ECTOMYCORRHIZAL FUNGAL COMMUNITIES OF DOUGLAS-FIR ON DIVERSE SOIL LITHOLOGIES OF CENTRAL BRITISH COLUMBIA

by

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Abstract:

Ectomycorrhizal (ECM) fungi are symbiotic partners of most conifers and improve host health by increasing access to nutrients and water in return for photosynthates. ECM fungi have been demonstrated to ameliorate the effects of some harsh soil chemical conditions on plants. Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) is found on and off extreme bedrock-derived soils in central British Columbia. The objectives of this study were to describe the soils and Douglas-fir forests found on the diverse lithologies of the Fort St. James area and to assess seedling health and ECM fungal communities of Douglas-fir grown on these soils by morphological and molecular means. Fifteen ECM morphotypes, 12 basidiomycetes and 3 ascomycetes were identified with *Tuber anniae* (Ascomycota) unique to ultramafic soils. Three morphotypes (E-strain, *Cenococcum geophilum*, and *Rhizopogon cf. villosulus*) were ubiquitous on all sites and no connection between parent material and ECM communities was established.
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1. Introduction and literature review

Within the rhizosphere, the area of soil directly influenced by plant roots, plants spread their roots to acquire water and nutrients. Forest ecosystems depend on the connection between above-ground inputs of sunlight, precipitation, litter fall from trees and the below-ground nutrient inputs from the soil. Mycorrhizal fungi form a mutualistic relationship by colonizing root systems, spreading networks of fungal hyphae throughout the soil (Smith & Read, 2008). Ectomycorrhizal fungi, a sub-group of mycorrhizal fungi, colonize a majority of forest tree species, including all conifers and most hardwoods found in British Columbia.

Soils are not uniform providers of nutrients and water to plants. Soil development is impacted by bedrock lithology, glacial activity, age and climate. Weathering of parent material releases trace minerals bonded to insoluble complexes that are important to plants. Weathering of some parent materials also releases potentially toxic elements into bio-available forms within the soil.

Within the soil, a broad consortium of bacteria, unicellular eukaryotes, invertebrates, and fungal organisms are housed, protected and supported. These organisms respond and adapt to changes in the substrate. This collection of interconnected organisms is largely responsible for the nutrient and water cycling that supports the above-ground ecosystem. The metabolic processes responsible for survival of below-ground organisms also bind and release materials vital to the proper functioning of above-ground organisms. The intersection between the immediate availability of nutrients in any given soil and the products of soil organisms also shapes the above-ground plant community.
Mycorrhizal fungal communities, often described in terms of functional guilds (see below), have been linked to increased health and vigor of plants, increased uptake of water and nutrients, drought resistance and translocation of soil nutrients into forms that are usually inaccessible to host plants (Parke et al., 1983; Li et al., 1991; Gonçalves et al., 2007, 2009). A functional guild represents a grouping of mycorrhizal fungi that share similarities in purpose for a specific host in a particular area (Molina et al., 1992; Massicotte et al., 1999). Stated simply, they act as if seeking a common goal (Perry et al., 1989).

Fungi are the plant's first direct contact with the soil surface and therefore are in closest proximity to damaging compounds within the soil. In extreme soils, these toxic conditions may derive from alkaline pH and heavy metals. Soil pH and nutrient uptake are often intertwined as nutrients become bio-available as pH goes up or down. Studies have documented a level of resistance to cellular toxicity caused by certain types of metals including zinc, nickel, copper, and aluminum, when host root systems are colonized by mycorrhizal fungi (Jones & Hutchinson, 1986, 1988; Wilkins, 1991; Jentschke & Godbold, 2000). Some studies have also shown mycorrhizal fungi communities have sensitivities to pH (Fujimura & Egger, 2012; Walker et al., 2014). By understanding some of the complexities of the influences of soil chemical characteristics on mycorrhizal fungi we can increase our knowledge of vast abiotic and biotic interactions that shape the soil system and ecosystem.

1.1. Mycorrhizal fungi

Mycorrhizal fungi form mutualistic relationships with plants through associations with the fine roots. These fungi share a phylogenetic lineage with saprotrophic fungi
(decomposers that use dead plant and animal matter within the soil as a carbon source) (Hibbett et al., 2000). Associations between roots and fungal hyphae were first described by German scientist Albert Bernhard Frank in the late 1800s (Frank, 1885). Fungal hyphae were discovered to penetrate, infect and envelop the roots of many plants in both long term and ephemeral unions. At the time of discovery, however, it was unclear whether or not the fungi were decomposing the plant.

Researchers in the early 20th century used improved microscopy to further characterise three proposed mycorrhizal categories based on morphology: endotrophic, ectotrophic, and ectendotrophic (Toumey & Korstian, 1947). Mycorrhizas are now broadly divided into seven designations based on morphology and host species: arbuscular, ericoid, arbutoid, orchid, monotropoid, ectomycorrhizas and ectendomycorrhizas (Peterson et al., 2004; Peterson & Massicotte, 2004). Of this group, ectomycorrhizas (ECM) are the most important to conifer tree species (Trappe, 1977), but are the least prevalent globally with only 3% of seed-bearing plants hosting ectomycorrhizal fungi (Moore et al., 2011).

1.2. Arbuscular and orchid mycorrhizas: individual guilds

Arbuscular mycorrhizas are characterized by a diagnostic feature called an arbuscule, made up of a combination of the root cell plasma membrane and tree-like projections of hyphae into the root cell. The arbuscule is the center for metabolite exchange between the symbionts. These fungi, comprising the phylum Glomeromycota, are common on crop species worldwide (Marschner & Dell, 1994; Kivlin et al., 2011). While not phylogenetically diverse, these fungi are essential to the success of many crop species as they supply needed minerals to the host plants.

Orchid mycorrhizal fungi first partner with germinating orchid seeds, which have
no fat or nutrient reserves of their own (Smith & Read, 2008). Fungal hyphae aid in success after germination by penetrating the orchid embryo, forming intracellular coils, and processing complex carbohydrates from the surrounding soil into simple sugars for the developing plant. Later, the orchid fungi will invade the roots and perform the same function (Rasmussen, 1995; Peterson & Massicotte, 2004). Both of these types represent individual functional guilds as they are usually restricted to their particular host (meaning orchid mycorrhizal fungi would not be found with arbuscular mycorrhiza on the same host).

1.3. Ericoid, arbutoid, ectendo and monotropoid mycorrhizas: shared guilds

Ericoid mycorrhizas are fungal-root associations between ascomycetes, such as *Rhizoscyphus ericae* (formerly *Hymenoscyphus ericae*) and plants within the order Ericales, including various genera such as *Vaccinium* and *Gaultheria* (McKechnie, 2009). Ericoid mycorrhizal fungi colonise the hair roots of the Ericales hosts, forming a loose mantle while also penetrating the epidermal root cells. Some mycorrhizal fungi are not exclusively ectomycorrhizal in form, but may also form arbutoid mycorrhizas when grown near plants in the *Ericaceae* family such as *Pyrola, Arbutus,* and *Arctostaphylos* (Massicotte *et al.*, 1993; Hagerman & Durall, 2004).

Ectendomycorrhizas are formed by fungi within the order Pezizales. Ascomycetes in the genus *Wilcoxina*, commonly grouped under the name E-strain, are the predominant ectendomycorrhizal fungi. These fungi can colonize a variety of hosts to somewhat different effect. For instance, *Wilcoxina* will form ectomycorrhizas with *Pseudotsuga menziestii* (Massicotte *et al.*, 1999) but will form ectendomycorrhizas with members of the *Larix* or *Pinus* genera (Yu *et al.*, 2001).
Monotropa is a genus of mycoheterotrophic achlorophyllous plants usually found in conifer forests which also has a suite of monotropoid mycorrhizal fungal associates. Both arbutoid and monotropoid mycorrhizal fungi often belong to the same functional guild as ectomycorrhizal fungi. All of these fungi can potentially form networks between their respective plants and make the trees and understory plants partners (Molina et al., 1992). In these cases, mycorrhizal fungi act as a conduit, passing carbohydrates from neighbouring trees to the plant (Simard et al., 1997).

1.4. Ectomycorrhizas

1.4.1. Structure and function

Ectomycorrhizas are a union between the external root surface of plants and fungal hyphae (Teste et al., 2009; Simard, 2009; O'Brien et al., 2011). Colonization of the fine roots of trees begins with the germination of fungal spores within the soil or from direct contact with fungal hyphae. Ectomycorrhizal fungal propagules are known to germinate in response to the presence of roots, and it is postulated that germination may be stimulated by the secretion of host root exudates (Molina et al., 1992). Fungal mycelia grow towards and weave together to encase the root tip in a mantle. Hyphae from the mantle push between the root epidermal and cortical cells to form the nutrient exchange structure known as the Hartig net (Peterson et al., 2004).

Tuberculate species of fungi have a different type of mycorrhizal fungal colonization shape: groups of fungal colonized tips with mantles will have a peridium, or rind, surrounding the root cluster. While research has not provided a clear function for these larger ectomycorrhizal structures, some experiments on Douglas-fir have shown that
they still obtain soil nutrients from rhizomorphs, long, thick, parallel strands of hyphae, extending into the soil (Zak, 1971).

Extraradical mycelia are long emanating hyphae that extend from the mantle into the surrounding soils. Mycelia increase the amount of soil exploration possible for a tree, allowing for greater uptake of minerals and water from the soil (Trappe, 1977; Teste et al., 2009). Rhizomorphs are used in the transport of absorbed nutrients. Ectomycorrhizal fungi have been shown to greatly increase the effective surface area of colonized root systems, allowing some levels of drought resistance (Parke et al., 1983).

Ectomycorrhizas differ from other types of mycorrhizas in the lack of live plant cell penetration by the fungal hyphae. Dying root cells can be punctured by fungal hyphae, but fungal hyphae remain on the surface of the live plant root, outside of the living cells (Yu et al., 2001). Once thought to be pathogenic (Tourney & Korstian, 1947), these fungi live in symbiosis with plants in exchange for a carbon source from the plant (Jones & Hutchinson, 1986; Teste et al., 2009). A variety of types of angiosperms and gymnosperms are potential hosts to ECM, but colonization is most common on perennial tree and shrub species (Moore et al., 2011). Ectomycorrhizal fungi have been the topic of much study because of the high timber value of their hosts (Hunt, 1992).

Basidiomycetes form the majority of ectomycorrhizal partnerships with many forest plants, though some ascomycetes and rare Zygomycetes are also ectomycorrhizal. It was estimated that as many as 6000 species of fungi worldwide can form ectomycorrhizal associations (Molina et al., 1992). Recent DNA and molecular evidence has raised this estimate to 20 000 potential species (Brundrett, 2009).

Host species influences the shape and structure of the Hartig net. Gymnosperms
usually have Hartig nets that penetrate between the root epidermal and cortical cells. In contrast, angiosperms form epidermal Hartig nets which do not extend into the cortical cells (Moore et al., 2011). Hartig net formation depends on the stage of growth of the root. Often the Hartig net is less developed at the growing apical end of the root tip (Massicotte et al., 1989). Mantle thickness is also influenced by the growth of the root, with uneven growth of mantles on older lateral roots compared to younger laterals (Massicotte et al. 1986).

1.4.2. Ecosystem impact and importance

Mycorrhizal fungi differ in the types of preferred hosts. Some are restricted to only one host (narrow host range), some can form partnerships with one family of plants (intermediate host range), and others can form partnerships with almost no restrictions (broad host range). ECM fungi are typically grouped between the intermediate and narrow host ranges, with some exceptions (Molina et al., 1992). Structure of colonized plant roots will vary depending on the species of plant and the species of fungi. The host plant can also determine the number of ECM species that can form mutualistic associations.

Most host trees will often have multiple species colonizing their root systems at one time (Massicotte et al., 1999; Robertson, 2003; Branco & Ree, 2010). Single roots can also be co-infected by different species trying to establish a symbiosis with the host (Bruns, 1995). In the case of Douglas-fir, only a small number of the fungal partners are restricted to the species (e.g. *Rhizopogon* cf. *villosulus* and *Suillus caerulescens*) while the others are capable of forming mycorrhizas with other tree and shrub species (e.g. *Cenococcum geophilum* Fr., *Russula* spp., and *Tuber* spp.) thus expanding the potential functional guild (Molina et al., 1992). Access to broad host range fungi also provides a more secure source
of inoculum as other plants can retain the fungi in the event of a disturbance that damages
the networks connected to the original hosts (e.g. regeneration in a clear cut with all
Douglas-fir removed).

1.4.3. Development of modern methods of ECM identification

Ectomycorrhizal species have typically been described by morphotyping root tips. This process developed due to the lack of known fruiting bodies of a majority of ectomycorrhizal species (Smith & Read, 2008). Morphotyping involves visual analysis, complemented by the use of light microscopy, to identify the particular species colonizing a root system. Colour and rhizomorph presence are used as diagnostic features, but fungal mantle structure is often the best recognition factor (Ingleby et al., 1990). Agerer (1987) and Ingleby et al. (1990) pioneered the in-depth identification of fungi through morphological means. Improved methods of compound and electron microscopy aided greatly in the characterization of mycorrhizas (Massicotte et al., 1986, 1992). Agerer later compiled a 5 volume work consolidating all photographic and descriptive data on mycorrhizas from around the world (Agerer, 1987-2008).

Prior to advances in DNA technology, ectomycorrhizal fungi would often be cultured on agar-based medium in the lab in order to isolate specific genets for population studies (Dahlberg & Stenlid, 1994). Once pure cultures of ECM fungal mycelium were obtained, they could be compared based on somatic compatibility (Baar et al., 1994). In other words, mycelium that could interact positively were deemed to be related or of the same species. This process was very time consuming and incomplete as many groups of ectomycorrhizal fungi do not culture in a laboratory setting and some different species do not display incompatibility with each other (Smith & Read, 2008).
DNA methods have improved the identification of many fungi. Extensive work has been done with Terminal Restriction Fragment Length Polymorphisms (T-RFLP) in the 1990s and early 2000s (Erland et al., 1994, 1999; Allen et al., 2003; Moser et al., 2005). This process uses bacterial enzymes to cut polymerase chain reaction (PCR) amplified sequences of interest, usually the ITS DNA band (Schoch et al., 2012), and measure the resulting fragments on the basis of size using gel electrophoresis. DNA fragments of the same size will assort together and are therefore indistinguishable from each other during gel visualization. Different ECM species can produce DNA sequences of the same length which limits the resolution of this technique (Gonçalves et al., 2009).

Amplification of portions of the ribosomal DNA fragment ITS through PCR and the advent of Sanger sequencing systems allowed for greater differentiation between fungal species (Bruns et al., 1998; Horton et al., 1999; Branco & Ree, 2010). Instead of basing separate identifications on fragment size, the order of base pairs could be recorded using dye-tagged nucleotides and compared to fungal sequences housed at GenBank® or the purely fungal database UNITE using BLAST (Basic Local Alignment Search Tool) (Kõljalg et al., 2005). This approach depends on the accurate vouchering of fungal specimens and maintenance of sequence databases.

Recently, next-generation sequencing (NGS) has become a point of great interest in the study of ECM fungal DNA. The speed of the process is advantageous over previous sequencing methods. Massively parallel ('454') pyrosequencing, one of the three main platforms of NGS, can generate a large number of sequences up to 500 base pairs in length (Henrik Nilsson et al., 2011). Instead of using dye-terminators and linear processing, NGS runs sequencing reactions in parallel, greatly reducing the time required. NGS uses and
generates a very large volume of data which must be handled properly. However, it is important to note that NGS will still rely on proper curation of database information and is vulnerable to database errors, lack of information about recorded sequences, or simply information overload (Taylor & Harris, 2012). Given the possibilities, this method will most likely continue to be the focus of much research in the next decade.

1.5. Ecological influence of soil parent material

1.5.1. Pedogenesis in central British Columbia

Pedogenesis refers to the natural processes that lead to the formation of a soil (Schaetzl & Anderson, 2005). These processes are influenced by climate and time from last disturbance. Pedogenesis is directly linked to the weathering of the soil parent material, when biotic and abiotic factors break down the original physical and chemical structure of the parent material. Moist, warmer sites increase the rate of production of secondary minerals from parent material while cooler, drier sites retard weathering (Brady & Weil, 2008). Organic matter is also cycled faster or slower from deposition to decomposition dependent on climate (Schaetzl & Anderson, 2005). The blending of the organic and inorganic inputs creates the unique signature of the soil (Brady & Weil, 2008).

Most Canadian soils are comparatively young, sharing a recent history of glacial disturbance. Central British Columbia was under a thick layer of ice during the Late Wisconsinan glaciation approximately 20 000 years ago. Ice flowing eastward from the Coast Mountains generated large volumes of glacial till, glaciofluvial and glaciolacustrine deposits (Plouffe, 2001). Retreat of the glaciers led to ice-free conditions in central British Columbia by 10 000 years ago (Clague, 1981). Colder continental climate and moderately
dry conditions coupled with recent glaciation led to the development of high proportions of Luvisolic and Brunisolic soils in central British Columbia (Clayton et al., 1977; Meidinger et al., 1991).

Soils vary over extremely small spatial scales (Cheng et al., 2011). A pedon is the smallest unit that can be designated a soil. Pedons are usually approximately a square metre, though this area may increase up to several meters to obtain proper soil definition, and 1 or more meters deep (Brady & Weil, 2008). This variation extends into layers within the vertical structure of soils referred to as horizons (Soil Classification Working Group, 1998).

Horizons are visually distinct bands of different chemical and physical properties. Organic horizons, formed from litter deposition in forests are usually referred to as L, F, or H layers dependent on the degree of decomposition. Mineral soil horizons are classified as A, the uppermost horizon which is affected by leaching and organic matter deposition, B, the second horizon, enriched with clays and other weathering products, and C, the lowest horizon, usually unaffected by most pedogenic processes (Clayton et al., 1977). Secondary, lowercase letters are used to designate specifics of each horizon. Luvisols will often have a Bt layer (with high amounts of illuvial clay), while Brunisols may have a Bm (very little pedogenesis has occurred) (Soil Classification Working Group, 1998). These minute variations within pedons necessitate on-site sampling through soil pits to properly classify the conditions found in a particular area.

1.5.2. Nutrient mobility within soils

Weathering of parent material makes many minerals and nutrients more bioavailable. However, this mobility within the soil profile can also release more toxic
elements (Kierczak et al., 2007; Cheng et al., 2011). Conversely, atmospheric inputs of material, or newly released minerals, can be bonded to other compounds within the soil, which renders them insoluble or not readily bio-available (Schaetzl & Anderson, 2005; Brady et al., 2005).

Nitrogen, phosphorus and potassium are all essential to the survival of plants. Phosphorus, for example, is often difficult to access on soils with calcareous parent material (Kishchuk, 2000). High pH within the soil causes the formation of orthophosphate ions which in turn forms complexes with carbonates to create insoluble minerals (Thorne & Seatz, 1955). Both serpentine and ultramafic derived soils may be high in heavy metals which are phytotoxic. Soils derived from serpentinized bedrock may have readily available nickel due to the ease of weathering serpentine and olivine. Nickel is a metal that is beneficial in trace amounts, but toxic to plants at high levels (Renz & Shilts, 1980; Kierczak et al., 2007). Drainage, or lack thereof, also impacts translocation of nutrients and minerals throughout the soil horizons (Schaetzl & Anderson, 2005).

Biotic factors also play a role in the movement of nutrients. Bulk soil, or non-rhizosphere soil, is usually under the influence of water drainage patterns and parent material. The rhizosphere features complex interactions between plant roots, mycorrhizal fungi, soil bacteria and the soil itself. Saprotrophic fungi decompose much of the organic material deposited on top of the upper forest floor horizons (Rayner & Boddy, 1988).

Mycorrhizal fungi can transport these newly accessible nutrients to their host tree while also increasing nutrient accessibility themselves. Extracellular enzymes released by the fungi into the soil will mobilize complex molecules containing nitrogen and phosphorus within the soil organic matter (Courty et al., 2010). It is also hypothesized that
some species of ECM can prevent uptake of toxic metals and minerals, though specific mechanisms and evidence are not clear (Wilkins, 1991; Azcón & Barea, 1992). It is theorized that the fungi bind the metals into the mantle tissue, stopping the host plant from absorbing the metal compounds (Moore et al., 2011).

1.6. Douglas-fir in Central British Columbia

1.6.1. Habitat and range

Douglas-fir (Pseudotsuga menziesii) is a long-lived conifer found in western North America from Mexico to central British Columbia (Silen, 1978). It can live for upwards of 500 years (Hermann & Lavender, 1990), and the Interior Douglas-fir subspecies (Pseudotsuga menziesii var. glauca (Beissn.) Franco) has been documented at 625 years of age in the Chilcotin region of British Columbia (N. Thompson, pers. comm. 2015).

Mature Douglas-fir is easily recognizable by its thickly grooved reddish bark, spreading crown, scaled cones and single spaced needles. Thick cork-like bark makes the mature tree resistant to ground fires, allowing for greater survival compared to other tree and shrub species within the same habitat (Hermann & Lavender, 1990). Seeds disperse from the cones through wind and may be transported great distances. Trees can form almost pure stands through natural regeneration, or can be a component of mixed stands usually dependant on soil and climate. Douglas-fir prefers well-drained soils and is not successful in compacted or wet areas (Hermann & Lavender, 1990).

Douglas-fir can grow on slopes and valley sides that represent too dry an environment for competing species like hybrid white spruce (Picea glauca var. engelmannii (Parry) Boivin) and subalpine fir (Abies lasiocarpa (Hook) Nutt.). (Hermann
& Lavender, 1990; Delong, 1999). It is often found with understory plants such as Arctostaphylos uva-ursi (L.) Spreng. which may host the same species of ectomycorrhizal fungi as Douglas-fir (Hagerman et al., 2001; Hagerman & Durall, 2004). Douglas-fir seedlings are sensitive to frost damage and are said to have a preference for sunny, southwest-facing slopes in cooler areas (Delong, 1999; Griesbauer & Green, 2010). It is unclear whether soil, competition, or climate impacts the establishment of Douglas-fir more.

1.6.2. ECM associates

A wide variety of fungi that span coastal, montane and interior plateau ecosystems are available to Douglas-fir as fungal associates. Douglas-fir's long lifespan has led to the hypothesis that the trees act as mycorrhizal refugia, providing inoculum for trees and shrubs after disturbance (Wiensczyk & Gamiet, 2002; Simard, 2009). Approximately 205 to 2000 different species of fungi have been estimated to form mycorrhizal partnerships with Douglas-fir (Trappe, 1977; Molina et al., 1992).

Some of the most common genera of epigeous, or above-ground fruiting bodies, genera include: Cortinarius spp., Suillus spp., and Russula spp (Arora, 1986; Smith et al., 2002). Below-ground, or hypogeous, fruiting mycorrhizal fungal partners include Rhizopogon spp and Tuber spp (Zak, 1971; Hunt, 1992; Massicotte et al., 1994). Other species that do not display known fruiting bodies of any kind, such as Cenococcum spp., are also colonizers of Douglas-fir roots (Jones et al., 2010).

1.7. Current research into ECM on extreme parent material

Some species of ECM are hypothesized to provide a measure of resistance to
impacts of soil metals (Wilkinson & Dickinson, 1995). An understanding of the below-
ground fungal network of ECM communities grown on extreme soils can help shed light
on forest success in regions influenced by strong chemical signatures of parent material.
This in turn aids in the preservation and management of these areas in both forest
production and habitat conservation. For instance, if certain species of ectomycorrhizal
fungi improve growth on areas with harsh parent material, planting seedlings that are pre-
inoculated with those species could improve seeding performance. Continued research into
ECM fungi that survive and succeed on extreme substrate helps to illuminate a small facet
of the world of below-ground soil interactions.

1.7.1. Mycorrhizal fungi of serpentine and ultramafic-derived soils

Serpentine soil, a broad term that is often used to describe soil with both ultramafic
and serpentinized parent materials, has been a research topic for many decades as it relates
to ectomycorrhizal fungi. John Maas and Daniel Stuntz (1969) did pioneering research to
classify the presence of epigeous fungi growing on serpentine soils of the Washington
Cascade Mountains. While few sporocarps were found on serpentine, a greater proportion
of the fungi found on serpentine were ectomycorrhizal (Russula sp., Amanita sp. and
Suillus sp.), compared with the non-serpentine sites, which may indicate that both plants
and ectomycorrhizal fungi need each other to survive on serpentine soils. Since that time,
much research has been done to describe and quantify the species richness (the number of
species found) and diversity (the variety of species) of ECM fungi on serpentine areas
around the world.

A study by Moser et al. (2005) on Quercus garryana Dougl. (Fagaceae) (Garry
oak) in Oregon determined, by field sampling ECM root tips, that fungal communities did
not significantly differ between serpentine and non-serpentine sites. However, this research did find that 43% of the 74 morphotypes in the study were unique to serpentine soils. Many of these unique morphotypes were rare and present as a single root tip, which were therefore not included in statistical analysis by the researchers. This study used morphological techniques coupled with the analysis of restriction fragment length polymorphisms (RFLP) (Moser et al., 2005). Gladish et al. (2010) studied conifer (Pinus ponderosa Douglas ex C.Lawson and Pinus Jeffreyi Balf.) ECM communities of Oregon serpentine soils by field sampling six sites (3 paired serpentine and non-serpentine). In this study, both ectomycorrhizas and hypogeous sporocarps associated with conifers were compared using DNA sequencing, instead of RFLP to identify fungi. Less hypogeous fungi diversity was found on serpentine soils which the researchers concluded may be more related to host populations than soil conditions. When comparing mycorrhizal fungi on serpentine and non-serpentine areas, the researchers concluded that limitations in the host species dispersal were driving fungal diversity, as opposed to differences in the underlying soil (Gladish et al., 2010).

Gonçalves et al. (2007) focused on Cenococcum geophilum isolates from a serpentine and non-serpentine site in Portugal using amplified fragment length polymorphisms (AFLP). AFLP uses a process similar to RFLP, but instead the data are usually treated as presence-absence. The serpentine isolates were not affected by nickel addition to growth media compared to non-serpentine isolates which may indicate resistance to nickel toxicity. However, all isolates were genetically distinct from one another and both serpentine and non-serpentine isolates were equal in levels of genetic diversity (Gonçalves et al., 2007). This is in contrast to a previous study conducted in
Maryland, USA which used similar techniques to show that serpentine *Cenococcum geophilum* isolates were more similar to each other than to isolates from non-serpentine soils (Panaccione *et al*., 2001).

Branco and Ree (2010) conducted several studies on serpentine ECM in oak forests in Portugal. These oaks were located on serpentine soils and harboured ECM fungal communities that had little overlap with non-serpentine communities, with only 15% of sampled species shared between sites (*Inocybe* sp., *Tricholoma* sp, *Cenococcum* sp. and others). Though there was low overlap between the fungi sampled, many species were documented only once and therefore, both serpentine and non-serpentine sites were not significantly different (Branco & Ree, 2010). Branco also conducted another study using reciprocal transplantation of seedlings into both serpentine and non-serpentine soils to compare the ECM grown on *Quercus ilex* spp. *bailota* (holm oak). Serpentine soils produced a higher fungal richness than non-serpentine soils, but were less rich and diverse than the previous study. Fungi were also shared between both soils, which leads Branco to conclude that fungi have a tolerance to the extremes within the soil (Branco, 2010).

Recently in Deer Isle, Maine, Davoodian *et al*., (2012) compared the arbuscular mycorrhizal communities of the host *Hypericum perforatum* L. (St. John’s wort) between soils derived from serpentine and granite parent materials. In this case, number of species was not considered; instead, colonization was compared in proportion to the entire root system. Percent colonization did not differ between serpentine and granite sites at any of the sampling times. Colonization did differ for both sites during stages of flowering, with the highest level found post-flower (Davoodian *et al*., 2012).

Research on serpentine soils to date is summarized in a review by Southworth *et al*.
(2014). They conclude that the majority of studies conducted indicate that ECM fungal communities of serpentine soils do not differ from those on non-serpentine soils. Some species, including *Cenococcum geophilum*, may be locally adapted to serpentine sites and hypogeous fungi are less prevalent on serpentine soils (Southworth *et al.*, 2014). However, some studies of ECM do indicate that the harshness of serpentine soils may increase biodiversity or richness instead of hindering it (Branco & Ree, 2010; Branco, 2010). However, this review and these studies also demonstrate the variability often found when conducting mycorrhizal research and the confounding problem of rare species.

1.7.2. Mycorrhizal fungi of calcareous soils

Arbuscular mycorrhizal (AM) fungi of calcareous soils have had much attention from an agricultural perspective. Studies have shown that cereal and legume crops grown in calcareous soil benefit from root inoculation with AM fungi (Kothari *et al.*, 1991; Li *et al.*, 1991). Low levels of accessible phosphorus in calcareous soils can be compensated for by inoculation with AM fungi (Chen *et al.*, 2003; Feng *et al.*, 2003). Commercial varieties of AM inoculum are now widely available and little attention is given to the biodiversity of the root systems of these crop species.

Ectomycorrhizal research involving calcareous soils is much rarer. Much of the work focuses on the survival of plant species based on the presence or absence of mycorrhizal associates. A majority of these studies pre-date the now common molecular DNA techniques used in parallel with morphotyping for identifications. Few of the studies in question describe the ECM community in great detail. An exception is a study conducted on eight *Salix* spp. (*S. reticulata* L. (reticulate willow), *S. herbacea* L. (least willow), *S. myrsinites* L. (myrtle willow), *S. glauca* L. (northern willow), *S. phyllicifolia
(L.) sm. (tea-leaved willow), *S. lanata* L. (woolly willow), *S. hastata* L. (halbert-leaved willow), *S. nigricans* Sm. (dark leaved willow)) grown in Norwegian calcareous and non-calcareous soils described prolific *Cenococcum geophilum* and 6 species of ECM including the genus *Tuber* and *Laccaria* through morphotyping. This study was an exploration of mycorrhizal conditions of *Salix* spp. at both boreal and alpine ecosystems (Dhillion, 1994).

Presence of ECM fungi has been connected to the survival of plants grown on calcareous soils or analogues. A study by Le Tacon (1978) on *Pinus nigra* J.F. Arnold (black pine) and *Picea excelsa* Link (*Picea abies* (L.) H. Karst-Norway spruce) compared uncolonized and seedlings already colonized by ECM fungi by planting them in sand substrate laced with calcium carbonate (CaCO₃). *Picea excelsa* was able to tolerate the increased levels of calcium without ECM, but *Pinus nigra* required colonization to survive. While this substrate was not a calcareous soil, the author used the addition of CaCO₃ to mirror naturally occurring levels of calcium within the soil (Le Tacon, 1978). Another species of pine, *Pinus halepensis* Miller (Aleppo pine), was also shown to require ECM fungal partners to survive on calcareous soils. Seedlings planted in sterile calcareous soil did not develop ectomycorrhizas and were stunted and stressed compared to seedlings planted in unsterile soil which were successful in partnering with ECM fungi (Piou, 1979). In both cases, survival was directly tied to the presence of mycorrhizal fungi.

Laypeyrie and Chilvers (1985) conducted a study involving both ecto and endomycorrhizal fungi comparing the success of sterile and non-sterile calcareous and acidic soils amended with potting mix and additional CaCO₃. *Eucalyptus dumosa* (White Mallee) grown in sterile acidic soil displayed no signs of stress, whereas the calcareous grown trees displayed very slow and inhibited growth on the sterile substrate. However,
when the sterile substrate was inoculated with unsterile soil, no difference in growth was observed between the acidic and calcareous soils, leading the researchers to conclude that ECM fungi were necessary for reducing the stress caused by calcareous soils (Lapeyrie & Chilvers, 1985). In another study, *Cenococcum graniforme* (=*geophilum*) sclerotia were used to inoculate sterile calcareous soil and successfully prevent plant death and chlorosis of leaves in *Helianthemum chamaecistus* (=*nummularium* or Common rock-rose) (Kianmehr, 1978). These two studies point to the potential for ECM fungi to protect the host plant from the damages associated with calcareous soils. While specific mechanisms are not documented in these studies, it is likely that limiting nutrients, such as phosphorus, are made more accessible to the host plants through the ECM fungi (Lapeyrie, 1990).

While serpentine soils have been extensively studied worldwide as extreme, edaphic habitats, no research has been done on ECM diversity in soils derived from the Stuart Lake Belt ultramafic rocks. Calcareous rocks and deposits within the Fort St. James region have been documented by Plouffe (2000) and information on calcareous soils and ecosystem impacts throughout the south of British Columbia and west Alberta has been gathered by Kishchuk (2000) but likewise, no research on ECM populations has been conducted. Little is known of the impacts of the physical, biological and geochemical signature of soils in the Fort St. area on ECM diversity within the rhizosphere.
1.8. Objectives of study

As a prelude for my biology work, the first objective is to provide an initial assessment of soils found on the diverse lithologies in the Fort St. James area, including ultramafic and calcareous bedrock. These soils are distinct from other ultramafic and calcareous areas due to the relatively recent glaciation and to my knowledge, no other study has made such a comparison. While Douglas-fir is of high economic importance in central British Columbia, little research has been done on the stand conditions of the ultramafic and calcareous grown stands in the Fort St. James area. This study will provide a description of the Douglas-fir forest attributes and associated vegetation grown on the strongly contrasting soils derived from ultramafic, calcareous, and glacial parent material.

Both calcareous and serpentine soils have been observed to have severe health impacts on plants on unglaciated areas worldwide (Lapeyrie, 1990; Gladish et al., 2010), but no research has been done on the plant health impacts of ultramafic, calcareous and glacial-derived soils in the Fort St. James area. My study will use a greenhouse bioassay to preliminarily assess the health and growth of Douglas-fir grown in these extreme soils. Research on the ECM communities worldwide has shown that ECM fungi are usually equally diverse on and off serpentine soil (Southworth et al., 2014). However, no study has explored the ECM fungal communities of Douglas-fir on ultramafic-derived soils in central British Columbia, or compared those communities to calcareous and glacial derived fungal assemblages. My study will use morphological identification coupled with DNA sequencing to categorize and quantify the unknown ECM fungal communities of Douglas-fir.
1.9. References


2. Fort St. James: Soils and forest ecosystems related to diverse lithologies

2.1. Description of bedrock origins

2.1.1. Ultramafic bedrock, serpentine rocks and soils

British Columbia has several distinct ultramafic bedrock deposits formed by ocean crusts, or ophiolite complexes, accreting against the North American Craton (Bulmer, 1992; Plouffe, 2000). Accreted fragments of ocean crust, known as terranes, form a patchwork of distinct bedrock types in central British Columbia. The Trembleur ultramafic rocks of the Cache Creek terrane are the underlying parent material of the ultramafic soils located in Fort St. James. The Trembleur ultramafic rocks are made up of a combination of ultramafic and serpentinized rocks including harzburgite and peridotite, and serpentinite (Plouffe, 2000). These rocks were formed during the Carboniferous period, approximately 360 to 300 million years ago, and the Lower Jurassic period, approximately 200 to 175 million years ago (Plouffe, 2000; Cohen et al., 2013).

Ultramafic rocks are igneous, formed from molten parts of the earth’s mantle. These rocks are low in silica and usually dark in colour due to the high levels of iron and magnesium. Heavy metals such as nickel and chromium can be enriched within ultramafic deposits. Serpentinization is a metamorphic process that occurs when ultramafic deposits are exposed to water at temperatures less than 400°C. The original rocks are oxidized and hydrolyzed by the water, weakening the structure of the rock (Alexander et al., 2006). Both serpentine and ultramafic parent material contribute high levels of iron, magnesium, chromium and nickel to the soils developed on the deposits (Bulmer & Lavkulich, 1994).
2.1.2. Above and below-ground effects of ultramafic and serpentine bedrock

Serpentine soils are one of the most widely recognized examples of strong chemical signatures within soil conditions (Harrison & Rajakaruna, 2011). True serpentine soils reflect the chemical character of the serpentine rocks and parent material. While both serpentine and ultramafic parent materials are often grouped together, ultramafic rocks are more stable than serpentinized rocks and will often have a slightly different chemical signature within the soils horizons (Bulmer & Lavkulich, 1994). Serpentine and ultramafic soils worldwide often present a very visible change in ecosystem type at the location where the bedrock transitions from the harsher serpentine and ultramafic parent material to one with a weaker chemical signature (Kruckeberg, 2004; Alexander et al., 2006).

High levels of magnesium lead to a low Ca:Mg ratio within the soil producing strain on plants grown in serpentine areas (Brady et al., 2005; Fitzsimons & Miller, 2010; Armbruster, 2014). Nickel levels within all soil horizons on serpentine soils can potentially be phytotoxic, interfering with cellular metabolism and growth of plants (Miller & Cumming, 2000; Panaccione et al., 2001). Plants usually attempt to reduce accumulation of nickel by exclusion in the rooting zone but this too reduces vigor (Mesjasz-Przybyłowicz et al., 2007). ECM fungi may play a role in the prevention of absorption of nickel and other metals (Brown & Wilkins, 1985; Wilkins, 1991; Wilkinson & Dickinson, 1995). Nutrient stress leading to chlorosis is also a factor of ultramafic and serpentine ecosystems due to relatively alkaline pH, low phosphorus and potassium availability (Alves et al., 2011). The fragility of serpentinized rocks frequently leads to soil instability, mass wasting and water loss, which can damage or remove some plants completely (Cleaves et al., 1974; Schreier, 1989).
Plant endemism on serpentine soils has been well documented in California, Cuba, Italy and New Caledonia (Kruckeberg, 2004; Brady et al., 2005; D’Amico & Previtali, 2012). What little research has been done on British Columbia serpentine and ultramafic ecosystems has not shown the same level of above-ground ecosystem change, often described as barrens, found elsewhere (Alexander et al., 2006). There is some evidence that northern and western maidenhair fern, Adiantum pedatum L. and Adiantum aleuticum L. respectively, show preference for serpentine soils (Paris, 1991); however, this is still anecdotal in British Columbia. The “serpentine syndrome” does not seem to produce the same level of plant endemism in British Columbia as described in areas less recently glaciated.

Fungi also display sensitivity to changes in soil chemistry. Soil pH and phosphorus levels have been found to influence fungal community structure. In the same study, dolomitic soils differed in community structure from granitic sites (Fujimura & Egger, 2012). Nickel has been shown to inhibit germination of some types of fungi in vitro, though isolates from ultramafic soils were generally more tolerant of heavy metals (Amir & Pineau, 1998). These factors have led to the hypothesis that the below-ground environment is as taxing for mycorrhizal fungi as it is to plants. Several studies have been conducted on the ECM communities of serpentine soils outside Canada, but few have found statistically quantifiable changes in diversity between serpentine and non-serpentine sites (Southworth et al., 2014). Specific mechanisms of ECM fungal success on serpentine soils have also not yet been documented.
2.1.3. Calcareous bedrock and soils

Calcareous bedrock in British Columbia is high in primary carbonate minerals and is found in patches throughout the province (Kishchuk, 2000). Dolostone or marine sedimentary limestone deposits make up the bedrock found underneath the majority of calcareous soils found in British Columbia. The sedimentary deposits in the Fort St. James area date from the Carboniferous and lower Jurassic periods (Plouffe, 2000). Calcareous soils on grassland ecosystems outside of British Columbia have been studied extensively due to their potential value as farm or ranch land (Azcon-Aguilar et al., 1986; Kothari et al., 1991; Azcón & Barea, 1992) but little research has been done on the recently glaciated calcareous soils of central British Columbia, especially those underneath Douglas-fir forest.

2.1.4. Above and below-ground effects of calcareous bedrock

High levels of calcium and high pH within calcareous soils present a difficult growth environment for many plants (Lapeyrie, 1990). Iron deficiency, shown by chlorosis of leaves, is very common in many plants grown in calcareous soils (Loeppert et al., 1994). Plants in this condition are limited in their ability to transport iron from the roots to the leaves and the rest of the plant. This may be a consequence of the formation of iron oxide precipitates due to the alkaline conditions (Mengel and Geurtzen, 1986; Mengel, 1994). Dissolved iron within the soil may also be present in suboptimum concentrations (Lindsay & Schwab, 1982). Calcium coupled with high pH within calcareous soils will lead to the formation of $\text{HPO}_4^{2-}$ ions and other insoluble bindings with phosphates, rendering phosphorus inaccessible to plants (Lapeyrie, 1990; Azcón & Barea, 1992; Kishchuk,
2000). Other trace nutrients such as copper, manganese and zinc are less soluble in calcareous soils due to the formation of chemical complexes and the ionization caused by the increase in pH (Thorne & Seatz, 1955; Marschner & Marschner, 2012).

Mycorrhizal fungi are thought to ameliorate the effects of calcareous soils on plants leading to increased success (Piou, 1979; Li et al., 1991). Phosphorus uptake is greatly increased by the inclusion of ectomycorrhizal fungi (Lapeyrie & Chilvers, 1985). Iron uptake may also be increased by both microbial and ectomycorrhizal mobilization (Szaniszlo et al., 1981). However, the reasons for increased plant success with ectomycorrhizal fungi on calcareous soils have not been fully explored. Individual species or guilds of ectomycorrhizal fungi that contribute to plant health in calcareous soils are also not well characterized (Lapeyrie 1990).

2.2. History of glaciation

2.2.1. Glacial parent material and soils

The passage of the Cordilleran ice sheet shaped the topography and water courses of the study area. Pressure from the ice sheet fragmented and ground down many of the bedrock surfaces and other consolidated deposits in the Fort St. James area (Plouffe, 2001). These fragments were carried by the glacier and deposited as the ice sheet receded. Many deposits are in the form of eskers, the sandy remnants of glacial river beds. Glaciolacustrine deposits consist of sorted and stratified, fine-grained sediments deposited in glacial lakes. Unsorted, blended sediments, gravels, cobbles and boulders that were directly deposited by the glacier are known as till. Material deposited from underneath the glacial are known as basal till, while ablation till was left as the glacier melted (Brady &
Weil, 2008). In plateau lands with gentle topography, the chemical and mineralogical composition of glacial parent materials reflect a blending of sediments and bedrock fragments drawn from across a wide area.

2.3. Sub-Boreal Spruce zone biogeoclimatic zone

2.3.1. Moisture and temperature regime

The Sub-Boreal Spruce biogeoclimatic zone (SBS) encompasses most of north-central British Columbia (Figure 2.3.1.1) and is typified by a continental climate with moist, temperate summers with intermittent precipitation and long cold winters with heavy snow. Summers can reach temperatures of over 30 °C, but usually range from 20-25 °C. Winters have average temperatures of between -10 °C and -20 °C with lows of -40 °C. Precipitation data varies between long term and short term collecting sites, but often ranges between 415 to 1650 mm yearly due to variety of terrain. This precipitation is generally split between snow and rain throughout the year (Meidinger et al., 1991).

Within the study area, precipitation averages approximately 550 mm per year. This is divided into approximately 65% rain and 35% snow. Winters average -9.5 °C with lows of -49.5 °C. Summers average 15.4 °C with highs of 36.7 °C. Mean annual temperature is 3.5 °C with 96 frost-free days (Fort St. James weather station, Environment Canada, 2014).
Figure 2.3.1.1: Study area location with respect to the boundaries of the Sub-boreal Spruce biogeoclimatic zone and the range of Douglas-fir dominated stands.

2.3.2. Plant community structure

The SBS has large conifer populations of hybrid white spruce (*Picea glauca* var. *engelmannii* (Parry) Boivin) and subalpine fir (*Abies lasiocarpa* (Hook) Nutt.). These trees are well adapted to snow loading and can withstand cold, snowy winters. Both are opportunistic colonizers and have a wide range of potential habitats across many different soil types and drainages, but require more moisture than Douglas-fir (Burns & Honkala, 1990). The SBS forest type is typically found between elevations of 500 to 1300 m in the Fort St. James region (Hrinkevich & Lewis, 2011). Historically, lodgepole pine (*Pinus contorta* Dougl. ex. Loud var. *latifolia*) has also had a large presence in the interior with
near monoculture stands on drier areas. However, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) outbreak of the late 1990s and 2000s has killed approximately 90% of all mature lodgepole pine (Safranyik & Wilson, 2007). The species often remains as a component of the understory, especially on drier sites.

Hardwood species are also present in the SBS. Black cottonwood (*Populus trichocarpa* Torr. & Gray), balsam poplar (*Populus balsamifera* L.), trembling aspen (*Populus tremuloides* Michx.) and paper birch (*Betula papyrifera* Marshall) are present in mixedwood stands with many of the conifers in the SBS. Underbrush species are varied within the SBS depending on moisture and light regime. Species include kinnikinnick (*Arctostaphylos uva-ursi* L. Spreng), blueberries and black huckleberries (*Vaccinium* spp.), Devil's club (*Oplopanax horridus* (Sm.) Miq.) highbush cranberry (*Viburnum edule* (Michx.) Raf.) and wild roses (*Rosa* spp.) (Meidinger et al., 1991). Presence of these species is related to elevation and moisture levels at each site. For example, Devil’s club is more common on lower, moister sites, while huckleberries are more common on higher, drier sites.

2.3.3. Douglas-fir in the Fort St. James area

Interior Douglas-fir has the most extensive range of all conifer species west of the Rocky Mountains (Van Hooser et al., 1991). Douglas-fir in the northern part of its range can grow at elevations ranging from 550 m to 2440 m (Hermann & Lavender, 1990). Most stands of Douglas-fir in the study area were found above 750 m (Figure 2.3.3.1). Of the approximately 28 074 hectares of calcareous parent material in the study area, 4812 hectares are Douglas-fir leading stands, and of the 8998 hectares of ultramafic parent
material, 3358 hectares are Douglas-fir leading stands. Douglas-fir naturally regenerates better on areas with litter fall, compared to bare mineral soil (Ryker, 1975). Seedlings range from shade tolerant to shade intolerant (Province of BC, 1995), but generally grow well under semi-open canopy conditions (Day, 1998). Douglas-fir has also been shown to vary genetically both regionally (Hermann & Lavender, 1990) and over environmental gradients as it adapts to changing conditions (Rehfeldt, 1991).

Figure 2.3.3.1: Locations of Douglas-fir stands with respect to elevation within the study area.
2.4. Methods of soil collection

2.4.1. Site selection

Soils derived from three types of parent material were selected for this study: ultramafic, calcareous, and glacial. Six field sites, two for each soil type, were chosen. Field sites were first determined for candidacy by using bedrock geology maps of British Columbia (Plouffe, 2000; Erdmer & Cui, 2009). Sites that showed ultramafic or calcareous bedrock or glacial materials within less than a day of travel from Prince George were identified. Local knowledge from Ms. Joanne Vinnedge (Ministry of Water, Land and Air Protection) was used to isolate sites known to have mature Douglas-fir dominated stands, mostly in the area of Fort St. James. From the subset of sites that met the criteria for soil and tree population, sites that were further than 50 km away from each other were eliminated in order to reduce potential climatic variability (Figure 2.4.1.1). Sites were each visited in-person to determine Douglas-fir presence, accessibility and similarity of site aspect.
2.4.2. Transect layout and soil collection

Composite soil samples were taken from each site to accurately represent the average condition. Three transects 30 m in length were established parallel to the contour spaced at a distance of 5 m at mid-slope. Approximately 4 L of the mineral soil were taken to a depth of 20 cm through both the A and B horizons at 6 m increments. All organic horizons were scraped away prior to collection. Large debris and rocks were removed from the soil in the field. All tools were sterilized with a 10% bleach solution prior to use on each site to prevent cross-contamination. Approximately 60 L of soil was collected per site and placed into sterilized 20 L buckets for transport back to UNBC.
2.4.3. Soil pits and horizon description

Soil pits were dug at all 6 sites to classify the soil into orders. Each pit was dug to a depth of 1 m or until parent material was encountered. A majority of sites allowed for a 1m pit, but some, like Murray-Ridge East had parent material within 75cm of the surface (see Appendix 1). The extent of fine roots was noted throughout both the A and B mineral horizons. Each soil was classified under the Canadian System of Soil Classification with field determination of Order and Great Group. Samples from each horizon were sent to the British Columbia Ministry of Environment Laboratory for determination of exchangeable cations, pH, iron, aluminum, and silicon (by sodium citrate-dithionite, sodium pyrophosphate and acid ammonium oxalate extractions) and particle analysis was conducted by Maxxam Laboratories BC.

While organic horizons were measured and used in the soil classification of each site, organic material was not collected with the mineral soil for the greenhouse bioassay (Chapter 3). ECM populations are known to have differential vertical distributions tied to rooting depth, therefore sampling several of the upper horizons was implemented to increase the chances of a variable population (Pickles & Pither, 2014). Mineral horizons were also chosen to better represent the chemical signature of the soil as organic layers are less influence by parent material.

2.4.4. Soils preparation and analysis

Soils were homogenized and sieved using a 5mm sieve to remove large particulates. A sub-sample of each soil was sent to ALS Geochemistry-Vancouver for whole rock, or total elemental analysis. Major elements were extracted using lithium borate
fusion and measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Trace elements, including chromium, were extracted by the same protocol, but instead inductively coupled plasma mass spectrometry (ICP-MS) was used for detection. Nickel and any remaining metals in the soil were isolated by a four-acid digestion (perchloric, nitric, hydrofluoric and hydrochloric acids) followed by ICP-AES. Samples were also sent to the BC ministry of environment lab to determine cation exchange capacity (CEC) by the BaCl₂ extraction method. Measurements of pH were conducted at UNBC by suspending 10g of soil in water and measuring the pH with a pH meter. This process was also repeated using a 0.01 M CaCl₂ suspension as it gives a slightly lower pH value (Schofield and Taylor 1955).

2.4.5. Statistical analysis of soil data

As each soil sample sent to the lab was a composite, only one measurement was taken. However, for the purposes of comparing sites by parent material, data from sites sharing the same parent material were pooled and compared using a one-way ANOVA (StataCorp., 2011) to determine significant difference between sites \((\alpha=0.05)\). Pair-wise mean comparisons were carried out using Tukey’s honestly significant difference (HSD) post-hoc test \((\alpha=0.05)\).

2.5. Methods of vegetation and forest survey

2.5.1. Site characteristics

Sites were accessed during late September and early October 12-14th 2012 and September 26-28th 2013. Understory vascular and non-vascular plants and mosses overlaying the soil organic horizon were identified using *Plants of Northern British*
The presence and identity of fungal sporocarps were also recorded whenever possible. Lichens were identified on both ground and trees. For all non-tree species, presence and absence were the only measures taken.

2.5.2. Host stand characteristics

Douglas-fir age was determined through the use of an increment borer on at least 10 trees per site. Each core was visually crossdated (Stokes & Smiley, 1968), ring width was measured in the program WinDENDRO (Regent Instruments, 2012) and crossdating was confirmed using the software COFECHA (Holmes, 1983).

Three circular fixed area plots of both 1/40th hectare and 1/250th hectare were used to count stems per hectare on each site. Trees greater than 15 cm in diameter at breast height (DBH) were included in counts for the 1/40th ha, while trees less than 15 cm DBH were counted in the 1/250th ha. All trees within a plot were measured for DBH. Plot level data were transformed to a per hectare basis (one tree on a 1/40th ha is equal to 40 trees per hectare). These values were then summed for each plot and then averaged over the three plots. Basal area was calculated by using the area of a circle formula $A = \pi r^2$. Basal area per tree values were summed and averaged across each site.

2.5.3. Statistical analysis of forest inventory data

Stand data were compared statistically (basal area and trees per hectare), and qualitatively (age classes) between each site (StataCorp., 2011). Mean basal area and mean trees per hectare were compared using a one-way ANOVA to determine significant difference between sites ($\alpha=0.05$). Means were compared using Tukey’s HSD post-hoc test ($\alpha=0.05$).
2.6. Soil analysis

2.6.1. Soil chemical and texture data

Soils derived from ultramafic and serpentinized rocks are expected to have elevated levels of some heavy metals in comparison with soils derived from other forms of parent material. Similarity was found in nickel and chromium concentrations between sites derived from the same parent material (Figure 2.6.1.1). On average, soils developed on ultramafic rocks in this study presented high levels of nickel and chromium, 1383 mg/kg and 3080 mg/kg respectively. These values were significantly higher than the glacial and calcareous derived soils which were well below 200 mg/kg for both metals (Figure 2.6.1.2).

Figure 2.6.1.1: Soil nickel and chromium concentrations for each site.
Both calcareous and ultramafic derived soils shared a higher cation exchange capacity (CEC) than glacial derived soils (Figure 2.6.1.3). These differences are not statistically significant, due to the amount of variation between individual sites (Figure 2.6.1.4). Both ultramafic and calcareous soils differed by over 10 cmol (+) kg\(^{-1}\) between sites.
Figure 2.6.1.3: Soil cation exchange capacity by BaCl₂ extraction for each site.

Figure 2.6.1.4: Mean cation exchange capacity by BaCl₂ extraction of each soil type with standard error of the mean.
Exchangeable calcium: magnesium (Ca:Mg) ratio was relatively low for ultramafic derived soils and high for calcareous soils. Glacial parent material had Ca:Mg ratio between that of ultramafic and calcareous. However, given the very large variation between the calcareous sites (Figure 2.6.1.4), these values do not differ significantly. This variation is due to the difference between the Ca:Mg ratio between both calcareous sites (Figure 2.6.1.5). It is important to note that the low Ca:Mg ratio for Pinchi hill does not reflect a deficit of calcium within the soil, but instead an unusually high level of magnesium (App. 1).

![Graph showing soil exchangeable calcium: magnesium ratio for each site.]

Figure 2.6.1.5: Soil exchangeable calcium: magnesium ratio for each site.
Soil pH differed significantly for all soil types. While no soils in this study were truly alkaline, calcareous and ultramafic derived soils had the highest pH with values of 6.7 and 5.9 respectively. Glacial soil had the lowest pH with a mean value of 5.2 (Figure 2.6.1.8). Sites that shared parent material had very similar pH values compared to each other (Figure 2.6.1.7).
Figure 2.6.1.7: Soil pH (H₂O) for each site.

Figure 2.6.1.8: Mean soil pH (H₂O) with standard error of the mean.
Soil particle size was determined for each homogenized sample (Table 2.6.1.1).

Each soil had a slightly different texture, but all belong to the loamy designation (Soil Classification Working Group, 1998).

Table 2.6.1.1: Soil texture data from each sample location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Parent Material</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuzkwa Rd</td>
<td>Limestone-Calcareous</td>
<td>Silt Loam</td>
</tr>
<tr>
<td>Pinchi Hill</td>
<td>Limestone-Calcareous</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>Spencer's Ridge</td>
<td>Glaciofluvial-Glacial</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td>Tezzeron</td>
<td>Till-Glacial</td>
<td>Loam</td>
</tr>
<tr>
<td>Murray Ridge - East</td>
<td>Ultramafic</td>
<td>Loam</td>
</tr>
<tr>
<td>Pinchi Mountain</td>
<td>Ultramafic</td>
<td>Clay Loam</td>
</tr>
</tbody>
</table>
2.7. **Forest overstory and understory data**

2.7.1. Douglas-fir stand age, basal area, and understory data

Almost all sites sampled had one or more age classes represented. Pinchi Mountain is the exception from this list (Table 2.7.1.1). This variety in ages suggests that there were several periods of disturbance, removing almost all mature trees within the stand, leaving only a few survivors. In the case of Murray Ridge, this disturbance history resulted in 4 separate cohorts of trees, with some dating as far back as 1596.

Dates reported here do not include missing rings when pith was missed. This underestimates the age of trees when pith was missed by 5 to 15 years. Cohorts were bounded by trees where pith was hit, allowing for accurate dating of the tree's establishment. The designation of age cohorts is such that trees with missing rings that were dated a decade older than cohort boundaries were placed into the next cohort.

<table>
<thead>
<tr>
<th>Location</th>
<th>Cohort A</th>
<th>Cohort B</th>
<th>Cohort C</th>
<th>Cohort D</th>
<th>Cohort 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuzkwa Rd</td>
<td>1893-1907</td>
<td>1814-1833</td>
<td>1778</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pinchi Hill</td>
<td>1898-1915</td>
<td>1798-1837</td>
<td>1730-1774</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tezzeron</td>
<td>1895-1918</td>
<td>1716</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Murray Ridge - East</td>
<td>1851-1877</td>
<td>1836</td>
<td>1780-1792</td>
<td>1596-1650</td>
<td>-</td>
</tr>
<tr>
<td>Pinchi Mountain</td>
<td>1895-1909</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spencer's Ridge</td>
<td>1900-1910</td>
<td>1734</td>
<td>-</td>
<td>-</td>
<td>1947</td>
</tr>
</tbody>
</table>
Vegetation surveys of all field sites showed similarity in the types of overstory and understory trees present. Douglas-fir, hybrid white spruce (*Picea glauca var. engelmannii* (Parry) Boivin), trembling aspen (*Populus tremuloides* Michx.) and lodgepole pine (*Pinus contorta* Dougl. ex. Loud var. *latifolia*) were found on all sites (Table 2.7.1.2). Understory plants including highbush cranberry (*Viburnum edule* (Michx.) Raf.), wild rose (*Rosa* spp.) and a variety of *Vaccinium* species (Table 2.7.1.3-4). All sites had dominant forest of conifers with some level of understory represented. While ultramafic sites did not lack in the number of species found, these areas did appear more scrub-like, with smaller plants of the same species when compared to other sites. Of the fungal sporocarps noted, few were ectomycorrhizal and are therefore not reported.

Table 2.7.1.2: Selected vegetation survey results, trees (+' indicates presence).

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Common Name</th>
<th>Kuzkwa Rd</th>
<th>Pinchi Hill</th>
<th>Spencer's Ridge</th>
<th>Tezzeron Ridge</th>
<th>Murray Ridge</th>
<th>Pinchi Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Abies lasiocarpa</em> (Hooker) Nuttall</td>
<td>Subalpine Fir</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Acer glabrum</em> Torr.</td>
<td>Douglas Maple</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Alnus viridis</em> subsp. <em>sinuata</em></td>
<td>Sitka Alder</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Betula papyrifera</em> Marshall</td>
<td>Paper Birch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Picea engelmannii</em> × <em>glauca</em></td>
<td>Hybrid White Spruce</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pinus contorta</em> subsp. <em>latifolia</em></td>
<td>Lodgepole Pine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus pensylvanica</em> L.f.</td>
<td>Pin Cherry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Prunus virginiana</em> L. <em>Pseudotsuga menziesii</em> var. <em>glauca</em> (Beissn.) Franco</td>
<td>Chokecherry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2.7.1.3: Selected vegetation survey results, shrubs (‘+’ indicates presence).

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Common Name</th>
<th>Kuzkwa Rd</th>
<th>Calcareous</th>
<th>Glacial</th>
<th>Serpentine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arctostaphylos uva-ursi</em> L.</td>
<td>Kinnikinnick</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Spreng</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Cornus canadensis</em> L.</td>
<td>Bunchberry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Fragaria virginiana</em> Duchesne</td>
<td>Strawberry</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>Goodyera oblongifolia</em> Raf.</td>
<td>Western</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Goodyera repens</em> (L.) R. Br.</td>
<td>Dwarf</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Pyrola asarifolia</em> Michx.</td>
<td>Bog</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Maianthemum racemosum</em> (L.) Link</td>
<td>False</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

53
Table 2.7.1.4: Selected vegetation survey results, herbaceous plants ('+' indicates presence).

<table>
<thead>
<tr>
<th>Latin Name</th>
<th>Common Name</th>
<th>Kuzkwa Rd</th>
<th>Pinchi Hill</th>
<th>Spencer's Ridge</th>
<th>Tezzeron</th>
<th>Murray Ridge</th>
<th>Pinchi Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Spiraea betulifolia</em> L.</td>
<td>Birch-leaved Spirea</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Ribes lacustre</em> (Pers.) Poir.</td>
<td>Prickly Currant</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>Rosa acicularis</em> Lindl.</td>
<td>Prickly Wild Rose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Rubus parviflorus</em> Nutt.</td>
<td>Thimbleberry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Salix sp.</em></td>
<td>Willow</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Shepherdia canadensis</em> (L.) Nutt.</td>
<td>Soopolallie</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Vaccinium caespitosum</em> Michx.</td>
<td>Dwarf Bilberry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Vaccinium membranaceum</em> Douglas ex Torr.</td>
<td>Black Huckleberry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Viburnum edule</em> (Michx.) Raf.</td>
<td>High Bush Cranberry</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Stand density tended toward higher values for glacial derived sites compared to calcareous and ultramafic derived sites. Both Murray Ridge and Pinchi Mountain have a significantly lower number of stems per hectare (all species) than the Tezzeron glacial site. While calcareous and ultramafic sites did display lower stand density, the data are variable. This variability is best shown on the Tezzeron site which while having the largest count, also had the greatest standard deviation (Figure 2.7.1.1).

Figure 2.7.1.1: Stand density for all study sites. Groups that do not share letter designations are significant.
Mean basal area (all species) differed between field sites, with higher values on glacial sites compared to both ultramafic and calcareous derived sites. However, no significant differences were found between sites. Standing dead basal area of lodgepole pine and Douglas-fir is also reported for Spencer’s Ridge as it had a large proportion of dead trees present. All other sites had small numbers of dead trees present as wind-throw (Figure 2.7.1.2).

Figure 2.7.1.2: Mean basal area of all trees per site (Douglas-fir, hybrid white spruce and subalpine fir-including standing dead) showing standard deviation.
2.7.2. Evidence of fire

Figure 2.7.2.1 shows a photograph of a standing dead, fire-killed stump on Pinchi hill. The trees standing to the left and right of the fire-killed stump were cored and crossdated. Since both younger trees originated between 1898-1897, it can be inferred that the fire removed most of the overstory and younger, weaker trees prior to the sampled trees establishment. Given the silvics of Douglas-fir, this fire can be dated 5 to 15 years previous, in the early 1890s or mid to late 1880s.

Figure 2.7.2.1: Photo of a standing fire-killed tree (Pinchi Hill) with younger regenerated trees. Above are the two cores from the trees standing to the left and right which established post-fire.
2.8. Discussion of field data

2.8.1. Soil properties

Sites with soil developed on parent material with distinctive chemical signatures reflected those chemical signatures within the horizons sampled in this study. Ultramafic-derived soils had the characteristic presence of high levels of nickel and chromium (Figures 2.6.1.1 and 2.6.2.2). Both Murray Ridge and Pinchi Mountain also had the low calcium to magnesium ratio expected of ultramafic soils. Calcareous-derived soil in contrast to ultramafic soil had very high calcium to magnesium ratio (Figures 2.6.1.5 and 2.6.1.6). Both calcareous and ultramafic soils had much higher pH than glacial-derived sites (Figures 2.6.1.7 and 2.6.1.8).

These variations within the soil chemical conditions are known to contribute to stress on the plants that grow on these soils (Kruckeberg, 1979; Mengel and Geurtzen, 1986; Pillon et al., 2010). In contrast to the “barrens” described in serpentine areas in other countries, and the harshness of calcareous areas, the sites sampled in this study had mature forests comparable to each other and surrounding ecosystems found within central British Columbia.

2.8.2. Forest conditions

Much of the above-ground data on the established forest indicates that all sites have experienced disturbance at one time or another. Logged stumps and presumed remains of old skid roads point to logging around the turn of the 20th century. The Fort St. James area has been influenced by European settlers for the past 200 years, leading to highgrading of stands (removal of the largest, most desirable trees) which creates patchy stands of trees
(MacGregor, 2002). However fire is likely the main historical cause of the variety of age classes found at the field sites that pre-date settlement. The characteristic variability of fire may have left patches of forest unburned. Larger, more robust trees may have been better able to withstand the flames. These trees would in turn provide the seed source for the next generation of trees and retain ECM inoculum within their root systems.

The small scope of this study can provide limited inference about the expected differences between forest ecosystems that grow on extreme soils. However, given the above data on trees per hectare and mean basal area per site, a more in-depth look at soil origin may be warranted. While the data are extremely variable, on-site visual surveys of vegetation and assessments of tree volume show that with a larger data set, significant differences between sites with a milder parent material (non-ultramafic and non-calcareous) may become apparent.

Knowledge of the limitations of sites depending on soil type could be helpful in informing forestry practices on treatment of specific sites. For example, a site that is ultramafic or calcareous may not produce the same volume of wood in the same timeframe as glacial soils. If that is known to forest planners, harvesting and replanting schedules may be modified to account for the expected changes. Ultramafic and calcareous soils may also host unique communities of below-ground organisms, including ECM fungi, which may not be apparent to the above-ground observations.

2.8.3. The use of sites of the same parent material as replicates

While sites did segregate according to parent material, data were variable for measures including cation exchange capacity (Figure 2.6.1.3), Ca:Mg ratio (Figure 2.6.1.5) and soil texture (Table 2.6.1.1). Sites that shared parent material often had differences in
one or more of these measures. For instance, Kuzkwa Rd and Pinchi Hill are a silt loam and a sandy loam respectively. Pinchi Hill will therefore have a higher level of drainage and potentially less nutrients within the soil compared to Kuzkwa Rd (Schaetzl & Anderson, 2005) even though they are both calcareous soils. Likewise both ultramafic sites have different textures with Murray Ridge-East having a loam texture and Pinchi Mountain having a clay loam texture. In this case, the clay in the Pinchi Mountain soil will cause increased water holding (Brady & Weil, 2008) compared to Murray Ridge-East. The change in nutrient balance and water holding capacity may impact the health and success of plants grown in those soils and may also impact the composition of the below-ground fungal communities. For this reason, while sites share parent material, they are not true replicates of one another and can be compared in general terms, but not pooled for statistical comparisons.

2.9. Summary

This study provides a first comparison of the strongly contrasting chemical differences found on the soils of the Fort St. James area. No other study in this region links the below-ground chemical and physical differences in soil to the above-ground conditions. Ultramafic soils present potentially toxic levels of chromium and nickel to Douglas-fir and the ecosystems found in the Fort St. James area, but these plant communities do not show the same level of stress seen outside of Canada. Mature trees are still capable of growing and establishing stands that appear to be only slightly less vigorous than comparable glacial sites. Calcareous soils likewise have extremely high levels of calcium within the soil, but do not show depauperate landscapes compared with glacial areas.
The lack of strain apparent on these ecosystems may be related to several ameliorating factors. The geological youth of these sites may mean that the soils have not had time to develop the same level of chemical signature compared to older areas in Europe and the Southern United States. As the individual sites with Douglas-fir represent drier areas, it is possible that the trees are more responsive to the favourable climate than to the individual soil conditions. The combination of drier climate, slope position and glacial blending of extreme parent materials and mixed sediments may have produced soil that is balanced between chemical harshness and beneficial conditions (e.g. well drained and enriched with organic material). It is also possible that the presence of ECM fungal partners and rhizosphere organisms may be acting as a first line of defence preventing uptake of damaging levels of calcium, nickel and chromium. In the following chapter, these communities of ECM fungi will be compared to ascertain if strongly contrasting soil signatures produce equally contrasting populations of ECM fungi.
2.10. References


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3. Characterization of *Pseudotsuga menziesii* var. *glauca* ectomycorrhizal fungi communities on ultramafic and calcareous soils in central British Columbia

3.1. Douglas-fir fungal partners

3.1.1. Douglas-fir and ECM in the field

Douglas-fir is an important part of western interior forest ecosystems from Mexico to central British Columbia. It is also known to form ectomycorrhizas (ECM) with a variety of species of fungi depending on the region (Smith *et al.*, 2002). In field studies, Douglas-fir has been found in partnership with members of the genera *Rhizopogon*, *Cenococcum*, *Russula*, and others (Trappe, 1977; Molina *et al.*, 1992; Goodman & Trofymow, 1998). ECM fungi are vital to the survival of Douglas-fir and may help in the perpetuation of stands over time (Simard, 2009). ECM species may be segregated by depth within the soil column and will differ over a variety of soil conditions (Pickles & Pither, 2014; Walker *et al.*, 2014).

In field studies of disturbed areas (e.g. logged areas, severe windthrow), a few morphotypes (a grouping based on morphology) are usually dominant, with a variety of ECM species that are present in low levels (Lang *et al.*, 2011; Pickles & Pither, 2014). Some of the common symbionts of Douglas-fir in disturbed areas include E-strain (usually of the genus *Wilcoxina*), MRA (*Mycelium radicis atrovirens*), and *Cenococcum geophilum*, all of which are broad host range fungi and capable of colonizing other hosts (pine and spruce) (Jones *et al.*, 1998; Barker *et al.*, 2013).

Areas that are not recently disturbed may produce a larger variety of morphotypes and species due to the retention of a living mycorrhizal network (Smith & Read, 2008).
study by Smith et al. (2002) of ECM fungal sporocarps in younger and old growth Douglas-fir stands in Oregon found that as stand age increased, so too did the likelihood of finding unique or rare species. The authors postulated that this might be due to the age of the stand, producing conditions favorable to ECM fungi that fruit rarely. In mature stands of Douglas-fir, the age of the root system may alter the composition of the community (Smith et al., 2002).

This diversity within the community, however, does not usually aid in differentiation between sites as rare morphotypes are not easily included in statistical analysis (Robertson, 2003; Branco & Ree, 2010). In morphotyping studies, rare types are often recorded and described, but not included in statistical analyses (Jonsson et al., 1999). However, while these tips do not help distinguish between sites or treatments in a quantitative fashion, they are informative from a community composition standpoint and have value in both field and greenhouse studies.

3.1.2. Douglas-fir and ECM in greenhouse conditions

Douglas-fir is often grown in a nursery under greenhouse conditions to produce seedlings for tree planting in forestry. Greenhouse Douglas-fir is often reticent to form mycorrhizas and may be very slow to achieve colonization, taking over 10 months (Kazantseva et al., 2010; Pickles et al., 2015). Species found in greenhouse studies mirror those in the field, but rare species are not as common. Often the community will show stronger levels of colonization, but a slightly less diverse group of species (Branco, 2010). Species often include E-strain and Rhizopogon spp., however, greenhouse contaminants from existing spore loads can also form ectomycorrhizas with Douglas-fir. For example, members of the Thelephora genus are often found in both the field and greenhouse settings.
High rates of ECM colonization are helpful in ascertaining potential differences between sites as it enables a greater statistical comparison of ECM communities. A much higher number of root tips are generated; higher numbers in turn increase statistical power (Magurran, 2004). While some species of ECM may not be as prevalent, or as visible due to absence of pre-existing hosts (Jones et al., 1998), growing ECM and their hosts in a nursery can provide higher resolution for the few types of fungi that may be more prevalent. ECM root tips generated from a greenhouse study also can be quickly processed and analyzed using morphological and molecular techniques (see section 1.4.3) in a controlled environment.

3.2. Methods of greenhouse bioassay

3.2.1. Seed preparation

Seedlot provenances (CONIFEX 53613 and 30822) were selected from trees within 50 km of the Fort St. James area (CONIFEX, Fort St. James). Parent trees from these seedlots were located in areas overlain with deeper glacial deposits from the late Wisconsinan Fraser Glaciation (Plouffe, 2000). Lack of strong chemical signatures within the soil minimized the risk of planting with stock that has had selective environmental pressures from soil chemical properties, such as elevated heavy metal concentrations.

All Douglas-fir seeds were surface sterilized to remove potential pathogens prior to planting. Seeds were submerged in 3% H₂O₂ with light agitation for 2 hours to ensure no contaminants remained on the seed coat. Autoclaved deionized water was used to triple rinse the seeds prior to soaking in fresh sterile deionized water for 24 hours. Seeds were
triple rinsed again with sterile water, dried with paper towels until seeds no longer adhered to one another and stored in sterile jars in a 2°C refrigerator for 21 days to cold stratify them to ensure germination (Kolotelo et al., 2001). Pre-soaked weight was recorded and post-soaked weight was monitored weekly to ensure the seeds did not desiccate prior to planting.

3.2.2. Seedling growth conditions

Following soil collection and transport to UNBC (see section 2.4.2), samples were stored at 4°C to preserve the fungal propagules within the soil until use. Soils were sieved through a 5mm mesh to remove pebbles and coarse woody debris. Homogenization of the soil was conducted with sterile equipment to ensure an even distribution of fungal inoculum within the soil sample. Homogenized soil was potted into 262mL Cone-tainer™ cells.

All cells were treated with AC Vertex™ fungicide prior to planting to remove any greenhouse contaminants. Potting tools were also treated with AC Vertex™ fungicide between site soils to prevent cross contamination. All tools and cells were well rinsed to remove residual fungicide prior to planting. The bottom of each cell was filled with 9 autoclaved sterile clay balls to ensure proper drainage. Each soil was potted by hand into 160 cells, 80 cells for each Douglas-fir seedlot. Cell trays were shaken to ensure even distribution of soil and then tamped down with a wooden dowel to remove air pockets. Each cell was planted with one seed and topped with a layer of heat-treated and rinsed forestry sand (coarse grit) to weigh seeds down during watering. In total, 960 cells were filled and planted on December 5, 2012.

Seeds were germinated under sodium lamps in the University of Northern BC
Enhanced Forestry Laboratory greenhouse-Pod B. Germination temperature was 25°C with a 16 hours light (day) and 8 hours darkness (night) regime. Light began at 8:00 am and finished at 10:00 pm to match the time of sunrise. Trays were watered on a 1 to 2 day schedule during germination and placed on a greenhouse grid bench to allow for complete drainage. Water pressure was reduced by using a 1 gallon/minute mist-head hose to prevent seeds from being dislodged from under the forestry sand. Germination was complete by January 18, 2013.

Counts were taken of the seedlings as germination progressed. Death of new seedlings and failure to germinate were also recorded. Temperature in the greenhouse pod was reduced to 22°C to prevent stressing the seedlings. Watering was also reduced to twice per week after germination to reduce the risk of damping off. Reducing the amount of water in the soil helps to prevent damping off as the microorganisms responsible for damping off thrive in wet conditions (Hartley & Pierce, 1917).

Watering was changed to a 2 gallon/minute rain shower head six weeks after planting. Seedling trays were rotated within the greenhouse pod to moderate any potential lighting or temperature anomalies within the pod. Periodic counts of seedlings still living were conducted to assess survival rate until the time of biomass sampling. Observation of yellowing foliage in May 2013 after 5 months of growth necessitated the application of water-soluble Tune-up fertilizer. This fertilizer has a 20-10-20 ratio of nitrogen, phosphorus and potassium (200 ppm, 100 ppm, 200 ppm respectively). Watering with fertilizer was conducted once weekly for a period of 2 months until foliage returned to green.
3.2.3. Seedling growth and biomass

Seedlings ($n=594$) were destructively harvested for biomass sampling at 10 months (40 weeks) of age. Height and mean basal diameter were recorded to the nearest millimeter and the nearest hundredth of a millimeter respectively using a standard 30 cm ruler and digital calipers. Root and shoot biomass were assessed by separating the roots and shoot of each seedling at the root collar and drying at 70°C for three days (Hagerman & Durall, 2004). Seedling roots and shoots were weighed separately on a Sartorius Micro scale to the nearest milligram. Root and shoot weights were added together to produce total biomass for each seedling.

3.2.4. Collection of seedling foliar samples for chemical analysis

After seedlings were oven-dried, a sub-sample of needles was harvested to quantify potential uptake of metals from the soil to the seedlings. Two grams of needles were required per sample, each made up of composites from multiple trees grown on each soil type. Microwave digestion was used to prepare samples for inductively coupled plasma (ICP) spectrometry. Two samples were analyzed for each site (12 in total) at the BC Ministry of Environment Analytical Chemistry Laboratory.

3.2.5. Mycorrhizal colonization

Seedlings take weeks to months to become fully colonized by ectomycorrhizal fungi (Erland & Finlay, 1992) and greenhouse grown Douglas-fir is especially reticent to form ECM associations (Castellano & Molina, 1989; Kazantseva et al., 2010). Periodic checks for fungal gnats were used as a non-invasive method of checking for colonization. The gnats feed on the fungi and only appear after the trees are fully colonized (H.
Massicotte, pers. comm. 2013). After 7 months of growth, a few gnats were observed and 3 seedlings were randomly selected to assess the progress of fungal colonization. These seedlings were gently removed from the cell to not disturb any fungal tissue on the roots. The roots were gently washed in cool tap water and fine roots were removed. Any lateral root tips that showed any sign of colonization were also removed.

A dissecting microscope was used to assess the presence or absence of fungal tissue on the roots. Squash mounts of root tips were also used to assess the thickness of any fungal mantles or hyphae. Due to the very low number of observed fungal tips (less than 10%), the seedlings were allowed to grow 3 months longer. More fungal gnats were observed in the greenhouse at this time, and a single seedling was subsampled to check for fungal colonization. A much higher level of colonization was detected (approximately 80%) and both morphotyping and biomass sampling was initiated.

3.2.6. Morphology analysis

Morphotyping is the process of identifying ectomycorrhizal fungal types using a set of microscopic characteristics. From 714 surviving seedlings, 120 were randomly subsampled for morphotyping, 10 from each seedlot and soil type. Each seedling was removed from the cell and gently shaken to remove the majority of soil particles. Roots were rinsed under cool free flowing tap water to dislodge the remaining soil. A stainless steel dish was filled with tepid deionized water and the roots were soaked with gentle agitation for 1-2 minutes at a time to ensure the roots were completely clean. The soaking process was repeated until the water remained clear (Massicotte et al., 1994).

Cleaned roots were separated from the shoot at the root collar and floated in deionized water in a stainless steel dish with a numbered centimetre grid in the bottom.
Grid patches were selected using a random number list that corresponded with the numbers on the grid. Larger roots were clipped and the root mass was dispersed evenly over the grid. Approximately 200 lateral and fine roots were cut from the root mass in 2 cm patches and placed in a glass petri dish filled with deionized water. Remaining roots were stored in the stainless steel tray in a 2°C refrigerator in case additional sampling was required, whether due to an incorrect number of tips or the discovery of a rare morphotype. A dissecting microscope was used to assess the visual traits with the tips floating in deionized water in a petri dish. The entire root system of a seedling was counted if the total number of roots was less than 200. During the course of the experiment, 23039 root tips were counted and morphotyped (Ingleby et al., 1990; Goodman et al., 1996; Agerer, 1987-2008).

Each root tip was counted as either uncolonized or colonized. Uncolonized root tips either had mature root tissue and no evidence of fungal hyphae, very young and fast growing meristematic tissue, or a layer of unorganized fungal hyphae too thin to measure or visualize using a squash mount. Colonized tips were divided into morphotypes on the basis of visual and microscopic traits. Colour was assessed first as it is one of the simpler traits to categorize. Luster, or how the root tip reflects light, is also a diagnostic feature. Many types of mycorrhizal fungi have distinct emanating hyphae. The branching pattern of the root tip, whether it is single, clustered, or coralline in shape is also diagnostic (see Appendix 3 for full descriptions).

A compound microscope was used to view the cellular details of several representative root tips from each root system. Wet squash mounts were prepared using a single root tip. Fungal mantles are made up of many layers of hyphae with particular shapes that distinguish one from another. Mantles differ in cells interlocking with one
another, non-interlocking, have a net-like shape, or a stellate pattern. Fungal mantles or hyphae may be studded with crystalline exudates of many colours. Presence of hyphal clamps or anastomoses between hyphae also helps differentiate between morphotypes. Presence or absence of cystidia or hyphae, along with the hyphal and cell size, can help separate morphotypes as well. Root tips were assigned a particular morphotype based on a combination of the above characteristics, using the *Colour Atlas of Ectomycorrhizae* (Agerer, 1987-2008) and *Identification of Ectomycorrhizas* (Ingleby et al., 1990) labeled as Types A-O, and counted.

3.2.7. Statistical analysis of biomass and morphology data

Seedling biomass measurements (height, basal diameter, root:shoot ratio and total biomass) were compared using statistical methods (StataCorp., 2011). A one-way ANOVA was used to determine significant differences between sites (α=0.05). Pair-wise mean comparisons were made using Tukey’s honestly significant difference (HSD) post-hoc test (α=0.05). Seedlot was not included in the analysis as a preliminary data check produced no differences in any data measures (biomass or morphology), between seedlots.

Morphotypes were counted and frequency (number of seedlings with individual morphotypes) and percent abundance (proportion of the morphotype in the entire seedling community) were calculated using the total sample and partitioned site values. From the morphotype totals, rank abundance curves were generated using the log transformed relative abundance (number of root tips across entire study) of each morphotype plotted against morphotype rank as a comparison of ECM communities between sites (Robertson, 2003; Kindt & Coe, 2005). Mean morphotype abundance values were calculated (averaged across sites) and compared using a one-way ANOVA to determine the significant
difference between sites ($\alpha=0.05$). Pair-wise mean comparisons were made using Tukey's HSD post-hoc test ($\alpha=0.05$).

Several diversity indices were used to compare differences between ECM communities on different sites. These diversity indices are non-parametric and rely on counts of species and abundance data. For all indices, a high value is indicative of higher diversity or richness. Indices used were the Shannon, Shannon-Evenness, Margalef and the Gini-Simpson. The Shannon index measures species richness and relative abundance, while the Shannon-Evenness index is only a measure of relative abundance. The Margalef index is a measure of species richness and the Gini-Simpson index measures relative abundance, but is more sensitive to abundant types (Magurran, 1988, 2004). Diversity index values were generated for each site using pooled morphology data. For comparison, diversity index values were calculated using each seedling, then averaged across sites to generate mean index values. Mean index values were then compared using a one-way ANOVA with a post-hoc Tukey's HSD test.

Sites were compared using morphology data to generate clusters using a two-way cluster analysis in PC-ORD (McCune & Mefford, 2006). The amount of root tips for a particular morphotype was relativized using the maximum number of root tips for that type. Dendrograms were constructed using a farthest neighbour joining tree with both a presence-absence matrix and a relative abundance matrix.

3.3. Methods of molecular analysis

3.3.1. DNA extraction

Representative root tips of each morphotype were collected for DNA extraction
during the morphotyping process. Only root tips with healthy, distinct mantles and little to
no evidence of secondary fungal colonization were selected. Each tip, or cluster of tips,
was removed from the root mass and dried on clean paper towel before being placed into
an autoclaved Eppendorf tube. The tubes were stored in a -20°C freezer until DNA
extraction could begin. Due to the poor quality of the MRA or O type morphotype, no root
tips were deemed suitable for DNA extraction.

A pilot study was conducted using sporocarp tissue and DNeasy Plant Mini Kit
from Qiagen. This protocol involved lyophilizing the tissue using liquid nitrogen and a
mortar and pestle. Due to poor yields with the DNeasy Plant Mini Kit, the MO BIO
PowerSoil® DNA Isolation Kit was tested as a possible replacement. When the MO BIO
PowerSoil® DNA Isolation Kit was used on similar sporocarp tissue, the yields were
superior to the pilot study and this kit was adopted.

The low amount of fungal tissue on root tips and the toughness of fungal mantles
initially made it difficult to extract a useful amount of DNA from the root tips. After
researching a variety of methods, an additional heat step and an altered bead beating step
were added to the MO BIO PowerSoil® DNA Isolation Kit protocol (Koide, 2005). Prior
to bead beating (agitation with beads to lyse the cells), the reaction tubes were placed in
boiling water for 10 minutes to increase the activity of the cell lysis buffer. Two 2mm
zirconium oxide beads were added to the garnet beads in each reaction tube to crush the
fungal cells as well as cut them. This increased the amount of DNA released into solution.
Extracted DNA concentration was measured on a Thermo Scientific™ NanoDrop 1000.

3.3.2. DNA amplification

Fungal DNA extracts were used as the template for amplification through the
polymerase chain reaction (PCR). Initially a master mix protocol was implemented, using aliquots of Taq polymerase, dNTPs (ATP, GTP, TTP, CTP), MgCl₂, 10x buffer and nuclease free water, plus ITS3 primer and NLB4 primer (Kennedy et al., 2015) for a 25μL reaction with 1μL of template. Reactions were run in a BioRad DNA Thermocycler for the following times: Step 1: 95 °C for 5 minutes; Step 2: 94 °C for 30 seconds; Step 3: 55 °C for 30 seconds; Step 4: 72 °C for 45 seconds; Step 5: repeat step 2-4 40 times; Step 6: 72 °C for 5 minutes; Step 7: hold at 4 °C.

Resulting amplifications were visualized on a 1% agarose gel, made from 0.4g agarose, 40mL TBE buffer and 0.5 μL EtBr. Amplicons, or product DNA samples, from the initial reaction were extremely poor, producing smears on the agarose gel. BSA (bovine serum albumin) was added to the master mix, which helped improve yields (Kreader, 1996; Farell & Alexandre, 2012), however, the results were still highly variable. Due to this variation, Fisher Scientific 5 PRIME™ MasterMix™ PCR Mix was used instead of lab-made master mix. Fisher Scientific 5 PRIME™ MasterMix™ PCR Mix also produced less than adequate amplification and also necessitated the adding of BSA. All reactions were run as duplicates to increase the amount of DNA amplicon produced. In an effort to increase DNA yields, reaction times were also altered to: Step 1: 95 °C for 5 minutes; Step 2: 95 °C for 1 minute; Step 3: 52 °C for 1 minute; Step 4: 72 °C for 1 minute; Step 5: repeat step 2-4 29 times; Step 6: 72 °C for 5 minutes; Step 7: hold at 4 °C.

Samples that showed only one clear band when visualized on agarose were purified using QIAquick PCR Purification Kit. Samples that showed two or more bands were set aside for gel purification. A large 2% agarose gel was used to visualize the separate bands. Each separate band was cut out of the gel and purified by a QIAquick Gel Extraction Kit.
DNA concentration was determined by the use of both a NanoDrop 1000 and a Qubit® 2.0 Fluorometer.

3.3.3. DNA sequencing

Purified samples were sent to the UNBC Genetics lab where they were sequenced using an Applied Biosystems 3130xL. A total of 166 representative morphotype DNA samples were sent for sequencing. Samples that were successfully sequenced were checked using the CodonCode software (Pignone et al., 2006; CodonCodeCorperation, 2014) and identified using The Basic Local Alignment Search Tool (BLAST). Samples with a lower than 85% similarity match to GenBank sequences were discarded (Madden, 2002).

3.4. Experimental results

3.4.1. Growth and biomass of seedlings

Greenhouse grown Douglas-fir seedlings were measured for mean basal diameter prior to destructive sampling. Murray Ridge and Pinchi Hill were the only sites that were significantly different from all other groups (Figure 3.4.1.1). All measures of mean basal diameter were significantly different between sites that shared parent material.
Figure 3.4.1.1: Mean basal diameter of greenhouse grown Douglas-fir seedlings with standard deviation. Groups that do not share letter designations are significantly different ($p < 0.001$, $F = 58.05$, $n=594$).

Douglas-fir seedling height differed numerically between sites, with tallest seedlings on glacial soil and shortest on calcareous soil (Figure 3.4.1.2). Pinchi Hill was the only site that was significantly different from all others. Both ultramafic sites were not significantly different from each other, as were both glacial sites. However, Tezzeron (glacial) was also non-significant with Murray Ridge (ultramafic) and Kuzkwa (calcareous) was non-significant to both ultramafic sites.
Figure 3.4.1.2: Mean seedling height of greenhouse grown Douglas-fir seedlings with standard deviation. Groups that do not share letter designations are significantly different (p < 0.001, F=60.92, n=594).

Total biomass measurements were significantly different between groups that shared parent material, meaning that each pair of soil types (ultramafic, calcareous, and glacial) did not have similar biomass measurements (Figure 3.4.1.3). Tezzeron and Kuzkwa seedlings were not significantly different from each other, as were Spencer’s Ridge and Pinchi Mountain. Only Murray Ridge and Pinchi Hill were significantly different than all other seedlings. Pinchi Hill had the lowest amount of total biomass with a mean value of 1.3 g. Spencer’s Ridge and Pinchi Mountain had the highest numeric values, with 5.32 g and 4.85 g respectively.
Figure 3.4.1.3: Mean total biomass of greenhouse grown Douglas-fir seedlings with standard deviation. Groups that do not share letter designations are significantly different \( (p < 0.001, F= 118.33, n=594) \).

Root-shoot ratio was significantly different between soils sharing calcareous and ultramafic parent material, but did not differ significantly between glacial soils. Pinchi Hill and Murray Ridge were also non-significant compared to each other and to glacial soils. Pinchi Mountain and Kuzkwa were both non-significant to each other, but significantly different from the previous four sites (Figure 3.4.1.4). The evenness between root and shoot values masks the large difference in total biomass between all sites (Figure 3.4.1.5)
Figure 3.4.1.4: Mean root:shoot ratio of greenhouse grown Douglas-fir seedlings with standard deviation. Groups that do not share letter designations are significantly different (p <0.001, F= 14.22, n=594).

Figure 3.4.1.5: Mean root and shoot weight as part of total biomass of greenhouse grown Douglas-fir seedlings (n=594).
3.4.2. Foliar metal content of seedlings

Given the mass of tissue required for foliar metals analysis, it was not possible to generate enough replicate samples for an ANOVA. However, the data do manifest some numerical trends. Foliar active iron for all sites ranged from 64.8 mg/Kg to 97.4 mg/Kg with one exception. Pinchi Hill had the lowest level of active iron, with 39.5 mg/Kg (Figure 3.4.2.1).

Soils with both calcareous and glacial parent material produced seedlings with very low levels of nickel and chromium (<1.2 mg/Kg). Murray Ridge-East and Pinchi Mountain seedlings had nickel levels of 18.89 and 17.17 mg/Kg respectively (Figure 3.4.2.2). Needle chromium levels were likewise high on ultramafic derived sites compared to glacial and calcareous (Figure 3.4.2.3) with Murray Ridge levels at 1.2 mg/Kg and Pinchi Mountain levels at 1.07 mg/Kg.

![Mean foliar active iron levels from greenhouse grown Douglas-fir seedlings with standard deviation (n=2).](image-url)
Figure 3.4.2.2: Mean foliar concentrations of nickel from greenhouse grown Douglas-fir seedlings with standard deviation (n=2).

Figure 3.4.2.3: Mean foliar concentrations of chromium from greenhouse grown Douglas-fir seedling with standard deviation (n=2).
3.4.3. ECM abundance and frequency

Overall, 15 morphotypes were described based on a total of 120 seedlings, representing 22039 root tips. Of those 15 morphotypes (Appendix 3), 12 were basidiomycetes and 3 were ascomycetes. Many morphotypes were identified to the genus level including *Cenococcum*, *Rhizopogon*, and *Tuber*. Some of the morphotypes were only identifiable to families, such as Thelephoraceae. One morphotype, name Blonde because of a pale, thready mantle, was not identifiable to any taxonomic level. Of all root tips studied, only 12% (n= 2641) were classified as non-mycorrhizal. Pinchi Hill (calcareous) had the highest frequency of seedlings with uncolonized tips (90%) while Kuzkwa (calcareous) had the lowest frequency (50%).

Only three morphotypes (*Cenococcum geophilum*, *Rhizopogon cf. villosulus*, and E-strain) were ubiquitous on all soil types and present on a large number of seedlings. Of the three, E-strain was the most prevalent, found on 94.2 % of all seedlings morphotyped (Table 3.4.3.1). *Rhizopogon cf. villosulus* was the next most prevalent (80%) and *Cenococcum geophilum* was the third most prevalent (40%). All other morphotypes were found only on 6 seedlings or less and are considered rare.
Some morphotypes were limited to a few seedlings, but present in fairly large numbers, like the *Hebeloma-Amphimena* type (4 seedlings, 295 root tips). Others were present in very small numbers, including *Thelephoraceae/Tomentella*-3 and 4 (1 seedling, 6 root tips, 1 seedling, 7 root tips). Both *Thelephoraceae/Tomentella*-3 and *Thelephoraceae/Tomentella*-4 were restricted to Pinchi Mountain. *Russula/Lactarius*-2 was restricted to the Kuzkwa site. In contrast to ECM with more patchy distribution, *Tuber* was restricted to only ultramafic sites (Table 3.4.3.1 and Figures 3.4.3.1-F).

![Figure 3.4.3.1: Dissecting microscope photos of common and rare morphotypes. A: *Rhizopogon* cf. *villosulus*, B: E-strain, C: *Cenococcum geophilum*, D: *Hebeloma-Amphinema* type, E: *Russula/Lactarius*-2, and F: *Tuber* sp.](image)
Figure 3.4.3.2: Compound microscope images of ECM hyphal and mantle structures. Top left, A: *Hebeloma-Amphinema* like emanating hyphae, note the clamp connection, bottom left C: *Hebeloma-Amphinema* sporocarp type. Top right, B: E-strain inner mantle showing labyrinthic pattern, bottom right, *Hebeloma-Amphinema* sporocarp found fruiting in soil from Murray Ridge-East, D: *Rhizopogon cf. villosulus* emanating hyphae, note the elbow-type bend on the pigmented hypha (arrow).
In terms of abundance of mycorrhizal tips, only *Rhizopogon cf. villosulus* differed significantly between sites (*P*<0.001, *F*=8.35) ranging from a high of 31.5% on Kuzkwa (calcareous) to a low of 6.8% on Murray Ridge-East (ultramafic) (Table 3.4.3.1). E-strain was consistently abundant for all sites, ranging from 52% on Tezzeron (glacial) to 74.4% on Murray Ridge-East. While glacial and ultramafic derived soils were not significantly different, the calcareous derived sites (Kuzkwa and Pinchi Hill) were significantly different from one another. This pattern was repeated for the number of uncolonized tips as well. All other morphotypes had numerical, but non-significant, differences.
Table 3.4.3.1: Seedling level comparison of frequency and % abundance of ECM root tips with standard error of the mean (unshared letters within rows denote significance). ANOVA used for comparison of mean values with a post-hoc Tukey HSD test across sites. ECM morphotypes are arranged in decreasing frequency rank. The P value represents the significance level (α=0.05, n=20).

<table>
<thead>
<tr>
<th>ECM Morphotype</th>
<th>Treatment Effect</th>
<th>Kuzkwa Abundance</th>
<th>Pinchi Hill Abundance</th>
<th>Spencer's Ridge Abundance</th>
<th>Tezziar Abundance</th>
<th>Murray Ridge East Abundance</th>
<th>Pinchi Mountain Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>Freq</td>
<td>Abundance</td>
<td>Freq</td>
<td>Abundance</td>
<td>Freq</td>
</tr>
<tr>
<td>Uncolonized</td>
<td>6.53</td>
<td>0</td>
<td>42 (1.7)a</td>
<td>50</td>
<td>33.9 (9.2)c</td>
<td>90 8.2 (2.9)ab</td>
<td>75 26.7 (5.6)bc</td>
</tr>
<tr>
<td>E-strain</td>
<td>2.27</td>
<td>0.0518</td>
<td>53.8 (3.7)</td>
<td>100</td>
<td>54.1 (9.2)</td>
<td>75 59.7 (5.7)</td>
<td>95 52.0 (4.3)</td>
</tr>
<tr>
<td>Rhizopogon cf. villosulus</td>
<td>8.35</td>
<td>0</td>
<td>31.5 (4.0)</td>
<td>95</td>
<td>6.9 (3.8)</td>
<td>15</td>
<td>23.1 (3.5)</td>
</tr>
<tr>
<td>Thelephoraceae/Tomentella-1</td>
<td>0.9 (0.9)</td>
<td>5</td>
<td>2.4 (2.4)</td>
<td>5</td>
<td>4.8 (3.3)</td>
<td>20</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Hebeloma-Amphinema like</td>
<td>0.2 (0.2)</td>
<td>5</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>3.2 (3.2)</td>
<td>5</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Centococcus geophilum</td>
<td>3.3 (2.6)</td>
<td>45</td>
<td>0.3 (0.3)</td>
<td>5</td>
<td>1.0 (0.3)</td>
<td>70</td>
<td>0.9 (0.8)</td>
</tr>
<tr>
<td>Tuber sp.</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Rhizopogon subcaerulescens/ Suillus caerulescens</td>
<td>0.3 (0.3)</td>
<td>5</td>
<td>2.4 (1.8)</td>
<td>10</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.5 (0.5)</td>
</tr>
<tr>
<td>Blonde</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>3.7 (3.5)</td>
</tr>
<tr>
<td>Russula/ Lactarius-1</td>
<td>0.2 (0.2)</td>
<td>5</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Russula/ Lactarius-2</td>
<td>2.1 (1.4)</td>
<td>10</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>MRA</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>3.3 (3.0)</td>
<td>10</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Russula/Lactarius-3</td>
<td>1.8 (1.7)</td>
<td>10</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Thelephoraceae/Tomentella-2</td>
<td>1.7 (1.7)</td>
<td>5</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Thelephoraceae/Tomentella-4</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
<tr>
<td>Thelephoraceae/Tomentella-3</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>
The two highest ranked morphotypes were also the most abundant, together comprising 90.5% of the root tips sampled. E-strain colonized tips represented 68.2% of all tips sampled. *Rhizopogon cf. villosulus* was the second most prevalent and made up 22.4% of all sampled tips (Figure 3.4.3.3). Combined, all other root tips only made up 9.5% of the number sampled. Of these, nine morphotypes account for less than 1% of sampled root tips due to their rarity. While many other morphotypes displayed locally higher numbers on one soil compared to another, *Cenococcum geophilum* was the only morphotype within the minority 9.5% found on all six sites (Figure 3.4.3.4).
Figure 3.4.3.3: Number of ECM root tips of the two major morphotypes, \((n=22\ 039, \text{ both types together represent } 90.5\% \text{ of all root tips sampled})\).
Figure 3.4.3.4: Number of ECM root tips of the 13 minor morphotypes (n=22 039, these types together represent 9.5% of all root tips sampled).
3.4.4. Diversity indices and rank abundance curves

Rank abundance curves show biodiversity of the community and the relative role that each morphotype plays (Magurran, 2004; Kindt & Coe, 2005). Based on the sampled numbers, the rank abundance curve is plotted with an exponential curve (Figure 3.4.4.1). The two highest points represent the most frequent and abundant ECM fungi, while the two lowest points represent the least frequent and abundant colonizers. Relative abundance of each site is represented in Figure 3.4.4.2. For all sites the meeting of the x-axis and the curve represent the morphotypes that were not present on the site. The steepness of the curve indicates lower evenness of species, therefore, Kuzkwa has a more even distribution of species than Tezzeron.

![Figure 3.4.4.1: Combined log-transformed rank-abundance curve. E-strain begins the curve with the highest rank. Table 3.4.3.1 lists the morphotypes in their pooled rank order, corresponding to the y-axis of this figure.](image-url)
Figure 3.4.4.2: Site-level comparison rank-abundance curves of ECM morphotypes of each site. Log-transformed abundance is plotted against the morphotype rank of each site. A point intersecting with the x-axis indicates absence of the morphotype with the given rank.
Both E-strain and *Rhizopogon cf. villosulus* had very high percent abundance compared to the low abundance of other ECM fungi. Pinchi Hill had the highest proportion of uncolonized tips (over 47%, or 1422 root tips) with four trees completely uncolonized. E-strain is the most evenly abundant morphotype on all sites. Many of the other sites had numeric, but not statistically significant variation in levels of abundance. For example, *Rhizopogon subcaerulescens/Suillus caerulescens* was only present on Kuzkwa, Tezzeron, and Murray Ridge. Of these three occurrence of *Rhizopogon subcaerulescens/Suillus caerulescens*, Tezzeron and Murray Ridge had much higher percent abundances with 4.11% and 3.45% compared to only 0.2% for Kuzkwa (Figure 3.4.4.3).
Figure 3.4.4.3: Rank percent abundance of morphotypes per site. Morphotypes are ranked from lowest to highest abundance (proportion of the community made up by each morphotype per site). Non-mycorrhizal tips are also represented (n= 22 039).
The number of morphotypes found on seedlings from each site is an indicator of species richness. There is a wide range of species richness across sites. For instance, the glacial site Tezzeron had the lowest number of ECM with only 4 morphotypes identified. In contrast, Kuzkwa, a calcareous site, had the highest number of morphotypes with 10 ECM types. Both ultramafic sites had more morphotypes than the glacial sites and Pinchi Hill, and less than Kuzkwa (Figure 3.4.4.4).

Figure 3.4.4.4: Number of ECM morphotypes per site as a measure of species richness.
Four diversity indices were compared to gauge the level of diversity found on all sites. Site level comparisons used the number of morphotypes found on all seedlings of a particular site and pooled the data. Site level indices are therefore not statistically comparable. Seedling level comparisons use each individual seedling to generate an index value. These values are then pooled on the basis of site and analyzed using ANOVA (Robertson, 2003).

ECM diversity was usually highest at the site level on the Kuzkwa site, in contrast to the other calcareous site, Pinchi Hill, which usually had the lowest diversity. Mid-level diversity was found on both glacial and ultramafic derived sites (Table 3.4.4.1). ECM diversity compared at the seedling level was usually highest on the Kuzkwa site, in contrast to the other calcareous site, Pinchi Hill, which usually had the lowest diversity based on pooled values. Mid-level diversity was found on both glacial and ultramafic derived sites. All diversity indices showed significance between sites (P<0.001).

The Margalef index, which is a measure of species richness, gives Spencer’s Ridge as the site with the highest richness at the seedling level. Spencer’s Ridge had the highest mean number of mycorrhizal species per tree (3 species of ECM). This is in contrast to the site level species richness, as Kuzkwa had the higher number of morphotypes (10 species) compared to Spencer’s Ridge (6 species).
Mean Gini-Simpson index values showed a significant difference between both calcareous sites (Table 3.4.4.2). The mean Shannon and Shannon evenness indices also separated out Kuzkwa and Pinchi hill from each other. The Margalef index based on pooled ECM morphotypes showed a much more even level of diversity than the total site data. Both types of indices showed slightly higher levels of diversity on the Kuzkwa sites in comparison to all other. Pinchi hill also remained the site with the lowest diversity in most cases.

Table 3.4.4.1: Comparison of four diversity indices by using pooled ECM root totals from each tree (Gini-Simpson, Shannon, Shannon Evenness, and Margalef) for each site (n=20).

<table>
<thead>
<tr>
<th></th>
<th>Calcareous</th>
<th>Glacial</th>
<th>Ultramafic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kuzkwa</td>
<td>Pinchi</td>
<td>Spencer's Ridge</td>
</tr>
<tr>
<td>Gini-Simpson</td>
<td>0.58</td>
<td>0.48</td>
<td>0.52</td>
</tr>
<tr>
<td>Margalef</td>
<td>1.09</td>
<td>0.54</td>
<td>0.61</td>
</tr>
<tr>
<td>Shannon</td>
<td>1.13</td>
<td>0.94</td>
<td>1.02</td>
</tr>
<tr>
<td>Shannon Evenness</td>
<td>0.49</td>
<td>0.58</td>
<td>0.57</td>
</tr>
</tbody>
</table>
Table 3.4.4.2: Comparison of four diversity indices using individual tree data for each site (Gini-Simpson, Shannon, Shannon Evenness, and Margalef). Means were compared by ANOVA with a post-hoc Tukey HSD test. Unshared letters denote significance (n=20).

<table>
<thead>
<tr>
<th>Site</th>
<th>F</th>
<th>P</th>
<th>Species Richness</th>
<th>Gini-Simpson</th>
<th>Shannon</th>
<th>Shannon Evenness</th>
<th>Margalef</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>1</td>
<td>2.8 (0.2)ab</td>
<td>1.1 (0.2)</td>
<td>0.476</td>
<td>0.759 (0.049)b</td>
<td>0.779</td>
</tr>
<tr>
<td>Mean Calculareous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Glacial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Ultramafic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Murray Ridge-East</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Pinchi Mountain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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3.4.5. Two-way cluster analysis

Morphology data was compared using the relative Sørensen index to test for similarity between groups. Figure 3.4.5.1 shows clustering using presence-absence data (black and white), while Figure 3.4.5.2 shows clustering with relative abundance data (shading). Data were compiled into a dendrogram using a farthest neighbour clustering.

Sites appear do not cluster closely according to their parent material. Murray Ridge-east is most distant from all other sites. Pinchi Mountain and Tezzeron appear to have the greatest similarity to each other (Figure 3.4.5.1).

*Rhizopogon cf. villosulus* shares similarity between Tezzeron, Pinchi Mountain and Spencer’s Ridge. *Tuber* sp. is shared only between ultramafic sites, and is present in higher numbers on Murray Ridge-East. The two morphotypes with the highest abundance, *Rhizopogon cf. villosulus* and E-strain, are similar in distribution and are clustered together (Figure 3.4.5.2).
Matrix Coding
■ Presence □ Absence

Information Remaining (%) 0 25 50 75 100

Figure 3.4.5.1: Two-way cluster analysis using a relative Sørensen index showing presence-absence of fungal species for comparison and using furthest neighbour clustering. Sites are represented by two-letter codes, while morphotypes are represented by single-letter codes. The designation “Un” represents uncolonized root tips (n= 22 039).
Figure 3.4.5.2: Two-way cluster analysis using a relative Sørensen index showing relativized values for the number of ECM colonized root tips for comparison and using furthest neighbour clustering. Sites are represented by two-letter codes, while morphotypes are represented by single-letter codes. The designation “Un” represents uncolonized root tips (n=22 039).
3.4.6. Confirmation of identity using DNA

DNA extraction and amplification were used to further clarify the taxonomic resolution of the selected morphotypes for this study. Many of the morphotypes were identifiable to their genus level, including *Rhizopogon*, *Suillus*, and *Tomentella*. Species level identification was achieved for both the Tuber type (confirmed at *Tuber anniae*) and the *Hebeloma-Amphinema* type (*Inocybe abjecta*). Out of 166 DNA samples sent for sequencing, only 94 returned sequence data. The other 72 samples did not produce readable data. Of the 94 returned sequences, only 69 had a strong enough signal and little to no evidence of a contaminating DNA sequence.

Out of 14 morphotypes sent for sequencing, 8 produced usable sequence data. All other morphotypes tested did not sequence well enough to produce a readable DNA trace (Table 3.4.6.1). The morphotype *Rhizopogon subcaeruleascens/Suillus caeruleascens* encompassed both *Suillus caeruleascens* and *Suillus lakei*. The *Rhizopogon cf. villosulus* type also encompassed *Rhizopogon subclavitisporus, Rhizopogon pedicellus*, and *Rhizopogon villosulus*. These two morphotypes represent species-complexes and were common to all sites on which the original morphotype was found. Overall, a total of 18 ECM species were determined in this study using combined morphological and DNA methods.
Table 3.4.6.1: Comparison of DNA identity and morphotyping identification. DNA sequences were compared with sequences from BLAST.

<table>
<thead>
<tr>
<th>Morphotype Label</th>
<th>Possible Identity</th>
<th>DNA Confirmed Identify</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td><em>Thelephoraceae/Tomentella</em>-1</td>
<td><em>Tomentella</em> sp.</td>
</tr>
<tr>
<td></td>
<td><em>Rhizopogon subcaerulescens/Suillus caerulescens</em></td>
<td></td>
</tr>
<tr>
<td>Type C</td>
<td><em>Cenococcum geophilum</em></td>
<td><em>Cenococcum geophilum</em></td>
</tr>
<tr>
<td>Type D</td>
<td><em>Rhizopogon cf. villosulus</em></td>
<td><em>Rhizopogon subclavisporus, Rhizopogon pedicellus, Rhizopogon villosulus</em></td>
</tr>
<tr>
<td>Type E</td>
<td>E-strain</td>
<td><em>Wilcoxina</em> sp.</td>
</tr>
<tr>
<td>Type F</td>
<td><em>Thelephoraceae/Tomentella</em>-2</td>
<td>Poor signal</td>
</tr>
<tr>
<td>Type G</td>
<td><em>Hebeloma-Amphinema like</em></td>
<td><em>Inocybe abjecta</em></td>
</tr>
<tr>
<td>Type H</td>
<td><em>Thelephoraceae/Tomentella</em>-3</td>
<td>Poor Signal</td>
</tr>
<tr>
<td>Type I</td>
<td><em>Russula/Lactarius</em>-1</td>
<td>Poor Signal</td>
</tr>
<tr>
<td>Type J</td>
<td>Blonde</td>
<td>Poor Signal</td>
</tr>
<tr>
<td>Type K</td>
<td><em>Russula/Lactarius</em>-2</td>
<td>Poor Signal</td>
</tr>
<tr>
<td>Type L</td>
<td><em>Tuber sp.</em></td>
<td><em>Tuber anniae</em></td>
</tr>
<tr>
<td>Type M</td>
<td><em>Russula/Lactarius</em>-3</td>
<td>Poor Signal</td>
</tr>
<tr>
<td>Type N</td>
<td><em>Thelephoraceae/Tomentella</em>-4</td>
<td><em>Tomentella</em> sp.</td>
</tr>
<tr>
<td>Type O</td>
<td><em>Mycelium radicis atrovirens</em> (MRA)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

3.5. Discussion

3.5.1. Impacts on seedlings grown on ultramafic, calcareous, and glacial soils

Seedlings displayed varied biomass responses to the different soil types. For all biomass measures taken, trees grown in the same soil type often differed between sites. This further supported the choice to not pool data on the basis of soil type. Soils are inherently variable and therefore, other properties such as texture may be more important than chemical composition. For example, trees grown on Pinchi Hill (calcareous) displayed very slow and stunted growth compared to their calcareous Kuzkwa counterparts. Pinchi Hill seedlings had the smallest height and the smallest root mass (Figure 3.4.1.5). The root
systems of the seedlings were also extremely fragile and largely uncolonized (Figure 3.4.4.3). Trees grown on ultramafic derived soils did not display signs of growth stress (often described as serpentine syndrome) comparable to trees grown on Pinchi Hill.

Foliar metal assessment may shed some light on the changes in growth of the Pinchi Hill trees. Active iron levels within the needles of the Pinchi Hill seedlings were extremely low when compared with all other seedlings (Figure 3.4.2.1). Iron deficiency is often linked with leaf chlorosis and plant death (Loeppert et al., 1994; Kishchuk, 2000). While pH values were not extremely high on Pinchi Hill, the measure of % CaCO₃ equivalent was much higher than all other soils (see Appendix 1). It is possible that the specific balance of carbonate mineralization with the Pinchi Hill soils rendered iron largely inaccessible to the seedlings, resulting in low levels of iron availability.

The lack of ECM colonization may also be a factor in the lack of success for the Pinchi Hill seedlings. Without the fungi, iron may not have been properly mobilized to the plants, resulting in further stress. Kuzkwa seedlings, also grown in calcareous soils, displayed no signs of undue stress. However, Kuzkwa also hosted the largest complement of mycorrhizal species. These trees also had very few uncolonized tips compared to the Pinchi Hill trees (186 tips uncolonized compared to 1422 uncolonized tips).

It is impossible to identify the specific reasons for the relative lack of colonization on the Pinchi Hill seedlings, however, it is possible that some combination of factors within the soil severely retarded the seedlings' ability to photosynthesize. In this case, because the seedlings are poor providers of sugars for mycorrhizal fungi, there is little incentive for the fungi to colonize the root system. It has been shown in pinyon pine (Pinus edulis Engelm.) that seedlings under attack by defoliator insects have reduced levels of
mycorrhizal colonization due to the lower levels of photosynthates produced. When foliage returned to normal, the mycorrhizal communities likewise recovered (Del Vecchio et al., 1993; Gehring & Whitham, 2003). The inability of the seedlings to thrive may create a negative feedback loop where ECM fungi will not form partnerships due to the poor health of the plant, which remains poor due to the lack of nutrients supplied by the fungi.

Nickel and chromium levels within the foliage of seedlings grown on ultramafic soil were quite high (Figure 3.4.2.2-3). Nickel levels were much higher than chromium, data which corresponds to established research on serpentine soils (Gough et al., 1989). Though not statistically assessed in this study, ultramafic seedlings were observed to display a loss of epinastic, or dominant leader, control (Mattheck, 1990). Seedlings displayed bushy, bonsai-like foliage and often had extremely long lateral branches and short leaders with set apical buds (Figure 3.5.4.1). It is possible that this morphology resulted from the apical buds receiving more of the heavy metals along with nutrients and sugars usually supplied to the apical leader to encourage growth. This may have impeded metabolic actively of the leaders, halting growth. Due to the lack of tissue on leaders that had set bud, no analysis could be performed to assess potential differences in the heavy metal levels within different tree tissues.

3.5.2. Diversity of morphotypes and community structure

The three common species *Rhizopogon* cf. *villosulus*, *Cenococcum geophilum*, and E-strain all correspond to dominant ECM species found in many other studies on Douglas-fir (Parke et al., 1983; Hunt, 1992; Jones et al., 1998; Massicotte et al., 1999; Hagerman & Durall, 2004). Some species within the *Rhizopogon* genus are known to be common mycorrhizal associates of Douglas-fir and do not form associations with other potential
host species (Zak, 1971; Massicotte et al., 1994). E-strain and *Cenococcum geophilum* are both generalist species that form mycorrhizas with many species of trees (Yu et al., 2001; Bourne et al., 2014).

My research produced ectomycorrhizal distributions for Douglas-fir that are comparable to other areas sampled within central and eastern British Columbia. Pickles et al. (2015) found Douglas-fir grown in soils from around the province produced ECM roots dominated by species of *Rhizopogon* and Pyronemataceae (E-strain). A species of *Tuber* was also found in the Fort St. James area. The soils in their study were separated by a much larger geographic area, but in each case, the distribution of fungi matched the pattern found in our own study: dominance of one or two types with a few rare species.

Previous research indicates that mycorrhiza may benefit from mixed stands with succession of one species to another, divided into early-stage and late-stage (Jones et al., 1998; Massicotte et al., 1999). Early-stage ECM fungi are readily able to colonize host roots from soil inoculum in areas under recent disturbance where refuge plants are few. Late-stage fungi cannot form associations directly from spores, but instead require a pre-existing living host to support a living hyphal network that will colonize new seedlings root systems (Jones et al., 1998). Members of the genus *Russula*, for example, belong to the group of late-stage colonizers (Jones et al., 1998) and may have appeared less prevalent in this study because of the use of monoculture pots.

The three ubiquitous ECM morphotypes may represent early-stage colonizers, ones that are best adapted to colonize seedlings quickly. This adaptation explains the high numbers and widespread nature of these morphotypes. Out of the other observed morphotypes, MRA and *Thelephora* spp. are also part of the early-stage colonizers, and do
not require refuge trees to support an establishing ECM association. Since soil collection inherently disturbs the soil, this study may over represent the proportion of the early-stage fungi found on all soils.

Recent glaciation may also play a role in the low numbers of ECM fungi retrieved from these soils. Areas now populated by Douglas-fir were dominated by pine, spruce and fir immediately following the last glaciation (Hansen, 1955). The recent advance of Douglas-fir may mean that the ECM species that colonize its roots have not yet had enough time for speciation to occur. What ECM species that are currently present may instead be descendants of fungi with a broad host range that were capable of transitioning from being spruce and fir symbionts to Douglas-fir partners.

A common problem faced by mycologists studying ECM communities is the patchy distribution and the appearance of rare species (Branco, 2010; Molina et al., 2011; Barker et al., 2013). ECM species may be present on only one root tip, indicating that the species is present, but providing a very small contribution to a species diversity index or an ordination. Ultramafic soils in this study produced the rare type, *Tuber anniae*, but not in numbers that could be usefully compared across sites. To ecologists, it is perhaps more useful to document rare species of ectomycorrhizas and treat the data as purely observational and focus on the wider distributed species.

3.5.3. DNA sequence enhanced identification

Out of 14 morphotypes isolated for DNA sequencing, only 8 were deemed to have sufficiently clear sequences to confirm identification (n=69 sequences). Of those types visually grouped together, only *Rhizopogon subcaerulescens/Suillus caerulescens* and *Rhizopogon cf. villosulus* were found to represent 2 and 3 different species respectively.
Two of the three *Rhizopogon* species identified (*R. subclavitisporus* and *R. villosulus*) have been grouped previously in *Rhizopogon* subgen. *Villosuli* sect. *Vinicolores* (Grubisha et al., 2002) which confirms our previous identification. The remainder of morphotypes tested corresponded to a single type of DNA sequence. This identification process, while vital to separating species complexes, continues to have complications.

Contamination was a consistent problem in the DNA samples. Growth media in studies such as this is by necessity non-sterile. ECM fungi themselves are opportunistic and hyphae from multiple species were often observed on one root tip at the same time. While root tips were harvested from deionized water after first being thoroughly rinsed, removal of all secondary hyphae is impossible. Root tips that appeared clean under a dissecting scope often would show other hyphae when observed under the compound microscope. This leads to multiple signals within one DNA sample, and obscures the desired sequence. Multiple fungal samples are also difficult to differentiate and may not produce viable data (Avis et al., 2010). Some fungal DNA does not sequence well, as was the case with a small unknown ascomycete found on both Spencer’s Ridge (glacial) and Pinchi Hill (calcareous) soil (Figure 3.5.4.1).

A secondary concern is the vast number of DNA sequences available online. Higher resolution curation of DNA sequences is imperative for proper identification of collected sequences. For example, all types of *Thelephora* identified by DNA sequences in this study did not have a listed species name with the online sequence. Others, such as many of the E-strain samples either corresponded to only the genus *Wilcoxina* or the order *Pezizales*. Morphotyping remains a vital tool in species identification. If vouchered specimens of ECM fungi were carefully sequences and those sequences curated,
identification of species of interest would likely become simpler, increasing the resolution of ECM community studies.

3.5.4. Complications and limits to greenhouse studies

Greenhouse growing conditions were selected in order to optimize growth of Douglas-fir, based on previous greenhouse studies (Massicotte et al., 1999; Hagerman & Durall, 2004). Field studies can be difficult as the age of mycorrhizal roots is often difficult to access. The number of root tips recovered is often much lower than what is obtained in a greenhouse study, produced a more diverse group of fungi, but with far less resolution (Branco & Rec, 2010; Branco, 2010). While colonized roots may be found, the evidence for succession of ECM on the same root tip (Massicotte et al., 1999) leads to the problem of competing DNA signals during extraction procedures. However, even with relatively controlled conditions, things occasionally go awry. In the case of this experiment, two large stressors could have potentially impacted the health of the seedlings: growth conditions and pest presence.

Lighting and temperature were adjusted to encourage the seedlings to germinate and then were reduced to encourage steady growth. However, due to several power failures at the Enhanced Forestry Lab (EFL) at UNBC, temperature and lighting conditions were reset back to the 16:8 hours of light and darkness and 25°C of the germination conditions. This change may have been a contributing factor in the chlorotic tissue that was observed, leading to preventative application of fertilizer to prevent seedling mortality. An infestation of defoliating spider mites may have impacted the health of the remaining seedlings (Figure 3.5.4.1). Stressed and dying trees do not have the same mycorrhizal communities as healthy trees (Del Vecchio et al., 1993) therefore, morphotyping had to be done quickly.
Even with the expedient harvest and characterisation of the root systems, it is possible that the stress on the trees masked the manifestation of some of the more rare potential ECM species.

Figure 3.5.4.1: A: Seedling from Murray Ridge with lateral growth and a set apical bud. B: Seedling from Pinchi Mountain with a swollen and bent stem. C: Small, unidentified ascomycete, unconnected with any known morphotypes, found on two sites (Spencer's Ridge and Pinchi Hill). D: Mature spider mite, with shed exoskeletons, webs, eggs and egg cases.
3.6. Summary

Based on morphology and DNA analysis, 3 morphotypes are present on all sites, with 12 others present in patchy distributions across a variety of sites. Of these morphotypes, E-strain was present in the highest abundance. *Tuber anniae* was restricted to the ultramafic sites, but this may be an artifact of distribution of spores, as opposed to an indication of an endemic species. Both ultramafic sites had higher species richness than the glacial sites, while one of the calcareous sites (Kuzkwa) had the greatest number of morphotypes present and the highest species diversity. Extreme parent material did not usually produce low levels of ECM diversity with one exception (Pinchi Hill-calcareous).

Douglas-fir seedling biomass varied across all soil types. No one parent material produced consistently larger trees than another. Pinchi Hill (calcareous) seedlings were the smallest, averaging only 1.3 g in weight, showing fragile roots and low levels of ECM colonization. However, even with the variations in biomass, root:shoot ratios remained fairly consistent. Douglas-fir seedlings may also show some level of tolerance to nickel within the soil. Seedlings grown on ultramafic soil had elevated levels of foliar nickel compared with all other seedlings and still had comparable, or larger, total biomass than their glacial and calcareous counterparts. Calcareous parent material in one case (Pinchi Hill) caused perceived detrimental changes in biomass, but parent material in general did not largely affect seedling biomass.
3.7. References


Farell EM, Alexandre G. 2012. Bovine serum albumin further enhances the effects of organic solvents on increased yield of polymerase chain reaction of GC-rich templates. BMC research notes 5: 257.


Kennedy NM, Robertson SJ, Green DS, Scholefield SR, Arocena JM, Tackaberry LE, Massicotte HB, Egger KN. 2015. Site properties have a stronger influence than fire severity on ectomycorrhizal fungi and associated N-cycling bacteria in regenerating post-beetle-killed lodgepole pine forests. *Folia Microbiologica* 60: 1–12.


4. Conclusions

Interior Douglas-fir was used as a host to compare ECM diversity on ultramafic, calcareous and glacial-derived soils. ECM communities developed on greenhouse-grown seedlings were assessed using a combination of morphological identification and DNA sequencing. Ultramafic and calcareous derived soils did not produce fungal communities that were depauperate compared to other sites. Preliminary field assessments of the Douglas-fir forest conditions also did not show large differences between soil parent materials. Both Murray-Ridge East and Pinchi Mountain (ultramafic) produced seedlings that were of similar vigour and had more morphotypes compared to the glacial sites.

Kuzkwa (calcareous) also had healthy seedlings and the highest number of morphotypes. Excluding Pinchi Hill (calcareous), which was discussed above, both glacial sites had the lowest number of morphotypes and did not display stressed seedlings. The presence of more fungal species on the ultramafic sites and Kuzkwa may indicate that the trees require more fungal partners to survive on the harsher soil. The poorer qualities of the site may also reduce the ability of a few morphotypes to entirely dominate, leaving room for less prolific but more tolerant ECM species. Seedlings in this study shared common ECM fungi found in greenhouse studies of other parts of British Columbia, but did not have the same diversity of ECM as field studies on Douglas-fir have shown throughout North America.

Biomass of seedlings differed across sites of the same soil type with no clear pattern. While Douglas-fir appeared largely resistant to the chemical signatures within
the soil, Pinchi Hill was the only site to produce severely stunted and stressed trees, while also showing the least amount of ECM colonization. Based on these differences, knowledge of underlying bedrock and resultant soils will most likely be of some benefit to afforestation efforts, but therefore necessitates on the ground assessment of habitat. Given the uneven history of disturbance on all of the sites, it is unlikely that any site faced complete removal of all Douglas-fir and understory plants, and by extension, the removal of live ECM networks, in the last 100 years.

Given the extreme stress observed in the greenhouse-grown Pinchi Hill seedlings, it is possible that these sites with extreme soils may have difficulty with seedling recruitment after complete removal of all trees, whether by logging or by fire. Without mature trees or understory plants to house living mycorrhizal networks, new seedlings under the duress of high calcium levels may not be capable of initiating mycorrhizal relationships with the early-stage ECM species found in this study. Mature Douglas-fir are already often left as seed sources when logging in central BC, but special attention should be given to the distribution and protection of reserve trees and advance regeneration on soils derived from calcareous or ultramafic bedrocks (see Figure 2.3.3.1 for Douglas-fir stands in question).

Seedlings grown on ultramafic soils were also shown to have uptake of nickel into their foliage. While plants grown on serpentine soils have been known to transport nickel and chromium (to a lesser extent), it had not been demonstrated to happen in Douglas-fir in central British Columbia before now. With increasing interest in the conversion of logged timber to bio-fuel, trees containing heavy metals may be a hindrance and possibly a health hazard due to the release of heavy metals into biomass
furnaces and the atmosphere. Awareness of the bedrock underlying areas earmarked for harvest may improve safety and functionality of these new energy solutions.

Elucidation of the below-ground networks of ECM remains difficult to quantify. In future, studies similar to this one would be augmented with both a field and lab component. Next generation DNA sequencing (NGS) could be implemented to quickly characterise the ECM component of soils around seedlings grown in the field, compared with soils from greenhouse seedlings. However, it must be stressed that the use of NGS will amplify all DNA within the soil, and not differentiate between spores, inoculum, and active ECM. For this reason, morphotyping will remain a critical tool for characterising the fungal communities involved directly on root systems.

Repeated sampling over the course of several years may also improve the assessment of the ECM communities as different species may manifest over time. All DNA samples generated should be vouchered in a university herbarium if possible, photographed, and then sequenced. These sequences should only be added to those on GenBank if the ECM identity is clear. Morphotype descriptions, coupled with the host and growing conditions should also be made available. While DNA technology continues to improve, the information gathered from a holistic study will ultimately provide better resolution to questions of fungal diversity.
Appendices

Appendix 1: Soil profile descriptions and analytical data

Site: Kuzkwa (BC12-17)

Latitude: 54° 42' 54.3" N Longitude: 124° 38' 49.4" W Elevation: 806 mm

Aspect: 225° Slope: 70% Slope position: midslope

Vegetation: Mature Douglas-fir stand with sparse understory

Parent material: Limestone colluvium

Soil classification: Orthic Eutric Brunisol

Comments: most coarse fragments in Bmk and Cca have continuous mammillated pendants on their lower surfaces

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF (BC12-17-01)</td>
<td>2-0</td>
<td>Very dark brown (10YR 2/2 m); fresh and semi-decomposed Douglas-fir needle litter; abrupt, wavy boundary; 0.5-3.0 cm thick; slightly acid (pH 6.31).</td>
</tr>
<tr>
<td>Bmk (BC12-17-02)</td>
<td>0-25</td>
<td>Dark yellowish brown (10YR 3/4 m); silt loam; weak, fine subangular blocky; very friable; plentiful, very fine, fine, and medium, oblique roots; 60-70% angular gravel and cobbles; weakly effervescent; gradual, wavy boundary; 20-35 cm thick; mildly alkaline (pH 7.38).</td>
</tr>
<tr>
<td>Cca (BC12-17-03)</td>
<td>25-70+</td>
<td>Brown (10YR 5/3 m); loam; single grain; loose; few, very fine, fine, and medium, oblique roots; 80% angular gravel and cobbles; strongly effervescent; mildly alkaline (pH 7.45).</td>
</tr>
</tbody>
</table>
Table 3.5.4.1: Kuzkwa (calcareous) soil horizon chemical data showing Iron, Aluminum, and Silicon concentrations by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Org-C</th>
<th>Tot-N</th>
<th>Tot-S</th>
<th>CaCO₃-eq</th>
<th>pH (H₂O)</th>
<th>pH (CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-17-01</td>
<td>LF</td>
<td>2-0</td>
<td>38.79</td>
<td>1.360</td>
<td>0.1075</td>
<td>6.62</td>
<td>0.1075</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BC12-17-02</td>
<td>Bmk</td>
<td>0-25</td>
<td>35.9</td>
<td>50.3</td>
<td>13.8</td>
<td>1.99</td>
<td>0.087</td>
<td>0.0082</td>
<td>4.35</td>
<td>7.71</td>
<td>7.38</td>
</tr>
<tr>
<td>BC12-17-03</td>
<td>Cca</td>
<td>25-70+</td>
<td>41.8</td>
<td>47.2</td>
<td>11.0</td>
<td>1.34</td>
<td>0.056</td>
<td>0.0042</td>
<td>8.17</td>
<td>7.87</td>
<td>7.45</td>
</tr>
</tbody>
</table>

*Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.

**Exchangeable (cmol (+) / kg)**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al³⁺</th>
<th>Ca²⁺</th>
<th>Fe³⁺</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Mn²⁺</th>
<th>Na⁺</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-17-01</td>
<td>LF</td>
<td>2-0</td>
<td>0.031</td>
<td>70.75</td>
<td>0.007</td>
<td>0.97</td>
<td>2.62</td>
<td>0.244</td>
<td>0.054</td>
<td>74.68</td>
</tr>
<tr>
<td>BC12-17-02</td>
<td>Bmk</td>
<td>0-25</td>
<td>0.003</td>
<td>17.71</td>
<td>&lt; 0.001</td>
<td>0.13</td>
<td>0.49</td>
<td>0.001</td>
<td>0.020</td>
<td>18.36</td>
</tr>
<tr>
<td>BC12-17-03</td>
<td>Cca</td>
<td>25-70+</td>
<td>0.001</td>
<td>16.44</td>
<td>&lt; 0.001</td>
<td>0.07</td>
<td>0.41</td>
<td>&lt; 0.001</td>
<td>0.021</td>
<td>16.95</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al₀</th>
<th>Alₚ</th>
<th>Fe₀</th>
<th>Feₚ</th>
<th>Fe₄</th>
<th>Si₀</th>
<th>Alₚ + Feₚ</th>
<th>Fe₄/Fe₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-17-01</td>
<td>LF</td>
<td>2-0</td>
<td>0.041</td>
<td>0.243</td>
<td>0.072</td>
<td>0.328</td>
<td>1.372</td>
<td>0.081</td>
<td>0.112</td>
<td>0.24</td>
</tr>
<tr>
<td>BC12-17-02</td>
<td>Bmk</td>
<td>0-25</td>
<td>0.032</td>
<td>0.239</td>
<td>0.044</td>
<td>0.315</td>
<td>1.049</td>
<td>0.094</td>
<td>0.076</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.
Figure 3.5.4.1: Soil profile from Kuzkwa (calcareous) showing LF, Bmk, and Cca horizons.
Site: Pinchi Hill (BC10-12)

Latitude: 54° 34' 41.5" N  Longitude: 124° 29' 7.6" W  Elevation: 804 m

Aspect: 225°  Slope: 45%  Slope position: midslope

Vegetation: Open Douglas-fir forest, with shrubby understory

Parent material: Limestone / dolomite colluvium.

Soil classification: Orthic Melanie Brunisol

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF (BC10-12-02)</td>
<td>2-0</td>
<td>Douglas-fir needle litter and partially decomposed organic matter; abrupt, wavy boundary; 2 cm thick.</td>
</tr>
<tr>
<td>Ahk (BC10-12-01)</td>
<td>0-15</td>
<td>Very dark grayish brown (10YR 3/2 m); sandy loam; weak, fine and medium granular; very friable; abundant, very fine, fine, and medium, oblique roots; 50% angular gravel; weakly effervescent; gradual, wavy boundary; 12-20 cm thick; mildly alkaline (pH 7.47).</td>
</tr>
<tr>
<td>Bmk (BC10-12-02)</td>
<td>15-40</td>
<td>Brown (10YR 4/3 d); sandy loam; weak, fine and medium granular; very friable; abundant, very fine, fine, and medium, oblique roots; 70-80% angular gravels; weakly effervescent; gradual, wavy boundary; 20-30 cm thick; mildly alkaline (pH 7.65).</td>
</tr>
<tr>
<td>Ck (BC10-12-03)</td>
<td>40-65+</td>
<td>Brown (10YR 5/3 d); sandy loam*; single grain; very friable; plentiful, very fine, fine, and medium, oblique roots; 90% angular gravels and cobbles; moderately effervescent; mildly alkaline (pH 7.61).</td>
</tr>
</tbody>
</table>

*field texture estimate only; insufficient sample available for analysis.
Table 3.5.4.2: Pinchi Hill (calcareous) soil horizon chemical data showing Iron, Aluminum, and Silicon by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Org-C</th>
<th>Tot-N</th>
<th>Tot-S</th>
<th>CaCO$_3$-eq</th>
<th>pH</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC10-12-01</td>
<td>Ahk</td>
<td>0-15</td>
<td>69.3</td>
<td>25.8</td>
<td>5.0</td>
<td>8.31</td>
<td>0.413</td>
<td>0.0493</td>
<td>20.37</td>
<td>7.65</td>
<td>7.47</td>
</tr>
<tr>
<td>BC10-12-02</td>
<td>Bmk</td>
<td>15-40</td>
<td>64.2</td>
<td>27.4</td>
<td>8.4</td>
<td>3.22</td>
<td>0.217</td>
<td>0.0335</td>
<td>20.34</td>
<td>7.80</td>
<td>7.65</td>
</tr>
<tr>
<td>BC10-12-03</td>
<td>Ck</td>
<td>40-65+</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>3.95</td>
<td>0.187</td>
<td>0.0241</td>
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<td>7.72</td>
<td>7.61</td>
</tr>
</tbody>
</table>

**Exchangeable (cmol (+) / kg)**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al$_3^+$</th>
<th>Ca$^{2+}$</th>
<th>Fe$^{3+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Mn$^{2+}$</th>
<th>Na$^+$</th>
<th>Sum</th>
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<tbody>
<tr>
<td>BC10-12-01</td>
<td>Ahk</td>
<td>0-15</td>
<td>0.011</td>
<td>46.589</td>
<td>&lt;0.001</td>
<td>0.183</td>
<td>11.537</td>
<td>0.001</td>
<td>0.040</td>
<td>56.762</td>
</tr>
<tr>
<td>BC10-12-02</td>
<td>Bmk</td>
<td>15-40</td>
<td>0.002</td>
<td>23.504</td>
<td>&lt;0.001</td>
<td>0.063</td>
<td>6.026</td>
<td>&lt;0.001</td>
<td>0.080</td>
<td>29.676</td>
</tr>
<tr>
<td>BC10-12-03</td>
<td>Ck</td>
<td>40-65+</td>
<td>0.008</td>
<td>24.177</td>
<td>&lt;0.001</td>
<td>0.072</td>
<td>8.037</td>
<td>&lt;0.001</td>
<td>0.041</td>
<td>32.335</td>
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<table>
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<th>Sample No.</th>
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<th>Depth (cm)</th>
<th>Al$_p$</th>
<th>Al$_o$</th>
<th>Fep</th>
<th>Fe$_p$</th>
<th>Fe$_o$</th>
<th>Fe$_d$</th>
<th>Si$_o$</th>
<th>Al$_p$ + Fe$_o$</th>
<th>Fe$_d$/Fe$_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC10-12-01</td>
<td>Ahk</td>
<td>0-15</td>
<td>0.254</td>
<td>0.388</td>
<td>0.241</td>
<td>0.426</td>
<td>0.792</td>
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<td>0.495</td>
<td>0.54</td>
<td>0.543</td>
</tr>
<tr>
<td>BC10-12-02</td>
<td>Bmk</td>
<td>15-40</td>
<td>0.175</td>
<td>0.260</td>
<td>0.094</td>
<td>0.168</td>
<td>0.544</td>
<td>0.036</td>
<td>0.259</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>BC10-12-03</td>
<td>Ck</td>
<td>40-65+</td>
<td>0.138</td>
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<td>0.082</td>
<td>0.162</td>
<td>0.542</td>
<td>0.031</td>
<td>0.220</td>
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</tbody>
</table>

*Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.
Figure 3.5.4.2: Soil profile from Pinchi Hill (calcareous) showing LF, Ahk, Bmk and Ck horizons.
Site: Spencer’s Ridge (BC12-19)

Latitude: 54° 21' 28.7" N  Longitude: 124° 20' 9.7" W  Elevation: 821 m

Aspect: n/a  Slope: level  Slope position: crest

Vegetation: Mature Douglas-fir forest, with some paper birch

Parent material: Gravelly sandy glaciofluvial ridge (esker)

Soil classification: Orthic Dystric Brunisol

Comments: Discontinuous, broken Aej horizon < 1 cm thick.

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ln (BC12-19-01)</td>
<td>4-3</td>
<td>Fresh Douglas-fir and paper birch leaf litter, minor feathermoss cover; abrupt, smooth boundary; 1 cm thick; extremely acid (pH 4.10).</td>
</tr>
<tr>
<td>Fm (BC12-19-02)</td>
<td>3-0</td>
<td>Very dark brown (7.5 YR 2.5/2 m); semi-decomposed organic matter, with pockets of brown rotted wood up to 5 cm thick; abundant mycelia; compact matted; abundant, very fine and fine, horizontal and oblique roots; abrupt, wavy boundary; 2-5 cm thick; strongly acid (pH 5.53).</td>
</tr>
<tr>
<td>Bm (BC12-19-03)</td>
<td>0-40</td>
<td>Brown (7.5YR 4/4 m); loamy sand; weak, fine and medium subangular blocky; very friable; abundant, very fine, fine, and medium, oblique, and few, coarse, oblique roots; 50% rounded gravel and cobbles; gradual, wavy boundary; 35-45 cm thick; very strongly acid (pH 4.91).</td>
</tr>
<tr>
<td>BC (BC12-19-04)</td>
<td>40-60</td>
<td>Yellowish brown (10YR 5/4 m); loamy sand; single grain; very friable; plentiful, very fine, fine, and medium, oblique, and few coarse, oblique roots; 60-70% rounded gravel and cobbles; gradual, wavy boundary; very strongly acid (pH 4.57).</td>
</tr>
<tr>
<td>C (BC12-19-05)</td>
<td>60-85+</td>
<td>Light yellowish gray (2.5Y 6/2 m); sand; single grain; loose; few, fine, medium, and coarse, oblique roots; 60-70% rounded gravel and cobbles; strongly acid (pH 5.10).</td>
</tr>
</tbody>
</table>
Table 3.5.4.3: Spencer’s Ridge (glacial) soil horizon chemical data showing Iron, Aluminum, and Silicon by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Org-C</th>
<th>Tot-N</th>
<th>Tot-S</th>
<th>CaCO$_3$-eq</th>
<th>pH (H$_2$O)</th>
<th>pH (CaCl$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-19-01</td>
<td>Ln</td>
<td>4-3</td>
<td></td>
<td></td>
<td></td>
<td>52.90</td>
<td>0.711</td>
<td>0.0746</td>
<td>4.28</td>
<td>4.10</td>
<td></td>
</tr>
<tr>
<td>BC12-19-02</td>
<td>Fm</td>
<td>3-0</td>
<td></td>
<td></td>
<td></td>
<td>47.18</td>
<td>1.572</td>
<td>0.1500</td>
<td>5.86</td>
<td>5.53</td>
<td></td>
</tr>
<tr>
<td>BC12-19-03</td>
<td>Bm</td>
<td>0-40</td>
<td>62.2</td>
<td>13.9</td>
<td>3.8</td>
<td>0.63</td>
<td>0.044</td>
<td>0.0048</td>
<td>5.57</td>
<td>4.91</td>
<td></td>
</tr>
<tr>
<td>BC12-19-04</td>
<td>BC</td>
<td>40-60</td>
<td>78.0</td>
<td>18.5</td>
<td>3.5</td>
<td>0.44</td>
<td>0.024</td>
<td>0.0057</td>
<td>5.29</td>
<td>4.57</td>
<td></td>
</tr>
<tr>
<td>BC12-19-05</td>
<td>C</td>
<td>60-85+</td>
<td>93.7</td>
<td>4.8</td>
<td>1.6</td>
<td>0.40</td>
<td>0.021</td>
<td>0.0038</td>
<td>5.85</td>
<td>5.10</td>
<td></td>
</tr>
</tbody>
</table>

Exchangeable cmol (+) / kg

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al$^{3+}$</th>
<th>Ca$^{2+}$</th>
<th>Fe$^{3+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Mn$^{2+}$</th>
<th>Na$^+$</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-19-01</td>
<td>Ln</td>
<td>4-3</td>
<td>0.168</td>
<td>14.88</td>
<td>0.008</td>
<td>6.42</td>
<td>5.51</td>
<td>3.025</td>
<td>0.079</td>
<td>30.09</td>
</tr>
<tr>
<td>BC12-19-02</td>
<td>Fm</td>
<td>3-0</td>
<td>0.208</td>
<td>83.28</td>
<td>0.004</td>
<td>2.77</td>
<td>6.20</td>
<td>1.434</td>
<td>0.064</td>
<td>93.77</td>
</tr>
<tr>
<td>BC12-19-03</td>
<td>Bm</td>
<td>0-40</td>
<td>0.283</td>
<td>3.22</td>
<td>0.008</td>
<td>0.18</td>
<td>0.40</td>
<td>0.024</td>
<td>0.019</td>
<td>4.13</td>
</tr>
<tr>
<td>BC12-19-04</td>
<td>BC</td>
<td>40-60</td>
<td>0.398</td>
<td>2.97</td>
<td>0.009</td>
<td>0.18</td>
<td>0.54</td>
<td>0.022</td>
<td>0.033</td>
<td>4.05</td>
</tr>
<tr>
<td>BC12-19-05</td>
<td>C</td>
<td>60-85+</td>
<td>0.062</td>
<td>3.21</td>
<td>0.002</td>
<td>0.17</td>
<td>0.53</td>
<td>0.015</td>
<td>0.024</td>
<td>4.01</td>
</tr>
</tbody>
</table>

|$^*$Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.
Figure 3.5.4.3: Soil profile from Spencer's Ridge (glacial) showing Ln, Fm, Bm, BC, and C horizons.
Site: Tezzeron (BC12-16)

Latitude: 54° 43' 3.8" N  Longitude: 124° 20' 50.1" W  Elevation: 868 m

Aspect: n/a  Slope: level  Slope position: crest

Vegetation: Mature Douglas-fir forest

Parent material: Fine gravelly morainal blanket over bedrock ridge.

Soil classification: Brunisolic Gray Luvisol

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/Ln (BC12-16-01)</td>
<td>5-3</td>
<td>Feathermoss and needle litter; abrupt, smooth boundary; 1-3 cm thick; extremely acid (pH 4.36).</td>
</tr>
<tr>
<td>Fm (BC12-16-02)</td>
<td>3-0</td>
<td>Very dark brown (7.5YR 2.5/3 m); abundant, very fine, fine, medium, and coarse oblique roots; abundant mycelia; compact matted; abrupt wavy boundary; 2-4 cm thick; very strongly acid (pH 4.94).</td>
</tr>
<tr>
<td>Ae (BC12-16-03)</td>
<td>0-25</td>
<td>Brown (10YR 5/3 m); loam; weak, fine and medium subangular blocky; very friable; abundant, very fine, fine, and medium, oblique, and plentiful, coarse; horizontal roots; 30% angular gravel and cobbles; gradual, irregular boundary; 10-30 cm thick; extremely acid (pH 4.50).</td>
</tr>
<tr>
<td>Bm (BC12-16-04)</td>
<td>10-30</td>
<td>Brown (7.5YR 4/4 m); loam; weak, fine subangular blocky; loose; plentiful, very fine, fine, and medium, oblique roots; 70% angular gravel [predominantly reddish oxidized sandstone]; gradual, broken boundary; 0-25 cm thick; extremely acid (pH 4.52).</td>
</tr>
<tr>
<td>Bt (BC12-16-05)</td>
<td>30-75+</td>
<td>Brown (7.5YR 5/4 m); loam; strong, fine and medium subangular blocky; firm; few, fine and medium, oblique roots; abundant clay films; 50% angular gravel; medium acid (pH 5.55).</td>
</tr>
</tbody>
</table>
Table 3.5.4.4: Tezzeron (glacial) soil horizon chemical data showing Iron, Aluminum, and Silicon by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Org-C</th>
<th>Tot-N</th>
<th>Tot-S</th>
<th>CaCO₃-eq</th>
<th>pH (H₂O)</th>
<th>pH (CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-16-01</td>
<td>S/Ln</td>
<td>5-3</td>
<td></td>
<td></td>
<td></td>
<td>54.00</td>
<td>0.918</td>
<td>0.086</td>
<td></td>
<td>4.54</td>
<td>4.30</td>
</tr>
<tr>
<td>BC12-16-02</td>
<td>Fm</td>
<td>3-0</td>
<td></td>
<td></td>
<td></td>
<td>50.45</td>
<td>1.628</td>
<td>0.151</td>
<td></td>
<td>5.26</td>
<td>4.94</td>
</tr>
<tr>
<td>BC12-16-03</td>
<td>Ae</td>
<td>0-25</td>
<td></td>
<td></td>
<td></td>
<td>44.0</td>
<td>1.628</td>
<td>0.151</td>
<td></td>
<td>5.11</td>
<td>4.50</td>
</tr>
<tr>
<td>BC12-16-04</td>
<td>Bm</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td>44.0</td>
<td>1.628</td>
<td>0.151</td>
<td></td>
<td>5.10</td>
<td>4.52</td>
</tr>
<tr>
<td>BC12-16-05</td>
<td>Bt</td>
<td>30-75+</td>
<td></td>
<td></td>
<td></td>
<td>46.5</td>
<td>1.628</td>
<td>0.151</td>
<td></td>
<td>6.26</td>
<td>5.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al³⁺</th>
<th>Ca⁴⁺</th>
<th>Fe³⁺</th>
<th>K⁺</th>
<th>Mg²⁺</th>
<th>Mn²⁺</th>
<th>Na⁺</th>
<th>Sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-16-01</td>
<td>S/Ln</td>
<td>5-3</td>
<td>0.068</td>
<td>20.75</td>
<td>0.005</td>
<td>6.21</td>
<td>7.52</td>
<td>2.308</td>
<td>0.095</td>
<td>36.97</td>
</tr>
<tr>
<td>BC12-16-02</td>
<td>Fm</td>
<td>3-0</td>
<td>0.045</td>
<td>63.38</td>
<td>0.006</td>
<td>4.41</td>
<td>7.33</td>
<td>2.901</td>
<td>0.129</td>
<td>78.21</td>
</tr>
<tr>
<td>BC12-16-03</td>
<td>Ae</td>
<td>0-25</td>
<td>0.369</td>
<td>3.95</td>
<td>0.022</td>
<td>0.17</td>
<td>0.74</td>
<td>0.032</td>
<td>0.024</td>
<td>5.30</td>
</tr>
<tr>
<td>BC12-16-04</td>
<td>Bm</td>
<td>10-30</td>
<td>0.379</td>
<td>4.05</td>
<td>0.010</td>
<td>0.21</td>
<td>1.06</td>
<td>0.072</td>
<td>0.020</td>
<td>6.60</td>
</tr>
<tr>
<td>BC12-16-05</td>
<td>Bt</td>
<td>30-75+</td>
<td>0.010</td>
<td>13.46</td>
<td>&lt;0.001</td>
<td>0.28</td>
<td>3.44</td>
<td>0.015</td>
<td>0.046</td>
<td>17.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Al₀</th>
<th>Al₀</th>
<th>Fe₀</th>
<th>Fe₀</th>
<th>Fe₀</th>
<th>Fe₀</th>
<th>Si₀</th>
<th>Al₀ + Fe₀</th>
<th>Fe₀/Fe₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-16-01</td>
<td>S/Ln</td>
<td>5-3</td>
<td>0.067</td>
<td>0.121</td>
<td>0.112</td>
<td>0.408</td>
<td>1.901</td>
<td>0.022</td>
<td>0.179</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>BC12-16-02</td>
<td>Fm</td>
<td>3-0</td>
<td>0.060</td>
<td>0.146</td>
<td>0.149</td>
<td>0.475</td>
<td>3.592</td>
<td>0.031</td>
<td>0.229</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>BC12-16-03</td>
<td>Ae</td>
<td>0-25</td>
<td>0.101</td>
<td>0.169</td>
<td>0.100</td>
<td>0.295</td>
<td>4.923</td>
<td>0.062</td>
<td>0.201</td>
<td>0.06</td>
<td></td>
</tr>
</tbody>
</table>

*Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate; o=oxalate, d=dithionite.
Figure 3.5.4.4: Soil profile from Tezzeron (glacial) showing S/Ln, Fm, Ae, Bm, and Bt horizons.
Site: Murray Ridge (BC12-18)

Latitude: 54° 29' 55.7" N  Longitude: 124° 7' 19.2" W  Elevation: 827 m

Aspect: 225°  Slope: 50%  Slope position: upper

Vegetation: Open stand of scattered, low productivity Douglas-fir, with mossy and lichen-dominated forest floor, 5-10% exposure of rock outcrop.

Parent material: Shallow colluvial veneer over hummocky ultramafic bedrock.

Soil classification: Orthic Eutric Brunisol

<table>
<thead>
<tr>
<th>Horizon (Sample) (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/L (not sampled)</td>
<td>2-1</td>
<td>Feathermoss and lichen, with scattering of Douglas-fir needle litter.</td>
</tr>
<tr>
<td>Fm (BC12-18-01)</td>
<td>1-0</td>
<td>Black (10YR 2/1 m); semi-decomposed moss, lichen, and needle litter; abundant mycelia; non-compact matted; plentiful, very fine and fine horizontal roots; abrupt, wavy boundary; 1-2 cm thick; strongly acid (pH 5.23).</td>
</tr>
<tr>
<td>Ahej (BC12-18-02)</td>
<td>0-1</td>
<td>Brown (10YR 5/3 m); silt loam*; weak, fine subangular blocky; friable; abundant, very fine, fine, and medium, oblique roots; clear, broken boundary; 0-2 cm thick; strongly acid (pH 5.33).</td>
</tr>
<tr>
<td>Bm (BC12-18-03)</td>
<td>1-30</td>
<td>Brown (10YR 4/3 m); loam; weak, fine subangular blocky; friable; abundant, very fine, fine, and medium, oblique roots; abrupt, irregular boundary; 20-40 cm thick; slightly acid (pH 6.17).</td>
</tr>
<tr>
<td>R</td>
<td>30+</td>
<td>Ultramafic bedrock.</td>
</tr>
</tbody>
</table>

*field texture estimate only; insufficient sample available for analysis.
Table 3.5.4.5: Murray Ridge-East (ultramafic) soil horizon chemical data showing Iron, Aluminum, and Silicon by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Org-C</th>
<th>Tot-N</th>
<th>Tot-S</th>
<th>CaCO3-eq</th>
<th>pH (H2O)</th>
<th>pH (CaCl2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC12-18-01</td>
<td>Fm</td>
<td>1-0</td>
<td></td>
<td></td>
<td></td>
<td>49.05</td>
<td>1.766</td>
<td>0.1334</td>
<td>5.65</td>
<td>5.65</td>
<td></td>
</tr>
<tr>
<td>BC12-18-02</td>
<td>Ahej</td>
<td>0-1</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>12.52</td>
<td>0.500</td>
<td>0.0437</td>
<td>5.64</td>
<td>5.64</td>
<td></td>
</tr>
<tr>
<td>BC12-18-03</td>
<td>Bm</td>
<td>1-30</td>
<td>35.3</td>
<td>41.7</td>
<td>23.0</td>
<td>3.04</td>
<td>0.175</td>
<td>0.0215</td>
<td>6.69</td>
<td>6.69</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Exchangeable (cmol (+) / kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fe3+</td>
</tr>
<tr>
<td>BC12-18-01</td>
<td>Fm</td>
<td>1-0</td>
<td>0.019</td>
</tr>
<tr>
<td>BC12-18-02</td>
<td>Ahej</td>
<td>0-1</td>
<td>0.019</td>
</tr>
<tr>
<td>BC12-18-03</td>
<td>Bm</td>
<td>1-30</td>
<td>0.002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Alp</td>
</tr>
<tr>
<td>BC12-18-01</td>
<td>Fm</td>
<td>1-0</td>
<td>0.075</td>
</tr>
<tr>
<td>BC12-18-02</td>
<td>Ahej</td>
<td>0-1</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.
Figure 3.5.4.5: Soil profile for Murray Ridge-East (ultramafic) showing S/Ln, Fm, Ahej, Bm, and R horizons
Site: Pinchi Mountain (BC11-06)

Latitude: 54° 38.973' N  Longitude: 124° 29.293' W  Elevation: 961 m

Aspect: 180°  Slope: 40%  Slope position: midslope

Vegetation: Open Douglas-fir forest, with juniper, spiraea, grass understory

Parent material: Colluvial veneer over bedrock.

Soil classification: Orthic Eutric Brunisol

<table>
<thead>
<tr>
<th>Horizon (Sample)</th>
<th>Depth (cm)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF (BC11-06-01)</td>
<td>3-0</td>
<td>Shrub, tree, and herbaceous leaf litter, and semi-decomposed organic matter; non-compact matted; plentiful, very fine, fine, and medium, oblique and horizontal roots; abrupt, wavy boundary; 2-4 cm thick; medium acid (pH 5.68).</td>
</tr>
<tr>
<td>Ah (BC11-06-02)</td>
<td>0-10</td>
<td>Very dark brown (7.5YR 2.5/2 m); clay loam; weak, coarse granular; friable; plentiful, very fine, fine, and medium, oblique, and few, coarse, horizontal roots; 20% angular and subangular gravel; gradual, wavy boundary; 8-12 cm thick; slightly acid (pH 6.25).</td>
</tr>
<tr>
<td>Bm1 (BC11-06-03)</td>
<td>10-28</td>
<td>Brown (7.5YR 4/3 m); sandy clay loam; weak, fine and medium subangular blocky; friable; plentiful, very fine, fine, and medium, oblique roots; 20-30% angular and subangular gravel and cobbles; clear, wavy boundary; 15-20 cm thick; neutral (pH 6.58).</td>
</tr>
<tr>
<td>Bm2 (BC11-06-04)</td>
<td>28-53</td>
<td>Brown (10YR 4/3 m); sandy loam; single grain; friable; few, very fine, fine, and medium, oblique roots; 60-70% angular gravel; abrupt, wavy boundary; 15-30 cm thick; neutral (pH 6.95).</td>
</tr>
<tr>
<td>C (BC11-06-05)</td>
<td>53-70</td>
<td>Very dark gray (2.5Y 3/1 m); sandy loam; massive; friable; few, very fine, fine, and medium, oblique roots; 50-60% angular and subangular gravel; abrupt, wavy boundary; neutral (pH 6.77).</td>
</tr>
<tr>
<td>R</td>
<td>70+</td>
<td>Fractured bedrock.</td>
</tr>
</tbody>
</table>
Table 3.5.4.6: Pinchi Mountain (ultramafic) soil horizon major element oxides and chemical data showing Iron, Aluminum, and Silicon by sodium citrate-dithionite, sodium pyrophosphate, and acid ammonium oxalate extractions.*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>%</th>
<th>pH (H₂O)</th>
<th>pH (CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF</td>
<td>3-0</td>
<td>46.49 1.450 0.1244</td>
<td>5.85</td>
<td>5.68</td>
</tr>
<tr>
<td>BC11-06-02</td>
<td>Ah</td>
<td>0-10</td>
<td>44.5 28.5 27.0 5.17 0.245 0.0246</td>
<td>6.57</td>
<td>6.25</td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1</td>
<td>10-28</td>
<td>53.1 24.3 22.6 2.34 0.126 0.0124</td>
<td>6.83</td>
<td>6.58</td>
</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2</td>
<td>28-53</td>
<td>67.0 22.8 10.2 0.45 0.031 0.0050</td>
<td>7.30</td>
<td>6.95</td>
</tr>
<tr>
<td>BC11-06-05</td>
<td>C</td>
<td>53-70</td>
<td>58.1 35.0 7.0 0.66 0.040 0.0031</td>
<td>7.35</td>
<td>6.77</td>
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</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>%</th>
<th>pH (H₂O)</th>
<th>pH (CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF</td>
<td>3-0</td>
<td>0.008 54.78 0.010 1.89 35.39 0.261 0.098</td>
<td>92.43</td>
<td></td>
</tr>
<tr>
<td>BC11-06-02</td>
<td>Ah</td>
<td>0-10</td>
<td>0.005 13.31 &lt; 0.001 0.41 24.34 0.050 &lt; 0.001</td>
<td>38.10</td>
<td></td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1</td>
<td>10-28</td>
<td>0.078 0.131 0.418 1.283 3.370 0.222 0.496</td>
<td>23.97</td>
<td></td>
</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2</td>
<td>28-53</td>
<td>&lt; 0.001 1.89 &lt; 0.001 0.08 8.60 0.007 &lt; 0.001</td>
<td>10.74</td>
<td></td>
</tr>
<tr>
<td>BC11-06-05</td>
<td>C</td>
<td>53-70</td>
<td>&lt; 0.001 5.19 &lt; 0.001 0.08 43.56 0.006 &lt; 0.001</td>
<td>48.80</td>
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</tr>
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</table>

Major Element Oxides:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>%</th>
<th>pH (H₂O)</th>
<th>pH (CaCl₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF</td>
<td>3-0</td>
<td>0.070 0.171 0.530 1.838 4.239 0.236 0.600</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td>BC11-06-02</td>
<td>Ah</td>
<td>0-10</td>
<td>0.078 0.131 0.418 1.283 3.370 0.222 0.496</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1</td>
<td>10-28</td>
<td>0.006 0.108 0.049 0.618 1.499 0.150 0.055</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2</td>
<td>28-53</td>
<td>0.006 0.453 0.067 1.471 2.027 0.238 0.073</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

*Org-C=organic carbon, Tot-N= total nitrogen, Tot-S=total sulfur, LOI=loss on ignition. Subscripts for Al, Fe, and Si extractions: p = pyrophosphate, o=oxalate, d=dithionite.
Table 3.5.4.7: Pinchi Mountain (ultramafic) soil horizon minor elements

**Minor Elements:**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Ag (ppm)</th>
<th>Ba</th>
<th>Ca (ppm)</th>
<th>Co</th>
<th>Cr (ppm)</th>
<th>Cs (ppm)</th>
<th>Cu</th>
<th>Dy</th>
<th>Er</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF 3-0</td>
<td>4</td>
<td>492</td>
<td>13.0</td>
<td>182.0</td>
<td>5740</td>
<td>1.06</td>
<td>29</td>
<td>0.84</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>BC11-06-02</td>
<td>Ah 0-10</td>
<td>1</td>
<td>168.5</td>
<td>9.5</td>
<td>149.5</td>
<td>4570</td>
<td>0.68</td>
<td>26</td>
<td>0.72</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1 10-28</td>
<td>1</td>
<td>320</td>
<td>14.8</td>
<td>109.0</td>
<td>3240</td>
<td>0.82</td>
<td>27</td>
<td>1.27</td>
<td>0.79</td>
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</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2 28-53</td>
<td>1</td>
<td>153</td>
<td>18.8</td>
<td>123.5</td>
<td>3040</td>
<td>0.64</td>
<td>49</td>
<td>1.67</td>
<td>0.91</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Eu</th>
<th>Ga</th>
<th>Gd</th>
<th>Hf</th>
<th>Ho</th>
<th>La</th>
<th>Lu</th>
<th>Mo</th>
<th>Nb</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF 3-0</td>
<td></td>
<td>0.28</td>
<td>4.8</td>
<td>1.02</td>
<td>1.2</td>
<td>0.16</td>
<td>6.4</td>
<td>0.06</td>
<td>&lt;2</td>
<td>4.9</td>
</tr>
<tr>
<td>BC11-06-02</td>
<td>Ah 0-10</td>
<td>1</td>
<td>0.26</td>
<td>3.6</td>
<td>0.97</td>
<td>1.1</td>
<td>0.14</td>
<td>4.8</td>
<td>0.06</td>
<td>&lt;2</td>
<td>3.9</td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1 10-28</td>
<td>1</td>
<td>0.37</td>
<td>5.5</td>
<td>1.50</td>
<td>1.6</td>
<td>0.26</td>
<td>7.6</td>
<td>0.11</td>
<td>&lt;2</td>
<td>5.0</td>
</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2 28-53</td>
<td>1</td>
<td>0.75</td>
<td>8.2</td>
<td>2.31</td>
<td>1.8</td>
<td>0.32</td>
<td>9.4</td>
<td>0.10</td>
<td>&lt;2</td>
<td>7.6</td>
</tr>
<tr>
<td>BC11-06-05</td>
<td>C 53-70</td>
<td></td>
<td>6.2</td>
<td>2310</td>
<td>&lt;5</td>
<td>1.46</td>
<td>15.4</td>
<td>1.16</td>
<td>&lt;1</td>
<td>56.9</td>
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</tr>
<tr>
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<td>Ah 0-10</td>
<td>1</td>
<td>5.5</td>
<td>2870</td>
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<td>1.19</td>
<td>10.1</td>
<td>1.09</td>
<td>&lt;1</td>
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</tr>
<tr>
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<td>Bm2 28-53</td>
<td>1</td>
<td>8.1</td>
<td>2140</td>
<td>&lt;5</td>
<td>1.80</td>
<td>16.2</td>
<td>1.62</td>
<td>&lt;1</td>
<td>59.6</td>
<td>0.3</td>
</tr>
<tr>
<td>BC11-06-05</td>
<td>C 53-70</td>
<td></td>
<td>12.0</td>
<td>2100</td>
<td>&lt;5</td>
<td>2.50</td>
<td>5.1</td>
<td>2.42</td>
<td>1</td>
<td>19.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Horizon</th>
<th>Depth (cm)</th>
<th>Tb</th>
<th>Th</th>
<th>Tl</th>
<th>Tm</th>
<th>U</th>
<th>V</th>
<th>W</th>
<th>Y</th>
<th>Yb</th>
<th>Zn</th>
<th>Zr</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC11-06-01</td>
<td>LF 3-0</td>
<td>0.13</td>
<td>0.76</td>
<td>&lt;0.5</td>
<td>0.06</td>
<td>0.31</td>
<td>245</td>
<td>2</td>
<td>4.7</td>
<td>0.44</td>
<td>206</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>BC11-06-03</td>
<td>Bm1 10-28</td>
<td>0.14</td>
<td>0.97</td>
<td>&lt;0.5</td>
<td>0.06</td>
<td>0.31</td>
<td>245</td>
<td>2</td>
<td>4.7</td>
<td>0.44</td>
<td>206</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>BC11-06-04</td>
<td>Bm2 28-53</td>
<td>0.21</td>
<td>1.35</td>
<td>&lt;0.5</td>
<td>0.10</td>
<td>0.58</td>
<td>167</td>
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<td>7.6</td>
<td>0.71</td>
<td>67</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>BC11-06-05</td>
<td>C 53-70</td>
<td>0.29</td>
<td>1.18</td>
<td>&lt;0.5</td>
<td>0.12</td>
<td>0.35</td>
<td>162</td>
<td>1</td>
<td>9.0</td>
<td>0.74</td>
<td>91</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.5.4.6: Soil profile for Pinchi Mountain (ultramafic) showing LF, Ah, Bm1, Bm2, and C horizons
Appendix 2: Elemental analyses of soil composites used in greenhouse experiment

Table 3.5.4.8: Chemical and elemental data from soil homogenates

<table>
<thead>
<tr>
<th>Location</th>
<th>SiO₂</th>
<th>A2O₃</th>
<th>Fe₂O₃</th>
<th>CaO</th>
<th>MgO</th>
<th>Na₂O</th>
<th>K₂O</th>
<th>Cr₂O₃</th>
<th>TiO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidcwa Rd</td>
<td>51.2</td>
<td>9.17</td>
<td>4.84</td>
<td>6.17</td>
<td>3.76</td>
<td>1.24</td>
<td>1</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
<td>Pinchi Hill</td>
<td>66.6</td>
<td>11.9</td>
<td>5.05</td>
<td>1.64</td>
<td>1.1</td>
<td>2.91</td>
<td>1.58</td>
<td>0.02</td>
<td>0.68</td>
</tr>
<tr>
<td>Spencer's Ridge</td>
<td>68.6</td>
<td>11.35</td>
<td>4.35</td>
<td>1.38</td>
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<td>2.62</td>
<td>1.49</td>
<td>0.03</td>
<td>0.74</td>
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<td>4.04</td>
<td>2.41</td>
<td>1.25</td>
<td>1.24</td>
<td>1.1</td>
<td>0.02</td>
<td>0.53</td>
</tr>
<tr>
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<td>48.4</td>
<td>5.38</td>
<td>8.87</td>
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<td>15.95</td>
<td>1.01</td>
<td>0.62</td>
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<td>0.37</td>
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<td>0.66</td>
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</table>

<table>
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<th>Location</th>
<th>MnO</th>
<th>P₂O₅</th>
<th>SrO</th>
<th>BaO</th>
<th>C</th>
<th>S</th>
<th>Ba</th>
<th>Ce</th>
<th>Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidcwa Rd</td>
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<td>0.02</td>
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<td>0.01</td>
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<tr>
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<td>0.04</td>
<td>0.11</td>
<td>2.28</td>
<td>0.01</td>
<td>932</td>
<td>27.1</td>
<td>160</td>
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<tr>
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<td>0.24</td>
<td>0.03</td>
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<td>0.19</td>
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<td>0.07</td>
<td>3.63</td>
<td>0.02</td>
<td>610</td>
<td>31.6</td>
<td>160</td>
</tr>
<tr>
<td>Murray Ridge - East</td>
<td>0.2</td>
<td>0.19</td>
<td>0.01</td>
<td>0.05</td>
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<table>
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<th>Dy</th>
<th>Er</th>
<th>Eu</th>
<th>Ga</th>
<th>Gd</th>
<th>Hf</th>
<th>Ho</th>
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<tr>
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<td>1.69</td>
<td>0.76</td>
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<td>4</td>
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<tr>
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<td>1.68</td>
<td>1.01</td>
<td>0.45</td>
<td>6.8</td>
<td>1.72</td>
<td>1.8</td>
<td>0.35</td>
<td>8.5</td>
</tr>
<tr>
<td>Pinchi Mountain</td>
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<td>1.07</td>
<td>0.65</td>
<td>8.1</td>
<td>2.04</td>
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<td>0.34</td>
<td>9.6</td>
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<table>
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<th>Nd</th>
<th>Pr</th>
<th>Rb</th>
<th>Sm</th>
<th>Sn</th>
<th>Sr</th>
<th>Ta</th>
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<tr>
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<td>3.04</td>
<td>45.7</td>
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<td>54.6</td>
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<td>1</td>
<td>231</td>
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Table 3.5.4.9: Chemical and elemental data from soil homogenates (continued)*

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<th>Bi</th>
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<th>Sb</th>
<th>Se</th>
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<td>0.06</td>
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*LOI = loss on ignition.
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<tr>
<th>Morphotype Label</th>
<th>Composite Description</th>
<th>Possible Identity</th>
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<td>Type A</td>
<td>ECM Dark brown, monopodial with a reflective luster, straight and unbranched tips. Mantle has an interlocking to non-interlocking irregular synenchyma, 2-3 μm wide with pigment blotches and a deep golden brown color. ECM Unbranched, straight, monopodial to cluster-tuberculate, matte bright white surface with matte grey-purple rind. Cottony texture. Mantle structure obscured by crystals.</td>
<td>Thelephoraceae/Tomentella-1</td>
</tr>
<tr>
<td>Type B</td>
<td>Emanating hyphae hyaline and branched 2-4 μm wide with garnet colored crystal ornamentation and thick cell walls. Highly differentiated rhizomorphs, hyaline in color, +100 μm wide with garnet crystalline deposits. ECM Monopodial, very black with a stringy texture. Matte luster with copious emanating hyphae. Stellate patterned net synenchyma. Hyphal cells approximately 5 μm wide. Some sclerotia visible. ECM Monopodial to pinnate, silvery-white with a thick black/brown rind. Texture varies from reflective to matte and cottony. Mantle structure obscured by thick silver-white crystals. Many dark grey-brown branching rhizomorphs and emanating brown hyphae. Hyphae 2-3 μm thick with slight globules, thick cell walls, and knee-like bends. Rhizomorphs +90 μm thick with some gray-silver crystals, slight globular deposits, thick cell walls, and knee-like bends.</td>
<td>Rhizopogon subcaerulescens/Suillus caerulescens</td>
</tr>
<tr>
<td>Type C</td>
<td></td>
<td>Cenococcum geophilum</td>
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<tr>
<td>Type D</td>
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<td>Rhizopogon cf. villosulus</td>
</tr>
<tr>
<td>Type</td>
<td>Description</td>
<td>E-strain</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>E</td>
<td>ECM Reddish brown single monopodial with a smooth texture and reflective luster. Net prosenchyma with hyphae approximately 6-7 μm with some +13 μm wide. ECM Monopodial, pale yellow-brown. Smooth texture with a matte luster. Interlocking to non-interlocking irregular synenchyma with no pigment blotches. One emanating hyphae, unbranched, septate hyaline, clamps, 3μm in width.</td>
<td>Thelephoraceae/Tomentella-2</td>
</tr>
<tr>
<td>F</td>
<td>Pale yellow in color to hyaline, 1-3 μm in width. Septate with clamps at almost every septum. Differentiated rhizomorph with both septa and clamps. Pale golden yellow in color. ECM Monopodial, very dark brown with a shiny luster. Interlocking to non-interlocking synenchyma. Some pale brown emanating hyphae with septa and clamps, approximately 4 μm wide.</td>
<td>Hebeloma-Amphinema like</td>
</tr>
<tr>
<td>G</td>
<td>Pale yellow in color to hyaline, 1-3 μm in width. Septate with clamps at almost every septum. Differentiated rhizomorph with both septa and clamps. Pale golden yellow in color. ECM Monopodial, very dark brown with a shiny luster. Interlocking to non-interlocking synenchyma. Some pale brown emanating hyphae with septa and clamps, approximately 4 μm wide.</td>
<td>Thelephoraceae/Tomentella-3</td>
</tr>
<tr>
<td>H</td>
<td>Pale yellow in color to hyaline, 1-3 μm in width. Septate with clamps at almost every septum. Differentiated rhizomorph with both septa and clamps. Pale golden yellow in color. ECM Monopodial, very dark brown with a shiny luster. Interlocking to non-interlocking synenchyma. Some pale brown emanating hyphae with septa and clamps, approximately 4 μm wide.</td>
<td>Russula/Lactarius-1</td>
</tr>
<tr>
<td>I</td>
<td>Pale yellow in color to hyaline, 1-3 μm in width. Septate with clamps at almost every septum. Differentiated rhizomorph with both septa and clamps. Pale golden yellow in color. ECM Monopodial, very dark brown with a shiny luster. Interlocking to non-interlocking synenchyma. Some pale brown emanating hyphae with septa and clamps, approximately 4 μm wide.</td>
<td>Blonde</td>
</tr>
<tr>
<td>J</td>
<td>Pale yellow in color to hyaline, 1-3 μm in width. Septate with clamps at almost every septum. Differentiated rhizomorph with both septa and clamps. Pale golden yellow in color. ECM Monopodial, very dark brown with a shiny luster. Interlocking to non-interlocking synenchyma. Some pale brown emanating hyphae with septa and clamps, approximately 4 μm wide.</td>
<td>Blonde</td>
</tr>
<tr>
<td>Type</td>
<td>Description</td>
<td>Species</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>L</td>
<td>ECM Pale golden brown, smooth texture with a reflective luster. Branched, unbranched to monopodial pinnate, some tips very long. Fine cystidia on the tip.</td>
<td><em>Tuber</em></td>
</tr>
<tr>
<td>M</td>
<td>ECM Monopodial, very pale gold-brown. Very smooth texture with a reflective luster. Mantle with interlocking to non-interlocking irregular synenchyma, cells pale yellow under microscope 3-4 µm in width.</td>
<td><em>Russula</em>/<em>Lactarius</em>-3</td>
</tr>
<tr>
<td>N</td>
<td>ECM Dark brown, monopodial with a reflective luster, straight and unbranched tips. Mantle has an interlocking to non-interlocking irregular synenchyma, 2-3 µm wide with a visible Hartig net.</td>
<td><em>Thelephoraceae</em>/<em>Tomentella</em>-4</td>
</tr>
<tr>
<td>O</td>
<td>Mantle hyphae 2-3 µm in width. Branched emanating hyphae dark brown-grey in color, finely varicose with anastomoses.</td>
<td><em>Mycelium radicis atrovirens (MRA)</em></td>
</tr>
</tbody>
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