxa confidently identified here, twenty-one species and two genera are new reports with WBP. This brings the total number of species or genera identified with WBP to twenty-two genera and fifty to fifty-five species.

The taxonomic and ecological descriptions detailed above show three major ways that the WBP ectomycorrhizal fungal community described here diverges from that described in the Greater Yellowstone Ecosystem (Cripps & Antibus, 2011; Mohatt et al., 2008). The first two distinctions are taxonomic. At higher levels (order), the two communities are quite similar, but at levels below genus the communities diverge. The most striking example of this distinction is in the Suilloid group. The group is well represented in both communities but there are no overlapping species. This is surprising because there are species in this group identified in the Greater Yellowstone Ecosystem (e.g. *S. sibricus*, *S. discolor, S. plorans, S. placidus*) that are restricted to five-needled pines and have a Holarctic distributions, occurring with these hosts in North America, Europe and Asia (Cripps & Antibus, 2011); this species do not appear dispersal limited yet none of these five-needle specialists were identified here. The same pattern is observed in other orders as well: Atheiales, Argaricales and Russuales (though *Cortinarius* and *Russula* are complicated by likely not being sufficiently resolved with the ITS2 region alone). What drives this divergence is unclear; however, the Greater Yellowstone Ecosystem differs substantially biophysically from the region encompassed by Mount Revelstoke and Glacier National parks. Mixed WBP forests including subalpine fir and Engelmann spruce in a rainforest climate could drive differences in the fungal assemblages and ecological organization compared with the Greater Yellowstone Ecosystem.

The second taxonomic distinction is the Helotiales group. The identification of this group here is likely an artifact of the molecular approach used to identify the community. Pooling root tips by tree and amplifying extracted DNA using a universal fungal primer recovers all fungal taxa associated with the root samples. Secondary associates, like many of those in the Helotiales, are co-amplified and are often misreported as ectomycorrhizal (Tedersoo et al., 2009).

The third distinction is a marked shift in the dominant ecological guilds. The communities of Mohatt et al. (2008) and Cripps & Antibus (2011) were dominated by Suilloids (*Suillus, Rhizopogon* and *Chroogomphus*), *Cortinarius* species and *Cenococcum.* The communities identified here tend towards the generalist guild of wood decomposing fungi in the Atheliales (*Piloderma, Amphinema* and *Tylospora*). *Piloderma* is especially dominant and was the most abundant confirmed ectomycorrhizal taxa in both mature trees and natural seedlings. Two reasonable explanations for this shift are suggested here. The detection of the dominance of this group could be a function of methodology. All three of these genera are resupinate; previous descriptions have relied heavily on collections of sporocarps, and the sequencing of root tips employed here would better represent these resupinate taxa. The method we used to sample fine root tips could also explain this pattern; sampling was biased towards upper soil horizons and roots close to the bole of the tree with high organic content where wood decomposing fungi are expected.

The shift could also reflect the different forest types between this study system and the Greater Yellowstone Ecosystem. The Atheliales have been shown previously to be a dominant component of the soil ectomycorrhizal community in the Engelmann spruce-subalpine fir biogeoclimatic zone (Walker, Phillips, & Jones, 2014). The soil in the sites where sampling of mature trees and natural seedlings occurred had high organic matter content and regularly contained woody litter (unpublished data not reported here). *Piloderma* has been shown to have the ability to access organic nitrogen forms and pass

them to an ectomycorrhizal partner (Heinonsalo et al., 2015); associations with taxa capable of obtaining nutrients from organic pools may be an advantage in organic soils like those studied here.

These distinctions indicate the WBP ectomycorrhizal fungal community of Mount Revelstoke and Glacier National parks is unique from the community identified in the Greater Yellowstone Ecosystem and adapted to the region's forest type. This adaptation likely carries ecological implications; a shift to a more generalist community with ectomycorrhizal fungi shared with other tree species (Engelmann spruce and subalpine fir) could facilitate interspecific mycorrhizal networks (Simard et al., 2012) but could also signal the successional replacement of WBP by later successional species (Keane et al., 2012).

4.3. Comparing tree types

The three tree types represent naturally occurring mature WBP, naturally regenerated seedlings distributed in the mature stands and planted seedlings from a site representative of active recovery efforts. Interpreting differences between these samples has important implications for WBP ecology and recovery efforts.

In the tree community, tests for differences in diversity and composition revealed no or variable differences between the tree types; there was, however, a trend of decreasing diversity from mature trees to naturally regenerated seedlings to planted seedlings. Moreover, qualitative compositional differences show distinct communities formed on each tree type. Mature trees and naturally regenerated seedlings were diverse, hosting generalist (*Piloderma, Amphinema, Tylospora, Cenococcum*), Helotian (*Meliniomyces)*, Agaricales (C*ortinarius*) and Suilloid (*Rhizopogon, Suillus*) species. The planted seedlings hosted a simpler community of Helotian (*Meliniomyces, Pezeloma*), *Suillus* and *Russula* species. In the soil community there were evident differences in diversity and composition. Soils of mature trees and naturally regenerated seedlings had greater diversity and contained species from all the major ectomycorrhizal clades identified here. The soil community of

planted seedlings contained just four dominant genera: Helotian *Calyptrozyma, Pezeloma* and *Rhizocyphus* and *Amphinema*.

The Helotian taxa disproportionately influence the detection of differences between tree types. Specifically, Helotian taxa tend to drive similarities between sites. The Bray-Curtis dissimilarity metric (Bray & Curtis, 1957) used here to test compositional differences relies on counts of shared taxa between sample groups; widespread generalist taxa shared between tree types in the soil or tree communities will push the metric towards estimates of no compositional difference. The Helotian taxa fulfill these criteria. The contested ectomycorrhizal status and lack of understanding of the ecologies of these taxa questions their inclusion; they may represent non-ectomycorrhizal contaminants (Tedersoo et al., 2009). This issue is further complicated by differences in sample size. The planted seedling soil and fine root tip samples consistently failed to amplify sufficient DNA to pass filtering and rarefying steps and some planted seedlings lacked colonized root tips completely (no sample was taken for these cases). This overstates the taxa identified in the samples that did amplify and fails to incorporate the fact that a failed amplification, or the lack of colonized root tips, is itself a result. If the Helotian taxa are removed from the community and low colonization and amplification rates are accounted for, two distinct communities resolve on natural and planted trees.

Two mechanisms might explain the creation of these two communities. The most obvious is the influence of site. The site where planted seedlings were collected differs from the sampling sites of mature trees and naturally regenerated seedlings in that naturally occurring WBP is absent or rare. In addition, the planted site was burned in a low to moderate severity wildfire in summer 2003 (K. Macauley, Parks Canada, personal communication, 18 March 2020). The influence of wildfire on ectomycorrhizal fungal communities is poorly understood. It is established that fire can reduce species richness, reduce colonization and cause shifts in composition by direct effects on the fungal community and indirect effects on the associated plant community (Dove & Hart, 2017; Taudière, Richard, &

Carcaillet, 2017). Persistent compositional shifts have been shown repeatedly in pine systems post fire (Karst, Randall, & Gehring, 2014). Specific to WBP, Trusty & Cripps (2011) demonstrated distinct ectomycorrhizal fungal communities on seedlings growing on burned and unburned soils irrespective of whether the seedlings occurred naturally or were planted. There was significant fire induced mortality of the coniferous tree community (subalpine fir and Engelmann spruce) at our sampling site that would have hosted a reservoir of ectomycorrhizal fungi. Seedlings were planted in amongst a plant community consisting mostly of ericaceous shrubs and herbs. Inoculum-source trees are important for ectomycorrhizal colonization (Teste, Simard, & Durall, 2009). The lack of source trees is compounded by the lack of naturally occurring WBP at or near the site. These WBP populations would have acted as reservoirs of the pine-specific Suilloid group. Suilloids are wind and animal dispersed and Trusty and Cripps (2011) inferred nearby WBP forests facilitated the colonization of seedlings occurring on a burned site with Suilloid taxa. The combination of fire and lack of source trees (including WBP) is likely a significant contributor to the low species diversity and distinct composition of the soil and tree communities associated with the planted seedlings.

The second mechanism that might drive the formation of distinct ectomycorrhizal communities on natural and planted WBP are innate differences between seedling types. Trusty and Cripps (2011) identify two of these – nursery fungi capable of excluding native fungi and differences in seedling root structure. In addition, intraspecific variation within a host species (i.e. different populations and genetic families of WBP) can influence ectomycorrhizal colonization (Karst, Jones, & Turkington, 2009). The planted seedlings sampled here were grown from seed sourced from populations within the two parks, making intraspecific genetic variation an unlikely driver. Our sampling lacked a control of nursery seedlings, so the influence of nursery fungi cannot be excluded, but there were no dominant taxa exclusive to planted seedlings. Fine root structure did differ substantially between planted and natural seedlings. Planted seedlings produced more root mass, finer roots and retained the shape of the original

planting container. This is the second observation of a lack of root egress in WBP planted seedlings (Trusty & Cripps, 2011). Seedling root structure is a second likely contributor to differences between tree types.

Disentangling the contributions of fire, inoculum sources and innate seedling differences to the formation of distinct ectomycorrhizal communities on natural and planted trees is a challenge. Fire and a lack of inoculum source manifest in reduced inoculum potential in the soil. This follows the pattern observed here, indicating these site differences may be the primary drivers.

4.4. Management implications

4.4.1. Inoculation of planted seedlings

The inoculation of planted WBP seedlings has shown variable results. In greenhouse experiments, inoculation has been shown to increase seedling size and nitrogen content (Jenkins et al., 2018). However, in the one monitored project that inoculated out-planted WBP seedlings, inoculation had no effect on seedling survival (Cripps et al., 2018; Lonergan et al., 2013). Inoculation has been shown to have positive effects in other species and forest systems, most notably in five-needled stone pines in Europe (reviewed in Cripps & Grimme, 2011).

Current management practices recommend inoculation at sites lacking soil inoculum of native WBP ectomycorrhizal fungi; this includes disturbed sites (e.g. severe burns, mountain pine beetle effected sites) and sites where WBP is absent (Cripps et al., 2018). In addition, current practices advocate using Suilloid taxa and locally sourced inoculum (Cripps & Grimme, 2011). Suilloid taxa are preferred because they are important in seedling establishment and are amenable to nursery inoculation. Locally sourced inoculum tends to have the highest success rates, which may be due in part to intraspecific genetic specificity (Cripps & Grimme, 2011; Karst et al., 2009). The candidate Suilloid fungi identified here that warrant investigation as potential inoculant taxa are: *Rhizopogon bacillisporus, R. brunneiniger*, *R. salebrosus*, *Suillus acidus*, *S. brevipes* and *S. punctatipes*. Initiating sporocarp surveys

paired with small inoculation experiments in the next few years to confirm the presence of these taxa and their performance as inoculants would be a useful to developing this management strategy in this portion of the WBP range.

4.4.2. Assessments of stand health

The WBP ectomycorrhizal fungal profile developed here also has the potential to be included in assessments of WBP stand health. Convincing evidence that supports the development of this management strategy comes from studies linking mountain pine beetle (*Dendroctonus ponderosae*) outbreaks to the ectomycorrhizal fungal community of lodgepole pine (*Pinus contorta* Doulg. ex Loud. var. *latifolia* Engelm.). Treu et al. (2014) show the abundance and diversity of ectomycorrhizal fungi is reduced in beetled effected stands. Karst et al. (2015) show that this reduction and compositional shift in ectomycorrhizal fungi has transgenerational effects on the health of regenerating seedlings; seedlings inoculated with fungi from soil collected in beetle-affected stands had lower concentrations of defense compounds (monoterpenes) than seedlings inoculated with fungi from soil collected in undisturbed stands. Notably, seedlings colonized exclusively by Helotian fungi produced the lowest concentration of monoterpenes. This is particularly relevant because Helotian fungi were dominant here. This guild of fungi may be an indicator of forests affected by pathogens.

The ectomycorrhizal community is reduced and shifted by forest pathogens and can perpetuate the negative health effects of these pathogens into successive generations (Karst et al., 2015). These patterns are likely to hold for the pathogens affecting WBP – mountain pine beetle and blister rust.

Integrating ectomycorrhizal fungi into assessments of WBP stand health requires an understanding of the indicator species and diversities that represent healthy and sick stands. The mature trees sampled here are part of a long-term study monitoring the health of WBP stands (Shepherd et al., 2018; Smith et al., 2008; Smith et al., 2013); this health data could be combined with the ectomycorrhizal fungal community data to identify indicator species and diversity thresholds.

Initiating experimental research, like that of Karst et al. (2015), would build upon the limitations inherent to the observational nature of this dataset. More generally, this discussion illustrates a research gap – understanding the relationship between the ectomycorrhizal fungal community, tree health and the pathogens primarily responsible for the decline of WBP, should be a research priority.

With measures in place to characterize healthy and sick ectomycorrhizal communities, ectomycorrhizal fungi could be integrated into assessments through sporocarp surveys or by the application of standardized molecular methods. Sporocarp surveys are limited in that they will miss non-fruiting species, do not necessarily reflect the below ground ectomycorrhizal community (Gardes & Bruns, 1996) and require experts capable of identifying sporocarps. Molecular methods are more costly (though not completely inaccessible) and require staff familiar with genetic data. Regardless of the method, developing this management strategy is warranted and would reinforce the importance of ectomycorrhizal fungi to WBP recovery.

4.4.3. Selection of planting sites

The detection of a distinct community on planted seedlings has clear implications for the selection of planting sites. Planted seedlings lacked ectomycorrhizal fungi or hosted a reduced set of the native fungal community and the soil surrounding them lacked the inoculum to provide that community. This result emphasizes the need to incorporate criteria related to ectomycorrhizal community development into planting site selection; a full set of these criteria are described in Keane et al. (2012) but two particularly important criteria are elaborated here: (1) fire history and (2) inoculum source trees.

Existing management recommendations prescribe sites affected by fire as planting sites, but this prescription needs to consider fire severity and time since fire. Increased fire severity amplifies compositional shifts in ectomycorrhizal communities; there are conflicting results on the effects of severity on abundance and diversity, but numerous reports indicate these may be reduced as well (Dove

& Hart, 2017; Karst et al., 2014). Dove & Hart (2017) performed a meta-analysis that suggests ectomycorrhizal fungal species richness and in-situ colonization can recover from fire on decadal timescales. Compositional shifts can persist for longer periods post fire (Karst et al., 2014; Taudière et al., 2017). Planting site selection should, however, focus on a specific component of the ectomycorrhizal community – the soil spore bank. The soil spore bank is the residual fungal tissues capable of germinating and colonizing planted seedlings and there is evidence that this component can survive high-severity fires (Glassman, Levine, DiRocco, Battles, & Bruns, 2016). However, the viability of this spore bank will decline in the years post fire (Keane et al., 2012). In summary, active out-planting considering the influence of fire history on the ectomycorrhizal community should: (1) select sites with low-moderate severity burn sites, (2) plant within a year of fire occurring and (3) if neither of the first two conditions are met, consider inoculation of seedlings (Keane et al., 2012).

Source trees are the second major consideration in planting site selection. This criterion needs to consider: (1) the type of source tree (WBP or other conifers) and (2) the proximity of source trees. WBP source trees are preferable; these trees will host the full WBP ectomycorrhizal fungal community and critically, the Suilloid taxa that may confer a competitive advantage over other conifers. Including other conifers as potential source trees is relevant in portions of the WBP range that feature mixed forests. The planting site studied here met the habitat requirements for planting, but WBP was rare at the site. Other sources of inoculum need to be considered in these contexts. Planting near live or recently killed subalpine fir or Engelmann spruce would still provide seedlings access to high-elevation conifer associates and generalist taxa.

Planting seedlings in close proximity to source trees will provide better access to the ectomycorrhizal community of the source tree. Teste et al. (2009) show that even over small spatial scales (0.5-5m from source) proximity can determine the richness and composition of the ectomycorrhizal community on planted seedlings. In contexts where no source trees are available at the

planting site (e.g. stand replacing burns), proximity of nearby WBP stands should be considered. Suilloid taxa from adjacent forests are capable of dispersing and colonizing seedlings on burn sites (Trusty & Cripps, 2011). Out-planting incorporating these source tree effects on ectomycorrhizal community development should: (1) select sites with WBP, (2) if WBP is absent, select sites with other conifer associates, (3) plant seedlings in close proximity to source trees, (4) if source trees are completely absent, select sites near existing WBP stands and (5) consider inoculation when colonization from source trees is unlikely.

Using site indicators like fire history and source trees is an indirect measure of the status of the ectomycorrhizal fungal community. An alternative approach is the development and integration of direct assessments of the community into site selection protocols. This builds upon the recommendation to integrate ectomycorrhizal fungi into assessments of stand health (see section 4.4.2.). Sporocarp surveys or molecular assessments of soil samples would provide working profiles to compare between planting sites. These assessments would benefit from a better understanding of the ectomycorrhizal fungal communities associated with healthy and sick stands (discussed section 4.4.2.) but even comparison against the existing community descriptions developed here and in the Greater Yellowstone Ecosystem (Cripps & Antibus, 2011; Mohatt et al., 2008) would be useful.

Active out-planting of putatively blister rust resistant seedlings is a core component of the WBP recovery strategy (Keane et al., 2012) . Better integrating site selection criteria that focus on ectomycorrhizal fungi is possible and much needed.

Chapter 5: Conclusion

Whitebark pine is an obligate ectomycorrhizal mutualist – this dependence on the mycorrhizal symbiose reiterates the importance of understanding the ectomycorrhizal fungal community with which it associates. This community is a critical component in the recovery of the tree species from its endangered status. This work makes contributions in species description and discovery, ectomycorrhizal ecology and applied management. The major fungal lineages identified in previous descriptive work (Cripps & Antibus, 2011; Mohatt et al., 2008) were confirmed at our sites: generalist ascomycetes (*Cenococcum, Wilcoxina*), Agaricales (*Cortinarius, Inocybe, Tricholoma, Hygrophorus*), Atheliales (*Piloderma, Amphinema, Tylospora*), Suilloids (*Suillus, Rhizopogon*), Russuales (*Lactarius, Russula*) and Theleporales (*Pseudotomentella, Tomentella*). Twenty-one new species and two new genera are added to the list of confirmed WBP ectomycorrhizal associates, mostly scattered within the dominant lineages. In addition, the ascomycetous Helotiales was identified as a significant component of the WBP root community, though the ectomycorrhizal status of this group is unknown. Relative to previously described communities, a major shift in the dominant ecological fungal guilds from the pine-specific Suilloid group to generalist species capable of associating with multiple hosts was detected. This shift aligns with the mixed coniferous species nature of the sites sampled here and may signal the successional replacement of WBP.

This work provides a list of WBP ectomycorrhizal fungal taxa that could be used in applied management as indicator species in the selection of planting sites or the assessment of stand health and in the inoculation of planted seedlings. In addition, this work clearly distinguished the ectomycorrhizal fungal communities occurring on planted seedling from those on naturally regenerated trees. Planted seedlings had lower colonization, diversity and largely lacked native ectomycorrhizal taxa. This emphasizes the importance of planting site selection criteria that incorporate the factors that support ectomycorrhizal community development.

The belowground fungal community is a central and important component of WBP ecology. This work contributes to describing and understanding this community.

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Supplementary Material

Supplementary Table 1. Confidence assessment of ectomycorrhizal taxa in the tree community. Confidence ratings are qualitative (low, medium, high). Annotations provide evidence for confidence designation. Numbers indicate frequently cited references: (1) (Mohatt et al., 2008), (2) (Cripps & Antibus, 2011). Other references are cited in annotations.

Taxa	Annotation
Ascomycota	
Dothideomycetes	
Mytilinidales	
Gloniaceae	
Cenococcum_unidentified	**Taxa:** *Cenococcum*; **Confidence:** high; **Reasoning:** Confirmed with WBP in (1) and (2).
Leotiomycetes	
Helotiales	
Helotiaceae	
Hymenoscyphus_tetracladius	**Taxa:** Helotiales; **Confidence:** medium; **Reasoning:** Identified in numerous samples confirming presence in/on roots. Ectomycorrhizal habit is not clear in Helotiales; the group includes pathogenic, root endophytic, and ericoid mycorrhizal taxa in addition to ectomycorrhizal taxa (Tedersoo et al, 2009).
Meliniomyces_bicolor	
Meliniomyces_variabilis	
Meliniomyces_unidentified	
Mycosymbioces_mycenaphila	
Mycosymbioces_unidentified	
Helotiales_fam_Incertae_sedis	
Acephala_unidentified	
Hyaloscyphaceae	
Pezizella_unidentified	
Leotiaceae	
Pezoloma_ciliifera	
Pezoloma_ericae	
Discinaceae	
Hydnotrya_unidentified	
Pezizomycetes	
Pezizales	
Pyronemataceae	
Wilcoxina	**Taxa:** *Wilcoxina*; **Confidence:** high; **Reasoning:** Confirmed previously with WBP (Trusty & Cripps, 2011).
Wilcoxina_unidentified	
Wilcoxina_rehmii	
Basidiomycota	
Agaricomycetes	
Agaricales	
Cortinariaceae	
Cortinarius_unidentified	**Taxa:** *Cortinarius*; **Confidence:** genus = high; species = low; **Reasoning:** *Cortinarius* species are diverse in WBP forests. The genus has been previously identified with WBP (1 & 2) and appears in numerous samples confirming its presence. Individual species, however, occur rarely in only a few samples. Often both the ITS region and the RPB2 region is needed to distinguish at the level of species (C. Cripps, personal communication, 30 July 2019).
Cortinarius_alboamarescens	
Cortinarius_barlowensis	
Cortinarius_borgsjoeensis	

	Cortinarius_boulderensis	
	Cortinarius_brunneus	
	Cortinarius_caesiobrunneus	
	Cortinarius_caperatus	
	Cortinarius_carabus	
	Cortinarius_cinnamomeus	
	Cortinarius_grosmorneensis	
	Cortinarius_lux-nymphae	
	Cortinarius_neofurvolaesus	
	Cortinarius_ochrophyllus	
	Cortinarius_privignus	
	Cortinarius_sarcoflammeus	
	Cortinarius_semisanguineus	
	Cortinarius_testaceofolius	
Entolomataceae		
	Entoloma_cetratum	**Taxa:** *Entoloma*; **Confidence:** low; **Reasoning:** *Entoloma* incudes fungi of both mycorrhizal and saprophytic trophic modes with some ambiguity (Kinoshita, Sasaki, & Nara, 2012). The genus has been identified from ectomycorrhizal samples of *Pinus* before (Pestaña Nieto & Santolamazza Carbone, 2009). Here, it is only present in a few samples and the literature does not support a confident designation as ectomycorrhizal; it is a possible soil contaminant.
Hygrophoraceae		
	Hygrophorus_purpurascens	**Taxa:** *Hygrophorus*; **Confidence:** medium; **Reasoning:** Genus has been identified in WBP forests before but not confirmed on root tips (1). Here it appears in only two samples. The nutritional modes of the Hygrophoraceae are highly debated. In the family, *Hygrophorus* is the only genus with recognized evidence demonstrating an ectomycorrhizal habit but this remains unclear. Numerous other species in the family have been demonstrated as potential bryophyte associates or saprophytic; the taxa is a potential contaminant (Seitzman et al., 2011).
Inocybaceae		
	Inocybe_soluta	**Taxa:** *Inocybe*; **Confidence:** medium; **Reasoning:** *Inocybe* has been confirmed with WBP before and with *Pinus flexilis* (1 & 2), but it only appears in one sample here.
Lyophyllaceae		
	Lyophyllum_shimeji	**Taxa:** *Lyophyllum shimeii*; **Confidence:** medium; **Reasoning:** *L. shimeii* has been identified as ectomycorrhizae with *Pinus* in Japan and East Asia before. The species requires extensive molecular techniques to distinguish it from closely related species of different habits (Visnovsky et al., 2014) and it only appears in two samples here. This gives medium confidence in its designation.
Tricholomataceae		
	Tricholoma_equestre	**Taxa:** *Tricholoma equestre*; **Confidence:** medium; **Reasoning:** *Tricholoma* has been confirmed in WBP forests before (1 & 2) but is only represented in one sample here.
Atheliales		
Atheliaceae		
	Amphinema_byssoides	**Taxa:** *Amphinema* and *Piloderma*; **Confidence:** high; **Reasoning:** Both genera are identified previously on WBP root tips and appear abundantly here.
	Amphinema_unidentified	
	Piloderma_bicolor	
	Piloderma_byssinum	
	Piloderma_sphaerosporum	
	Piloderma_unidentified	

Tylospora_fibrillosa	**Taxa:** *Tylospora*; **Confidence:** high; **Reasoning:** Appears in numerous samples and has been identified with *Pinus* elsewhere (Suz et al., 2017) (but not with WBP).
Boletales	
Gomphidiaceae	
Gomphidius_glutinosus	**Taxa:** *Gomphidius glutinosus*; **Confidence:** medium; **Reasoning:** Related genera in the Gomphidiaceae have been confirmed with WBP before (1 & 2) but only recovered in one sample here.
Rhizopogonaceae	
Rhizopogon_unidentified *Rhizopogon_bacillisporus* *Rhizopogon_brunneiniger* *Rhizopogon_salebrosus*	**Taxa:** *Rhizopogon*; **Confidence:** high; **Reasoning:** Confirmed with WBP previously and part of the pine or WBP specific Suilloid group (1 & 2).
Suillaceae	
Suillus_acidus *Suillus_brevipes* *Suillus_punctatipes* *Suillus_unidentified*	**Taxa:** *Suillus*; **Confidence:** high; **Reasoning:** Confirmed with WBP previously and part of the pine or WBP specific Suilloid group (1 & 2).
Cantharellales	
Cantharellales_fam_Incertae_sedis	
Sistotrema_unidentified	**Taxa:** *Sisotrema*; **Confidence:** medium; **Reasoning:** Genus considered ectomycorrhizal (Di Marino et al., 2008) but nature of root association not clear (Potvin et al., 2012). Identified in a reasonable number of samples.
Russulales	
Albatrellaceae	
Albatrellus_flettii *Leucogaster_unidentified* *Leucogaster_nudus* *Leucophleps_spinispora*	**Taxa:** *Albatrellus, Leucogaster*, and *Leucophelps*; **Confidence:** medium; **Reasoning:** Three genera are part of the ectomycorrhizal albatrellus lineage that associates with conifers (Tedersoo et al., 2010) but are only found in a few samples.
Russulaceae	
Lactarius_caespitosus *Lactarius_rufus* *Russula_adusta* *Russula_fragilis* *Russula_griseascens* *Russula_paludosa* *Russula_puellaris* *Russula_turci* *Russula_vesca* *Russula_xerampelina*	**Taxa:** *Lactarius* and *Russula*; **Confidence:** high; **Reasoning:** Both genera identified with WBP previously in (1) and (2) and are present in numerous samples.
Thelephorales	
Bankeraceae	
Phellodon_melaleucus	**Taxa:** *Phellodon*; **Confidence:** medium; **Reasoning:** Genus ectomycorrhizal with conifers (Tedersoo et al., 2010) and other species in the Theleporales (see Theleporaceae) have been identified with WBP (1 & 2) but only appears in one sample.
Thelephoraceae	
Pseudotomentella_unidentified *Pseudotomentella_mucidula* *Pseudotomentella_nigra* *Tomentella_sublilacina*	**Taxa:** *Pseudotomentella* & *Tomentella*; **Confidence:** high; **Reasoning:** Previously identified with WBP in (1) and (2) and present in a reasonable number of samples.

Mucoromycota Endogonomycetes Endogonales Endogonaceae *Endogone_unidentified* *Endogone_lactiflua*	**Taxa:** *Endogone*; **Confidence:** high; **Reasoning:** Genus ectomycorrhizal (Tedersoo et al., 2010) and identified previously with various *Pinus* species (e.g. Fassi, Fontana, & Trappe, 1969). Found here in numerous samples.