NATURAL REGENERATION POTENTIAL OF DOUGLAS-FIR FOLLOWING WILDFIRE AND CLEARCUT HARVESTING

by

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Abstract

The McLure fire of August, 2003, affected over 26,000 hectares in the interior of British Columbia. The study objective was to determine the impact of wildfire and clearcutting severity on Douglas-fir regeneration potential in the Interior Douglas-fir biogeoclimatic zone. The study design consisted of five treatments that compared a range of disturbance severities: high severity burn, low severity burn, clearcut, screefed clearcut, and undisturbed forest. At each of four replicate sites per treatment, 1000 seeds were sown in late spring of 2004. Natural regeneration potential was assessed by measuring seedling performance and mycorrhizal diversity over a three-year period. Overall, the treatments with the greatest disturbance severity had the greatest natural regeneration potential due to increased resource availability. Seedling survival was considerably higher in the burn and clearcut treatments than the undisturbed forest. Seedlings in the high severity burn had significantly greater shoot height, biomass, and foliar N and P content than those in the clearcut treatments. Seedlings regenerating in the burn treatments had the lowest ectomycorrhizal colonization in the first growing season but all seedlings in all treatments were colonized by the start of the second growing season. Increased disturbance severity, either by wildfire or clearcutting, led to a uniform ectomycorrhizal community dominated by Wilcoxina sp. In contrast, the undisturbed forest was dominated by a more diverse ectomycorrhizal community. The simplification of the ectomycorrhizal community did not negatively affect seedling growth or survival. The highest biomass accumulation and foliar nitrogen content occurred in the high severity burn and were associated with the lowest levels of mycorrhizal colonization and diversity. This supports the hypothesis that plants reduce their carbon investment in mycorrhizal fungi when growing under favorable conditions. Overall, the results
indicate that, given a seed source, the natural regeneration potential of Douglas-fir is high after both wildfire and clearcut harvesting.
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Co-authorship statement

In Chapter 1, I assisted in creating the study design and I conducted the research, including the data collection, statistical analysis, and manuscript writing. Dr. Suzanne Simard helped initiate the project, proposed the initial study design, and assisted in manuscript preparation.

In Chapter 2, I assisted in creating the study design and I conducted the research, including the data collection, statistical analysis, and manuscript writing. Dr. Suzanne Simard helped initiate the project, proposed the initial study design, and assisted in manuscript preparation. Dr. Melanie Jones also helped with initiating the research project and designing the study. Dr. Dan Durall facilitated the molecular analysis of mycorrhizal fungi.

In chapter 3, I designed the study and conducted the research, including the data collection, statistical analysis, and manuscript writing. Dr. Suzanne Simard assisted with the experimental design and manuscript preparation.
1. Introduction

1.1. Context

Few topics in forest ecology have more visceral appeal and dramatic impact than that of fire on forests ecosystems. The power of fire to transform by both restoring and destroying forest ecosystems is evident to all those who witness a wildfire and its aftermath. After the flames die down, however, the power of fire to shape forest ecosystems can be easily overlooked. In the summer of 2003, over 260,000 hectares in the province of British Columbia (B.C.) were affected by fire. The McLure fire was located near Kamloops B.C. and affected 26,420 hectares (Ministry of Forests 2003). The fires of 2003 served a reminder that fire remains one of the major shaping forces in interior B.C. The recent fires remind forest researchers and planners that there can be no full examination of the future of forests in interior B.C. without considering fire.

Before the advent of professional forestry, natural regeneration after wildfire was a major pathway for forest renewal in forests of western North America. Due to concerns over natural failures, artificial regeneration policies were instituted across western North America, including B.C. (Weetman and Vyse 1990, Hermann and Lavender 1999). However, natural regeneration continues to be a major source of forest renewal, adding diversity even in planted monocultures (Hermann and Lavender 1999). In recent decades, the desire to protect biodiversity has resulted in the development of so-called “emulation silviculture”, which seeks to base silvicultural practices on natural disturbance regimes to meet multiple management objectives, ranging from
timber production to protecting wildlife diversity (Long 2009). The effort to emulate natural disturbances raises the issue of how viability of natural regeneration of harvested areas compares to that after wildfire. Since this study was initiated, natural regeneration has received renewed attention due to the debate over post-harvest silvicultural practices on natural regeneration (Donato et al. 2006, Newton et al. 2006).

A better understanding of the role of soils and soil organisms in tree establishment and growth is one of the major research priorities in natural regeneration and wildfire research (Whelan 1995). Many conifers such as Douglas-fir and lodgepole pine that germinate after high-severity disturbances also form an obligatory, but horizontally transmitted, symbiosis with ectomycorrhizal (EM) fungi. Mycorrhizal and natural regeneration research intersect in two major areas: the effect of disturbances on mycorrhizal communities (Jones et al. 2003, Cairney and Bastias 2007) and the relationship between plant productivity and mycorrhizal colonization (Jonsson et al. 2001, Treseder 2004, Hoeksema et al. 2010). Researchers have been investigating the degree to which changes in mycorrhizal community caused by disturbances might alter forest successional pathways through their effects on individual tree productivity (Simard 2009).

1.2 Objectives and hypotheses

The overarching objective of this dissertation was to investigate the natural regeneration potential of Douglas-fir in response to common disturbances to the Interior Douglas-fir (IDF) biogeoclimatic zone. By experimentally seeding, we were able to focus on regeneration from germination until the third year of establishment. We assessed natural regeneration in four main areas: germination, survival, growth, and mycorrhizal colonization. Specifically, we tested three main hypotheses:
1. Increasing disturbance severity results in high natural regeneration potential (growth, survival, and biomass) due to an increase in light, moisture, and nutrient availability.

2. Increasing disturbance severity results in a simplified EM community that is slower to colonize seedlings due loss of inoculum in the forest floor and mature hosts.

3. Increased resource availability after wildfire reduces plant investment in EM colonization.

1.2 Literature Review
1.2.1 Fire as a disturbance: effects on vegetation and soils

Fire releases as heat a large amount of energy through the oxidation of organic matter (Fisher and Binkley, 2000). The extent to which fire releases energy is dependent on fuel consumption and the moisture levels of the fuel (Fisher and Binkley, 2000). In one sense, fire is a highly predictable event, where heating results in injury and death to plants and animals either directly or indirectly (Agee 1993). Conversely, researchers have found evidence of nonequilibrium long-term dynamics in the interactions between forests and fire (Franklin and Tolonen 2000).

The effects of wildfire on forest ecosystems are commonly assessed by determining the magnitude of the fire event, either by direct assessment of the heat released in a fire (fire intensity) or by post-hoc assessment of fire effects (fire severity) (Agee 1993). Fire severity is defined as the amount of damage to vegetation and degree of combustion of the forest floor. Fire creates a spectrum of severities that depend on a large number of factors, ranging from fuel loading to fuel moisture content (Neary et al., 1999). The severity spectrum runs from low severity, indicating a living remnant overstory with uneven combustion of the forest floor, to high severity, indicating full combustion of the forest floor, death of the overstory, and exposure
of mineral soil (Belillas and Feller, 1998). As a result of spatial variation in fire severity, seedlings and associated EMF can experience a wide variety of conditions in the above and belowground environment.

Fire affects the chemistry, biology and physical structure of soils and related processes (Agee, 1993). The effects range from temperature changes in the upper soil profile to changes in the physical properties, such as bulk density (Agee 1993). For the IDF zone, changes in soil hydrology are especially important because they can affect plant growth directly through changes in moisture availability, but also indirectly through their effects on nutrient cycling. The effects of fire on soil moisture levels and soil hydrology can leave the post-fire environment radically different than the pre-fire one. The loss of vegetative cover results in less canopy interception and soil retention of precipitation, and consequently, an increase in water loss from forest ecosystems (Neary et al., 1999). Water infiltration into soil can be reduced after a fire because the exposed mineral soil can be sealed by the action of raindrops (Neary et al., 1999) and by development of a water-repellent layer by hydrophobic organic compounds (DeBano, 2000). These changes can lead to soil drying and erosion, which in turn can lead to losses of nutrients and soil (Fisher and Binkley 2000). Although this might lead to increased desiccation at a site, removal of plant cover can also reduce evapotranspiration and possibly offset losses associated with water repellency (Neary et al., 1999).

1.2.2 Fire effects on nutrient cycling

Fire causes changes in nutrient cycles that directly affect the availability of nutrients to the mycorrhizal symbiosis. Fire is in essence a rapid decomposition process in that it can break down organic substrates into less complex and inorganic forms. Schimel and Bennett (2004) argue that the N cycle is driven by the depolymerization of N-containing polymers from organic
matter; this is because the deploymerization controls the availability of N. Fire can therefore have a major effect on N cycling because it releases N from polymerized forms of organic matter.

The fate of nutrients released by fire is crucial to understanding post-fire nutrient dynamics. Are most of the nutrients released from organic matter and subsequently lost, or does a significant percentage remain long enough to become available to plants? Nutrients are lost from fires through the combined action of five processes: oxidation, volatilization, convection of ash particles, leaching, and erosion (Fisher and Binkley, 2000). Significant N losses through volatilization begin to occur in the range of 175°C-200°C (Agee, 1993). Other key nutrients have higher volatilization points, such as inorganic P at around 800°C (Agee, 1993).

The oxidation of nutrients in the soil can lead to ready availability of inorganic ions for plant uptake, but the amount and duration of increased nutrient availability to any given organism depends on the interactions among nutrient losses, plant establishment, and competition. Within the first year or two, researchers have reported post-fire increases in available forms of nutrients such as N, P and Ca (Fisher and Binkley, 2000). In the case of N, any initial pulse of N typically lasts no more than a few growing seasons (Fisher and Binkley, 2000). In the short-term, research has indicated that fire can increase, decrease, or have no effect on available nitrogen (Agee, 1993). To clarify the issue, Wan et al., (2001) conducted a meta-analysis of 87 fire studies for short-term trends in N dynamics. They found that NH$_4^+$ and NO$_3^-$ levels increased after fire, but the elevated levels generally did not persist beyond the first year. Temporarily, increases in NH$_4^+$ levels occur first, followed by increases in NO$_3^-$ due to nitrification (Fisher and Binkley, 2000). The nitrate can be lost due to leaching (Agee, 1993). Nitrogen availability subsequent to the initial post-fire pulse depends on factors such as N
volatilized during the fire, changes in rates of mineralization by microorganisms, and
competition between plants and microbes (Fisher and Binkley, 2000). Smithwick et al. (2005)
found that predicting the effects of severe wildfires on post-fire N availability in coniferous
forests is difficult given the highly spatial variability of stand-replacing wildfires.

There is clearly an immediate loss of N to the atmosphere due to the action of fire (Agee,
1993). In their meta-analysis, Wan et al. (2001) found no significant effect of fire on total N in
soils because the amount lost was small compared to the total pool; however, they emphasized
that significant N losses may occur in the upper 2 cm. This may not have been detected in the
meta-analysis because of differences in sampling techniques between studies. Brais et al. (2000)
found that severe burns in a boreal forest significantly reduced forest floor nutrient levels, while
light and moderate burns did not.

1.2.3 Conifer regeneration and wildfire

Successful natural regeneration requires fulfillment of the so-called “natural reproduction
triangle”: sufficient seed source, suitable seed bed, and an environment compatible with
germination and seedling establishment (Roe et al. 1970). Wildfire, of course, has the potential to
reduce or eliminate seed sources, but uneven combustion at a local scale leaves seed banks, and
on the landscape scale, seed sources in close proximity to the burned sites (Donato et al. 2009).
The timeframe that natural regeneration occurs ranges from immediately post-disturbance to up
50 years, with complete failure possible on harsh sites (Gratkowski 1961, Weetman and Vyse
1990, Shatford et al. 2007).

The forest litter layer can inhibit seed germination by creating a mechanical barrier and
leaving seeds prone to desiccation (Caccia and Ballare 1998, Bai et al. 2004). Litter can also
contain germination-inhibiting allelochemicals (Mallik 2003). Disturbances that remove or reduce the forest floor are often required to create favourable microsites within seedbeds for conifer germination by reducing soil organic-matter depth, exposing mineral soil and reducing the abundance of competing vegetation (Greene et al. 1999). Reducing the depth of the forest floor can improve moisture and temperature conditions for germination (Herr et al. 1999, Klinka et al. 2000). The high moisture holding capacity of mineral-soil seedbeds, providing readily available water to seeds, is cited as one of the major advantages over organic seedbeds (Greene et al. 1999, Oleskog and Sahlen 2000).

The optimal seedbed for germination within a species varies with the environmental conditions (e.g., aspect) (Oswald et al. 1998). Douglas-fir prefers a bare mineral or mixed mineral/mineral seedbed (Klinka et al., 2000), but germinates successfully in seedbeds with litter in some environments (Ryker 1975). Burning the forest floor may reduce Douglas-fir germination compared to bare mineral-soil beds (Oswald et al. 1998). The exact effect of ash on seed germination is unclear but the increase in pH may lead to an increase in pathogenic damping-off fungi, which quickly kill newly germinated seedlings (Tarrant 1954) or to toxic environmental effects that inhibit germination. Unfavourable moisture conditions may also result from the hydrophobic nature of ash (Kemball et al. 2006).

After germination, newly emerged seedlings can be vulnerable due to limited carbohydrate reserves and small root systems, leading to high mortality rates in the first year of establishment (Tappeiner and Helms 1971, Canham et al. 1999). Other major factors limiting plant growth include winter temperature extremes, summer frost, and competition with pinegrass (Calamagrostis rubescens Buckl.) (Newsome, 1998). Summer droughts are a major cause of mortality (Dunne and Parker 1999, Simard et al. 2003). Because these climatic conditions are
common in southern the interior of B.C., they have the potential to limit regeneration. In the IDF zone, for example, mean annual precipitation ranges from 300 to 750 mm and moisture deficits are common during the growing season (Hope et al. 1991). Water stress has also been shown to negatively affect the uptake of nutrients (Larcher, 2001; Tuininga et al., 2002).

1.2.4 Fire, nutrients and seedling growth

There is clear evidence that wildfires provide a favourable environment for seedling establishment by meeting the requirements of regeneration triangle. Studies have found positive effects of wildfire on seedling establishment (Hermann and Lavender 1990, Oswald et al. 1998, Kembal et al. 2006) as well as growth (Hermann and Chilcote 1965, Oswald et al. 1998, Keyser et al. 2010). However, the optimal conditions for germination may conflict with subsequent growth and survival (Schupp 1995). Oswald et al (1998) found evidence for this conflict in Douglas-fir where germination was greater in bare mineral soil but growth and survival was higher in burned seedbeds.

Temperate forest ecosystems show evidence of widespread N limitation (Vitousek and Howarth 1991). Increased availability of nutrients, particularly nitrogen, have long been hypothesized to improve foliar nutrients of plants growing after a fire (St John and Rundel 1976). Although nutrients are typically not the primary limitation for newly germinated seedlings (Smith et al., 1997), seedlings will respond positively to nutrient additions (Larcher 2001). Studies of lodgepole pine have found that the foliar nutrient levels remain favourable for 5-15 years after the fire event (Romme et al. 2009, Turner et al. 2009). Fires can also have long-lasting effects on stand nutrient status by affecting stand density (Turner et al. 2009). Over time, however, foliage of Douglas-fir growing in dry forest ecosystems shows symptoms of foliar N deficiency (Moore et al. 2004). The short-term nutrient dynamics during Douglas-fir seedling
establishment are unclear since many of the studies that examined Douglas-fir post-fire dynamics did not report foliar nutrient levels of seedlings (Tappeiner and Helms 1971, Hermann and Lavender 1990, Oswald et al. 1998).

1.2.5 Effects of fire on ectomycorrhizae

Fire can affect soil microorganisms directly by killing them, or indirectly through changes in soil properties and changes in the plant community. Disruption of living organisms through protein degradation and tissue death begins in the range of 40–70°C (Neary et al., 1999). The starting point of biological disruption depends on the organism and the fire conditions (Neary et al., 1999). For EM fungi, the survival and/or regeneration of host vegetation is the major determinant of post-fire fungal population dynamics because most EMF have only limited saprotrophic abilities (Smith and Read, 1997). Mycorrhizal fungi can persist at a site through spores, on root tips of surviving trees, and perhaps on recently dead root tips. Mortality of mycorrhizal fungi is correlated with tree mortality and the degree of consumption of organic matter; the more severe a fire, the more re-colonization depends on spores than presence of EM hosts (Dahlberg, 2002). Several studies have confirmed the importance of spores and sclerotial for recolonization after a fire (Baar et al., 1999; Grogan et al., 2000).

Combustion of the organic layer has a major effect on the EMF community, even in lower intensity burns. In a prescribed burn study, Stendell et al. (1999) found that total EM biomass was reduced eight-fold in the forest floor but was unaffected in the mineral soil. Because of fire’s effect on the forest floor, mineral soil can be a refuge for EMF. Even during severe fires that result in the combustion of a large portion of the organic layer, it is likely that some EMF will survive in the mineral soil. Grogan et al. (2000) found evidence that most of the post-fire EMF colonization in a severely burned came from the upper mineral soil.
Fire has the potential to reduce or temporarily eliminate some mycorrhizal fungal species from a site (Bruns et al., 2002; Dahlberg, 2002; Grogan et al., 2000; Stendell et al., 1999). Severe fire can cause a noticeable change in the mycorrhizal fungal community, with an increase in ascomycetous fungi (Grogan et al., 2000). Bruns et al. (2002) analyzed genets of *Suillus pugens* and *Amanita francheti* in response to a stand-replacing fire, and found that *A. francheti* was largely eliminated by fire whereas *S. pugens* recolonized via spores. In a chronosequence study, Visser (1995) found that most EMF species that had colonized root tips of regenerating jack pine 6 years after a wildfire were still present in stands 122 years after fire. A number of EMF groups are commonly found in regenerating stands, including *Wicoxina* sp., *Rhizopogon* sp., MRA, and *Thelophora* sp. (Baar et al., 1999; Visser, 1995). Kranabetter (2004) suggested that EMF commonly found after disturbances, such as *Thelephora terrestris*, may benefit pioneer seedlings by increasing the proliferation of fine roots that help the plants obtain soil resources.

There is debate as to whether EM recovery after a disturbance is dictated more by inoculum presence or by changes in soil biology and chemistry caused by the disturbance (Jones et al., 2003). In a review of the effects of clearcutting on EM, Jones et al. (2003) argued that both factors are equally important. They argue that EM proliferating after a disturbance are better adapted to the disturbed than undisturbed environments. Studies have indicated that changes in the soil environment brought by disturbance, such as temperature and host age, also have the potential to affect mycorrhizal colonization (Jones et al., 2003; Kranabetter and Friesen, 2002; Parke et al., 1983).

The leading explanation for explaining the observed structure of EMF communities has been the niche differentiation theory, which views soil as an n-dimension hypervolume (Tilman 2004). Disturbances can potentially affect mycorrhizal communities by altering available niches,
such as altering soil depth through the disruption of the forest floor (Dickie 2002). Alternatively, neutral theory proposes that species distributions are not determined by trophically similar species traits or the traits of competitors but by stochastic drift in the densities of competitively identical (i.e., functionally equivalent) species (Hubbell 2001). At the very least, the influence of stochastic factors, such as climate, may make it difficult to detect underlying mechanisms responsible for the distribution of EM fungi through time and space (Twieg et al. 2007).

Researchers have hypothesized that plants invest more energy in mycorrhizae when key nutrients are limited and less energy when nutrient availability increases (Treseder 2004). Field fertilization studies have shown that increased N availability is associated with reduced mycorrhizal colonization (Treseder 2004). Lab studies have also shown that favorable nutrient availability can reduce EM colonization (Correa et al. 2008, Hobbie et al. 2009). The decline in colonization with increased nutrient availability has been associated with reduced belowground allocation (Lilleskov et al. 2002). Based on these trends, wildfire has the potential to alter mycorrhizal colonization by increasing nutrient availability.

1.2.6 Mycorrhizal colonization and seedling growth

Research has shown that mycorrhizal colonization can be beneficial to conifer seedling growth (Christy et al., 1982; Miller et al., 1998). Some conifer species have been shown to grow without mycorrhizal colonization for at least the first year of growth (Christy et al. 1982). Other researchers, in contrast, reported 100% seedling colonization within the first year after fire (Miller et al., 1998; Purdy et al., 2002). Nevertheless, even if colonization takes place within the first year of regeneration, there can be a gap of several months between the beginning of fine root tip development and EMF colonization (Miller et al., 1998).
Photosynthesis is the foundation of plant growth. Colonization by EMF has been shown to increase photosynthetic rates compared to plants that were non-mycorrhizal (Dosskey et al., 1990; Miller et al., 1998; Purdy et al., 2002; Smith and Read., 1997). Mycorrhizal enhancement of photosynthesis has been attributed to increased nutrient uptake and a strengthened carbon sink (Read and Smith 1997; Dosskey et al. 1990). As in other areas of mycorrhizal research, there is evidence that EMF differ in their abilities to affect plant processes. For example, (Dosskey et al., 1991) found strong evidence that plants inoculated with *Rhizopogon vinicolor* had enhanced photosynthesis rates whereas those inoculated with *Hebeloma crustuliniforma* and *Laccaria laccata* were unaffected. Some studies have shown that EMF colonization can increase photosynthetic rates under drought stress (Dixon and Hiolhiol, 1992; Parke et al., 1983).

Do increased photosynthetic rates translate into increased seedling growth? Plant growth may actually be reduced where increased photosynthetic rates do not compensate for the C demand of the EMF (Smith and Read, 1997). Field studies on EM fungi inoculations have shown variable results, ranging from increased to decreased growth (Jones et al., 2003). The relationship between mycorrhizal species diversity and plant growth is also not clear based on available evidence. From a theoretical point of view, the soil environment is an extremely heterogeneous environment (nutrient availability, pH, etc.). There are roughly 5000 species of EMF that exist (Molina, 1992). Studies have shown variation in EMF functional capabilities, and so it is logical to hypothesize that trees gain physiological versatility with increasing EM diversity (Jones et al., 1997). Baxter and Dighton (2001) found that greater mycorrhizal diversity on *Betula populifolia* seedlings resulted in increased growth of root biomass relative to shoot biomass but not an overall biomass gain. They also found that greater mycorrhizal diversity increased P uptake. However, the complexity of quantifying EMF diversity, and the difficulty of
culturing many EMF, has meant that assessments of functional diversity have been conducted on only a limited number of species and often lack realistic conditions. In one of the few field studies that manipulated EMF diversity, Simard et al., (1997) found that Douglas-fir seedlings with higher mycorrhizal diversity had greater photosynthetic and height growth rates.

1.2.7 Comparing fire and clearcuts

On a landscape level, wildfire is more variable than clearcut harvesting in spatial scale, pattern and frequency of occurrence (McRae et al., 2001). Both types of disturbance have the potential to change the organic layer and thereby alter the environment that germinated seedlings must grow and survive in. Along the continuum of fire severity, high severity burns with high or complete tree mortality have the most in common with clearcutting; as a result, the following comparison will focus on that difference. Both fire and clearcutting, followed by site preparation, have the potential to affect biomass and chemical processes of the forest floor, but fire differs in its alteration of soil organic matter by combustion. Because of combustion effects, fire commonly causes greater losses of forest floor than does clearcutting (McRae et al., 2001). Site preparation methods that involve displacement of the forest floor can further increase the effects of clearcut harvesting (Simard et al., 2003). Clearcuts and fire disturbances both have the potential to change nutrient availability and losses, but generalizations about the effect of harvesting on belowground processes are difficult to make. Reductions in stand biomass have the potential to lower nutrient levels but the effects vary among nutrients. For instance, the removal of trees can remove large amounts of P, K, Ca, and Mg, but, even in severely burned sites (if not salvage-logged), these nutrients are mostly retained immediately after a fire (McRae et al., 2001). Generalizations about the effects of harvesting on forest-floor N mineral dynamics, measured by gross or net mineralization, are difficult to make because harvesting alters N
dynamics in some coniferous ecosystems (so-called responsive sites) but not others (Grenon et al., 2004). In the responsive ecosystems, forest-floor nitrogen dynamics after harvesting are affected by a wide range of factors, such as soil moisture and soil available C, which affect the production/consumption ratio of mineral N (Grenon et al., 2004). Similarly, decomposition rates vary after harvest, with increases and decreases in rates, depending on site factors (Prescott et al., 2003).

1.3 Dissertation overview

In Chapter 2, I examine the effects of disturbance severity on natural regeneration potential of Douglas-fir by comparing the germination, survival, and growth (biomass and foliar nutrients) over the first growing season in five treatments: high severity burn, low severity burn, clearcut, screefed clearcut, and undisturbed forest. Natural regeneration potential was assessed for a three-year period in the high severity burn, clearcut, and screefed clearcut treatments.

In Chapter 3, we examined the effects of increasing disturbance severity on the ectomycorrhizal symbiosis by comparing de novo colonization and ectomycorrhizal diversity over a three-year period among the five treatments. We used morphotyping to identify EMF and molecular analysis to confirm their identity.

In Chapter 4, we used the growth data (biomass, foliar nutrients) from Chapter 2 and the mycorrhizal colonization and diversity data from Chapter 3 to examine the relationship between seedling growth variables and mycorrhizal colonization and diversity. Ordination and regression analysis was used to explore these relationships.
1.4 References


2. Natural regeneration potential of Douglas-fir along wildfire and clearcut severity gradients

2.1 Introduction

Natural regeneration plays a major role in forest renewal in Canada (Natural Resources Canada, 2008). Recent research and controversy around forest regeneration policy in western Canada (BC Ministry of Forests and Range 2009) and the United States, combined with predicted increases in the extent, frequency and severity of wildfire with climate change (Flannigan et al. 2000, Krawchuk et al. 2009), have heightened the need to re-assess the viability of natural regeneration after wildfire (Donato et al. 2006, Greene et al. 2007, Klenner et al. 2008). Even though planting has largely replaced natural regeneration over the past three decades in efforts to increase reliability and rapidity of regeneration following harvesting, natural regeneration following wildfire has continued to be the dominant process naturally shaping Canadian forests (Natural Resources Canada 2007). Even in clearcuts, recent studies show that natural regeneration can be abundant and result in adequate stocking in the interior Douglas-fir (Pseudostuga menziesii var. glauca (Beissn.) Franco) forests of western North America (Heineman et al. 2002, Shatford et al. 2007).

Wildfire has historically been an important disturbance creating the necessary conditions for natural regeneration in the dry interior forests of North America. In recent decades, the use of

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fire as an ecosystem restoration tool has highlighted the need for an improved assessment of the ecological implications of fire severity (Fule 2008). Prescribed fires have been advocated as a means to restore ponderosa pine ecosystems to historical conditions where fires were frequent and of low severity (Covington et al. 1997, Roccaforte et al. 2008). This approach has also been suggested for other forest ecosystems in dry interior western US and Canada (Klenner et al. 2008). However, available evidence suggests that the historic disturbance regime in interior Douglas-fir forests was one of mixed severity resulting from both natural and human-caused disturbances (Heyerdahl et al. 2006, Klenner et al. 2008). In the dry forests of southern interior British Columbia, a highly heterogeneous forest landscape has resulted from low to high severity fires, insect outbreaks, and timber harvesting using regeneration methods ranging from selection to clearcut systems (Klenner et al. 2008). Thus, any attempt to understand and manage these landscapes must take into account the historical mixed-severity disturbance regime.

Wildfires affect natural regeneration by altering the three necessary components of the so-called natural reproduction triangle: sufficient seed source, suitable seed bed, and an environment compatible with germination and seedling establishment (Roe et al. 1970). While extensive fires can reduce availability of nearby seed sources, they may also benefit germination and survival of naturally dispersed seed by reducing soil organic matter depth, exposing mineral soil and reducing the abundance of competing vegetation (Greene et al. 1999). Consequently, it has been hypothesized that an increase in fire severity may favour natural regeneration by enhancing conifer germination and survival through improved substrate quality and increased resource availability (Flannigan et al. 2000). These disturbance effects on soils and vegetation are thought to improve conditions for conifer establishment primarily by increasing soil temperature as well as soil water and nutrient availability to new germinants (Herr et al. 1999,
Another effect particularly important in N-limited forests is the short-term increase in nitrogen availability (i.e., assart effect) following fire (Wan et al. 2001).

The debate over natural regeneration also ties into research attempting to assess the extent to which clearcutting can emulate wildfire as a management tool (McRae et al. 2001). “Emulation silviculture” attempts to replicate the chemical, physical, and biological effects of natural disturbances in an effort to reduce the environmental impacts of harvesting (Crow and Perera 2004). Similar to wildfire, harvesting (i.e., through mechanical site preparation) can increase disturbance to the forest floor and improve seedling growing conditions in temperate and boreal forests, in part by removing competing vegetation (Haeussler et al. 1999, Sutherland and Foreman 2000). The mechanisms by which these disturbances affect seedling establishment conditions are not well understood, however. By investigating the mechanisms by which forest floor disturbance facilitates regeneration, the disturbance severity at which clearcut harvesting mimics wildfire can be determined.

The McLure fire occurred near Kamloops, British Columbia, and burned 26,420 hectares (Ministry of Forests 2004). The mixed severity wildfire occurred in a working forest, and resulted in a mosaic of low, medium and high severity burns inter-mixed with clearcuts. This presented an opportunity to examine natural regeneration following wildfire and clearcutting disturbances on comparable sites. The overall objective of our study was to experimentally assess the effects of disturbance severity on the natural regeneration potential of interior Douglas-fir. We defined disturbance severity as the amount of organic material consumed, either by wildfire (Johnstone and Chapin 2006) or by screefing (i.e., mechanically removing organic matter) in the clearcuts. By artificially providing seed, the mechanisms by which
regeneration success was limited were examined without the confounding effects of variable or inadequate seed sources (Greene et al. 1999).

We tested three hypotheses in this study to determine the extent to which disturbance severity affects natural regeneration potential. First, we hypothesized that increasing disturbance of the forest floor would increase germination by creating more favourable seedbeds. Secondly, we hypothesized that subsequent survival and growth of germinants would increase with increasing disturbance severity, mainly as a result of increased resource availability (light, soil water and nutrients). Thirdly, we hypothesized that removal of light and water limitations would unmask soil nutrient limitations to natural regeneration potential (assessed by survival, growth, and foliar nutrients). Hence, we predicted that natural regeneration potential in full light conditions would increase with increasing soil nutrient availability. Considering all three hypotheses together, we predicted that the high severity burn and screefed clearcut treatments would have the greatest natural regeneration potential.

2.2 Methods

2.2.1 Study area

The study was conducted at 700-1300 m elevation near Barriere, British Columbia, Canada (51’00N, 120’00W). Most sites occurred in the Thompson Dry, Cool Interior Douglas-fir (IDFdk2) variant of the Interior Douglas-fir (IDF) biogeoclimatic zone, but some sites were transitional to the Thompson Dry, Mild Montane Spruce (MSdm2) variant of the Montane Spruce (MS) biogeoclimatic zone. The IDF zone typically has warm, dry summers and cool
winters and the MS zone has short, warm summers and cold winters. The IDFdk2 variant occurs at 850-1130 m elevation and has an annual precipitation of 379 mm (range 295 to 452mm) and annual temperature of 4.8 °C (range 3.4 to 6.6 °C) (Lloyd et al. 1990). The MSdm2 variant occurs at 1275-1530 m, has an annual precipitation of 606 mm (range 523 to 669mm) and annual temperature of 2.8 °C (range 0.9 to 5.4 °C) (Lloyd et al. 1990). Soils at the study area were Brunisols and Luvisols of silty or sandy loam texture (Soil Classification Working Group 1998).

Prior to disturbance, the forests were dominated by mature interior Douglas-fir with increasing amounts of subordinate lodgepole pine (Pinus contorta var. latifolia (Engelm.) Critchfield) at higher elevations. Common understory species included Arctostaphlos uva-ursi (L.) Spreng., Linnaea borealis L., Lupinus arcticus S. Watson, and Calamagrostis rubescens Buckl. (Lloyd et al. 1990). The most common disturbances in the IDFdk2 and MSdm2 variants are wildfire, harvesting, insect outbreaks, windthrow and livestock grazing. The IDF and MS zones have a mixed severity fire regime, which includes patches of low to high severity disturbance and a variable fire return interval (Klenner et al. 2008).

2.2.2 Experimental design and treatments

In May 2004, 16 sites were located across a continuum of wildfire and harvesting disturbance severities, ranging from undisturbed forests to high severity burns and screefed clearcuts. The sites included five treatments: 1) high severity burn (HSB), 2) low severity burn (LSB), 3) screefed clear-cut (SCC), 4) clear-cut without screefing (CC), and 5) undisturbed forest (UF). These treatments were replicated four times in a completely randomized design,
with replicate sites located at least 1 km apart, except for the CC and SCC treatments, which were located in the same replicate clearcuts. Measured site characteristics are given in Table 2.1.

Burn severity was defined as the amount of organic material consumed by the fire (Johnstone and Chapin 2006). Burn severity was determined post-hoc based on depth of forest floor charring and exposure of mineral soil. At the HSB sites, the forest floor was completely consumed and the mineral soil exposed (depth of forest floor 0.7±0.2 cm). Only charred boles of fire-killed trees remained of the canopy and understory trees, which were removed prior to study initiation using a mechanical harvester. At the LSB sites, at least 95% of the canopy had survived the fire but 100% of the understory was killed (depth of forest floor 1.8±0.5 cm). We required that the HSB and LSB conditions occurred uniformly over an area at least 50m x 50m to qualify as a replicate site.

The undisturbed forest and clearcut sites were located either within the overall perimeter or at the immediate periphery (within 4 km) of the McLure fire. The undisturbed forests were dominated by mature Douglas-fir trees that were 100-200 years old. Forest floor depth averaged 4.9±0.6 cm. The clear-cut sites were harvested in the winter of 1993-1994 and were cleared of canopy cover over an area of at least 1.5 ha. In May 2004, two circular plots with the plot centers at least 5m apart within each clear-cut and randomly assigned the CC or SCC treatment. In the SCC treatment, all vegetation and forest floor was removed (screefed) down to the mineral soil using shovels and rakes (depth of forest floor 0 cm). In the CC treatment, the forest floor was left intact (depth of forest floor 3.8±0.4 cm).

Treatment effects were assessed within a single 15-m circular plot at each replicate site. Within each plot area, five seed beds (120 cm x 140 cm) were located. Wooden stakes were
driven into the ground to mark the four corners and center of each seedbed. Seeds of interior Douglas-fir (seedlot 48523, Tree Seed Center, Ministry of Forests, Surrey, B.C., Canada) were stratified at 4 °C for 3 weeks and sown into the seedbeds during the first two weeks of May, 2004. At each seedbed, seven to eight narrow rows (<1 cm wide) were dug through the forest floor (where it remained) to expose bare mineral soil. The seeds were covered by a thin layer of mineral soil and soil organic matter, if present. To reduce seed predation, the seedbeds were covered with two types of mesh (2.5-cm-diameter mesh and a finer mesh). The mesh was supported by the stakes and its edges were buried in the ground.

All vegetation establishing within and adjacent to the seed beds was clipped at the root collar and removed from the plots. There was little vegetation, except for some pinegrass at the clearcut sites and fireweed (Epilobium latifolium L.) at the HSB sites. Natural Douglas-fir regeneration was absent from the clearcut, UF and HSB sites, but some occurred near the seedbeds in the LSB sites, and were removed.

The original goal of the study was to compare seedling establishment, growth and survival among all treatments for three years after disturbance (July 2004 to September 2006). However, the number of replicates in the UF and LSB treatments was reduced from four in July, 2004, to one each by August, 2006, due to logging (2 LSB sites), cattle grazing (1 UF sites), poor growing conditions (2 UF sites), and infection by a fungal root pathogen, Rhizina sp. (1 LSB site). As a result, only the HSB, SCC and CC treatments (hereafter collectively referred to as the ‘no canopy treatments’) were compared for the full three-year period. One HSB site was excluded from this analysis because of germination failure. One UF site was excluded in the survival analysis in 2004 because of complete mortality resulting from physical damage by cows in August 2004.
2.2.3 Seedling responses

The seedbeds were monitored regularly for germination and survival. This was done by placing a wooden toothpick next to each newly germinated seedling and assigning the seedling a number based on the row and position within the row. Starting in May, 2004, the seedbeds were monitored weekly for germination. Seedling survival was then assessed twice a month from July until September, 2004, and May through September in 2005 and 2006. After each monthly count, a subset of seedlings was removed from the seed bed and assessed for shoot and root biomass and shoot height. Samples were oven-dried at 72 °C and weighed after reaching a constant mass. Seasonal survival (from July to September in 2004 and from May to September in 2005 and 2006) was calculated by dividing the number of seedlings counted at the end of the sampling season by the number of 2004 germinants. The number of seedlings sampled for biomass in the intervening months was subtracted from the previous count.

Foliage was sampled for nutrient concentration in October, 2004, and September, 2005 and 2006. From each sampled seedling, all needles were removed for analysis of foliar N and P concentration, and foliar biomass was measured to estimate nutrient content. The samples were sent for analysis to the Analytical Laboratory in Victoria, British Columbia (B.C. Ministry of Forests, Research Branch). Foliar N was analyzed by dry combustion with the Leco CHN-600 analyzer (LECO Corp., St. Joseph, MI). Foliar P was analyzed by inductively coupled plasma–atomic emission spectroscopy following microwave digestion (Kalra and Maynard 1991).

Seedling wood was assessed for natural abundance $\delta^{13}$C for a seasonally integrated proxy measure of relative water use efficiency (WUE). In September 2004, 2005 and 2006, the most
recent year’s growth was sampled by removing the terminal bud from the shoot and cutting out a small (approximately 1cm long) piece of wood tissue. The samples were weighed and placed in tin capsules. The samples were then combusted, CO₂ was liberated, and the gas was analyzed for $^{13}$C/$^{12}$C ratio with a continuous-flow isotope-ratio mass spectrometer. In 2004, the samples were sent to the Pacific Center for Isotopic and Geochemical Research at UBC. In 2005 and 2006, they were sent to the UC Davis Stable Isotope Laboratory (Davis, CA, USA). The C ratio ($\delta^{13}$C) was calculated as: $\delta^{13}$Csample ‰ = ([$^{13}$C/$^{12}$Csample] / [$^{13}$C/$^{12}$Cstandard] -1) x 1000. The standard used was Vienna-PeeDee Belemnite (V-PDB).

### 2.2.5 Soil nutrients, water and temperature

Mineral soils were sampled for soil nutrients to a depth of 10 cm using a hand-held soil corer. Mineral soil was collected in October 2004 and analyzed for total N, mineralizable N (as NH₄-N), available P (Bray), pH (water) and total C. The soil organic layer was also sampled for total N, mineralizable N (as NH₄-N), available P (Bray), pH (water) and total C. All samples were sent to the Analytical Laboratory in Victoria, British Columbia (B.C. Ministry of Forests and Range, Research Branch). Percentage total N was determined using the colorimetric method. Mineralizable N was determined after a two-week anaerobic incubation (Bremner 1996). Soil analysis methods followed Kalra and Maynard (1991).

Volume for soil bulk density was determined by inserting marbles into the holes created by the hand-held soil corer. The volume of the marbles was determined by water displacement in the laboratory. Mass was determined by oven-drying the fine fraction (<2 mm) at 105 °C. Soil C and N contents were determined by multiplying each nutrient concentration by bulk density.
Volumetric soil water content (%) was measured monthly from May to September in 2004, 2005 and 2006 using a time-domain reflectometry probe (Hydrosense CS620, Campbell Scientitific). At each measurement time, water content was sampled at three locations in each seedbed (centre and each end of the seedbed) by inserting the probe to 20 cm depth in the mineral soil. The three samples were then averaged to produce a value for seedbed soil water content.

Growing season light availability (400–700 nm) was assessed in at each replicate site using hemispherical canopy photography. The photographs were taken in July, 2006, during homogeneous clear sky conditions. Each photograph was taken at 1-m height using a Nikon digital camera with an attached Nikon FC-E8 fisheye converter lens set on a tripod. One photograph was taken in the center of each seedbed and the five values averaged per replicate site. The growing-season light availability (direct and diffuse light sources) was expressed as a percentage of full sun and was calculated from each photograph using the Gap Light Analyser (GLA) 2.0 software, following Frazer et al. (2000).

Soil temperature was measured using Stowaway Tidbit (Onset Computers) temperature loggers. The temperature loggers were placed at 10 cm depth in the mineral soil in the center of the experimental plots (outside of the seedbeds) in July 2004 and retrieved one year later. The data was downloaded and the temperature logger replaced into the soil; this procedure was repeated in 2005 and 2006. The data loggers were finally retrieved and downloaded in September 2006. Only 14 data loggers were available for use and assigned to site replicates as follows: four sites each in the HSB and the UF treatments, and three sites each in the CC and LSB treatments. Soil temperature was recorded at 20-minute intervals. For statistical analysis,
monthly average, minimum, and maximum temperatures were calculated. Data recorded during the first two weeks after datalogger instalment was not included in the analysis.

2.2.6 Statistical analysis

Statistical analysis was carried out using SAS ver. 9.1 (SAS Institute 2002). A generalized linear mixed model assuming a Poisson distribution was used to assess treatment effects on germination and survival (Littell 2006). A maximum likelihood approach (Proc Mixed) was used for analysis of treatment effects on soil nutrients, foliar nutrients, soil water content, $\delta^{13}$C and seedling growth responses. The effect of time on soil water content and growth was assessed by including time as a main effect in the model. Subsamples were included as random factors for analysis of seedling growth, $\delta^{13}$C, and foliar nutrients. For analysis of temperature data, repeated measures analysis was used, where the temperature probe was included in the mixed model as a random factor. Where significant treatment effects were found, differences between means were detected using the Tukey-Kramer multiple comparison test. For comparison of canopy openness in the LSB and UF treatments, significant differences were detected using a maximum likelihood approach (Proc Mixed).

2.3 Results

2.3.1 Germination and survival

Disturbance significantly affected Douglas-fir seed germination in 2004 ($F=4.09$, $P=0.0211$). Germination tended to be higher in all disturbance treatments than the undisturbed
forest, but the difference was significant only for the SCC treatment (Fig. 2.1). Any disturbance significantly reduced seedling mortality compared with the undisturbed forest during the first growing season (July to September, 2004), with survival rates exceeding 70% in all disturbance treatments (Table 2.2). In subsequent years (2005 and 2006), when the LSB and UF treatments were excluded due to loss of replicate sites, seedling survival was unaffected by treatment (F=0.46, P=0.6400), but the survival of the 2004 germinants significantly decreased with time (F=10, P=0.0056). For all three treatments combined, average seedling survival declined from 61% (se 6.5) in 2005 to only 30% (se 0.64) in 2006, with no evidence of a treatment X time interaction (F=0.06 P=0.940).

2.3.2 Biomass and height

Disturbance increased seedling shoot and root biomass and shoot height in 2004 (Table 2.2). Compared with the undisturbed forest, seedlings in 2004 had significantly greater shoot and root biomass in all disturbance treatments and greater shoot height in the CC and HSB treatments (Table 2). Over the remaining two years of the study period (2005-2006), seedlings in the HSB had significantly greater shoot biomass (Fig. 2.2) (F=12 P=0.0004), root biomass (data not shown) (F=11, P=0.0007), and shoot height (Fig. 2.3) (F=11, P=0.0043) than those in the CC and SCC treatments. Shoot biomass, root biomass, and height increased from 2005 to 2006 (F=300, P<0.0001 for shoot biomass; F= 380.0, P<0.0001 for root biomass (data not shown), F=380.0, P<0.0001 for shoot height (data not shown). There was no evidence of a treatment X time interaction for shoot biomass (F=0.34, P=0.8477), root biomass (F=0.48, P=0.753), or shoot height (F=0.48, P=0.3722).
2.3.3 Foliar nutrients

Disturbance had a small effect on seedling foliar N concentration and no effect on foliar P concentration in 2004 (Table 2.2). Because seedlings grew larger in the HSB than the other treatments, they had significantly higher N and P contents in 2004 (Table 2.2). The N:P ratios were similar among treatments in 2004 (data not shown).

When examining the treatments with no canopy remaining (i.e., HSB, CC and SCC), we found that both N and P concentration declined from 2005 to 2006 (F=14, P=0.0018 and F=8.53, P=0.0100, respectively) but these trends did not differ among treatments (F= 1.7, P=0.2454 and F=0.55, P=0.5900, respectively). Seedling N concentration fell from 2.4% (se 0.08) in 2005 to 1.2% (se 0.08) in 2006. Seedling P concentration fell from 0.30% (se 0.08) in 2004 to 0.18% (se 0.08) in 2006.

Foliar nutrient content did not change from 2005 to 2006 (F=0.02, P=0.8783 for N content, F=0.6, P=0.4499 for P content, and F=2.5, P=0.1303 for N:P ratio). There was also no evidence of a treatment effect on N content (F=0.64, P=0.5423), P content (F=0.11, P=0.8975), or N:P ratio (F=0.51, P=0.6106). Mean foliar N content for 2005 and 2006 was 0.26 grams (se 0.0062). Mean foliar P content for 2005 and 2006 was 0.05 grams (se 0.0003). Mean N:P ratio for 2005 and 2006 was 7.3 (se 0.33).

2.3.4 Stem δ\textsuperscript{13}C

Stem natural abundance δ\textsuperscript{13}C was affected by the presence of canopy cover during the first growing season. Seedlings in the LSB and UF treatments had significantly lower δ\textsuperscript{13}C
values than seedlings in the three ‘no-canopy’ treatments (HSB, CC, SCC), which were similar to each other (F=16, P<0.0001) (Fig. 2.4). When comparing the ‘no-canopy’ treatments only from 2005 to 2006, we found no effect of disturbance severity (F=1.5, P=0.2912) or time since disturbance (F=0.99, P=0.3229) on stem $\delta^{13}$C. Average $\delta^{13}$C values for the three ‘no-canopy’ treatments were -26.0‰ (se 0.30) in 2005 and -26.2‰ (se 0.26) in 2006.

2.3.5 Soil nutrients

Total C and N content, Bray P, and bulk density did not vary significantly among the five treatments in 2004 (P>0.05, Table 2.3). However, there was a strong tendency for screeching to reduce total C, total N, and mineralizable N compared to all other treatments (Table 2.3). Disturbance had a small effect on C:N ratio, which was significantly higher in the CC than the other four treatments (F=5.0, P=0.0133) (Table 2.3). In addition, there were significant treatment effects on mineralizable N and pH (Table 2.3). Mineralizable N was higher in the CC and UF than in the HSB, LSB, and SCC treatments in 2004 (F=11, P=0.0008) (Table 2.3). Soil pH was higher in the HSB and LSB than the two clearcuts treatments and intermediate in the UF (F=7.04, P=0.0013) (Table 2.3).

2.3.6 Soil water content

Compared to treatments without canopy cover (HSB, CC, SCC), those with canopy cover (LSB and UF) had significantly lower average and minimum soil water contents (Table 2.4). The SCC treatment had the highest and UF had the lowest average, maximum and minimum soil water content of all treatments, while the HSB was the driest of the ‘no-canopy’ treatments.
Treatment also affected maximum soil water content, which was higher in the clear-cut than the burned or UF treatments (Table 2.4).

All three measures of soil water content varied significantly among years (F=23, P<0.0001 for average soil water content; F=16, P<0.0001 for maximum soil water content; F=21, P<0.0001 for minimum soil water content). There was no evidence of a treatment X year interaction (F=1.03, P=0.4270 for average; F=1.01, P=0.4485 for maximum; and F=0.85, P=0.6043 for minimum soil water content). Average soil water content was lowest in 2004 (11%, se 0.90), highest in 2005 (18%, se 0.55) and intermediate in 2006 (15%, se 0.85). Maximum and minimum soil water content followed similar patterns, where 2004 was the driest growing season and 2005 the wettest (data not shown). Within years, soil water content declined significantly through the growing season, except that it increased slightly from June to July in the undisturbed forest and low severity burn (Fig. 2.5a and b).

2.3.7 Soil temperature

Neither mean nor minimum soil temperature was affected by treatment, but maximum temperature increased (but not significantly) following removal of canopy cover (Table 5). Monthly average, maximum, and minimum temperatures varied significantly over the growing season, with significantly higher temperatures in July and August (Table 2.5). There was no evidence of an interaction between treatment and month (F=1.1 P= 0.3711). Temperature varied significantly among years (F=4.4 P=0.0177). The average temperature was higher in 2004 (13.01°C, se 1.1) than in 2005 (12.6°C, se 1.1), but neither year was significantly different than 2006 (12.9 se 1.1). There was no evidence of an interaction between treatment and year (F= 0.41,
P=0.7453). There was no evidence of a yearly difference in maximum or minimum temperatures (F=2.6, P=0.0828 and F=0.89, P=0.4164, respectively) (data not shown).

2.3.7 Light

Canopy openness was 100% in the HSB, CC and SCC treatments. There was no significant difference in canopy openness between the LSB and UF treatments (F=1.8 P=0.1939). Mean canopy openness was 45% (se 15%) in the LSB treatment and 33% (se 9.5%) in the UF treatment.

2.4 Discussion

2.4.1 Disturbance effects on germination

We found that all disturbance types increased germination compared to the undisturbed forest, but was only significant in the case of the SCC treatment. The effect of disturbance severity was masked by the contrasting effects of clearcutting versus wildfire. Compared with the low severity disturbances, germination significantly increased with screeing in clearcuts but not with high severity burning in wildfires. Therefore, we accept our first hypothesis that increasing disturbance severity increases germination, but only through screeing in clearcuts and not through wildfire.

We expected a disturbance severity effect because reducing the depth of surface organic layers and exposure of mineral soil with disturbance can improve moisture and temperature conditions for germination (Herr et al. 1999, Klinka et al. 2000). Forest litter inhibits Douglas-fir
germination by creating a mechanical barrier and leaving seeds prone to desiccation (Caccia and Ballare 1998, Bai et al. 2004). Our method of creating a mixed organic /mineral seedbed across all treatments likely enhanced germination success, particularly in the undisturbed forest. If the seeds had been sown directly into the forest floor, we expect that germination in the undisturbed forest would have been much poorer due to lower soil temperatures and lower access to soil resources. Our results support other studies showing that interior Douglas-fir germinates well on mineral or mixed mineral/organic seedbeds (Klinka et al. 2000). The germination success in the clearcut and undisturbed forest treatment also suggests that the forest floor does not have to be entirely removed or severely disturbed to enhance germination; instead, only removal of the forest litter layer or mixing it with mineral soil is necessary.

The presence of ash on the severely burned seedbeds may have contributed to the weak germination response to increasing wildfire disturbance severity. Our results support other studies that ash in the seedbeds may reduce germination by increasing moisture and temperature fluctuations as well as lowering soil moisture holding capacity (Herr et al. 1999, Mallik 2003). The higher pH caused by ash in the burned forest floors may have contributed to germinant mortality by damping-off fungi (Tarrant 1954) or toxic environmental effects (Kemball et al. 2006). Higher maximum daytime temperatures in the high severity burn also likely contributed to reduced germination success compared with the screefed clearcut treatment.

2.4.2 Disturbance effects on seedling survival

Disturbance severity did not significantly affect seedling survival, and thus we rejected the first part of our second hypothesis. In 2004, survival was high in all treatments, where it
exceeded 50%. The high survival rates in 2004 indicate favourable survival conditions, even in the drier and closed canopy conditions of the undisturbed forest. In subsequent years, however, survival declined from various causes (ranging from logging to fungal disease) in the undisturbed forest and low severity burn to the extent that we could not continue to analyse these treatments. In the remaining treatments where there was no overhead canopy, seedling survival remained similar among treatments over the three years, with the greatest decline in the third year of the study. In these treatments, there was no difference in light or soil water availability to seedlings. Other studies in the interior Douglas-fir forests have found that reductions in soil water content, light availability and night-time temperatures and increases in daytime temperatures contribute to early seedling mortality (Simard et al. 2003).

2.4.3 Disturbance effects on seedling growth and physiology

Once germinants have established in dry interior Douglas-fir ecosystems, resource availability (light, water and nutrients) is considered to surpass microclimatic effects in limiting seedling growth (Newsome 1998; Simard et al. 2003). We expected canopy removal would improve both light and moisture conditions. When combined with a nutrient pulse, this would lead to increased growth relative to the LSB and UF treatments. In contrast to germination and survival, increasing disturbance severity significantly increased growth by increasing resource availability, leading us to accept our second hypothesis related to growth. From 2004 to 2006, seedlings growing in the high severity burn had significantly higher shoot height, shoot biomass and foliar nutrient content than those in the clearcut treatments, suggesting that burning increased resource availability to seedlings through combustion of the forest floor.
In this study, seedling productivity appeared to major driver of WUE patterns, which reflect the balance between assimilation and transpiration (Larcher 2001). The $\delta^{13}C$ values, used a proxy for WUE, indicated that light availability was the major driver in isotope depletion variation followed in importance by nutrient availability. The least depleted $\delta^{13}C$ values corresponded with both increased light availability and increased soil water availability, suggesting enhanced photosynthetic capacity lead to increased WUE (Cregg et al. 2000, Guy and Holowachuk 2001). Seedlings growing under closed canopy conditions (LSB and UF) were more depleted in $\delta^{13}C$ than those growing in the open in 2004, corresponding with low light and soil water availability. In this study, the effects of light and water availability are difficult to disentangle, but light was most likely the major limiting factor since the seedlings would not likely respond to increased water availability under a closed canopy.

Seedlings in the LSB treatment grew faster and had higher (but not significantly) $\delta^{13}C$ values than those in the UF, suggesting their productivity increased from a nutrient pulse. Even so, low light availability likely limited the benefits of increased nutrient availability, witnessed by the very high N concentration but relatively low N content of foliage. Increases in foliar N concentration have been associated with increasing WUE in Douglas-fir (Ripullone et al. 2004). In the case of the open-grown seedlings, the disparity between biomass and $\delta^{13}C$ values suggests the moderately drier conditions in the HSB treatments may have affected WUE. Productivity gains in the HSB seedlings may not have resulted in lower $\delta^{13}C$ values because the seedlings responded to drier conditions by reducing transpiration.

Taken as a whole, the results provide evidence that seedling growth under the canopy was limited both by low light and soil moisture availability, but that canopy removal increased seedling growth more so through increased light availability. Thus, we partially accept our third
hypothesis that canopy removal eased light and water limitations to seedling growth. Though seedling productivity appeared to drive $\delta^{13}C$ values, there may have also been a source effect on seedlings growing in close proximity to the forest floor because the air just above the forest floor is highly depleted in $\delta^{13}C (\approx -29\%)$ (Bauer et al. 2000), which can affect plant $\delta^{13}C$ values (Berry, 1997). Therefore, a source effect may have contributed to the depleted $\delta^{13}C$ levels observed in seedlings growing under canopy cover, especially in the UF where air circulation was most restricted.

When comparing growth among the three no-canopy treatments, we found evidence to support our third hypothesis that alleviating nutrient limitations with burning would lead to the greatest growth. The higher foliar N and P contents in the high severity burn than the clear-cut treatments suggest that combustion of the forest floor increased nutrient availability. Foliar N concentrations in 2004 and 2005 were well above the 1.4% critical value for limiting growth in Douglas-fir (Moore et al. 2004), but were approaching the critical level in 2006, suggesting that an N limitation was starting to develop with time. In contrast, foliar P concentration and N:P ratio remained above critical levels throughout the study period (Ballard 1986). Nevertheless, N:P declined by 19% from 2004 to 2006, providing further evidence for the onset of an N limitation in the final growing season.

The higher foliar N concentration in the HSB treatment is consistent with other studies showing increased nutrient availability for the first several years following wildfire or clear-cutting in interior Douglas-fir forests (Hope et al. 2003). This assart effect is often short-lived (1 to 2 years after fire) and generally occurs after forest fire (Wan et al. 2001). In addition to the direct nutrient release from combustion, the elevated pH levels in the high severity burn relative to clearcuts likely contributed to the increase in nutrient availability, particularly P (Caccia and
Ballare 1998). In clear-cuts, post-disturbance nutrient pulses are also expected but typically peak later than following wildfire (3 to 5 years after fire) (Keenan and Kimmins 1993); thus, our study may have been completed too early to capture any short-term pulse in the screefed clearcuts. Overall, we found little evidence of a correlation between growth and soil nutrients, but soil measurements do not always reflect plant-available nutrients (Turner et al. 2009) (see Chapter 4).

Based on previous research, we expected a stronger seedling response to screefing in the clearcuts, but it may have been masked by the slow vegetative recovery after harvest. Simard et al. (2003), for example, found that mechanical removal of pinegrass increased survival and growth of lodgepole pine seedlings by increasing soil water availability, but in their study, pinegrass cover was very high. Stark et al. (2006) examined seedbank and post-disturbance vegetation in three of the high severity burn and two of the clearcut sites used in the present study, and found that re-vegetation was sparse, with <25% of subplots occupied by vegetation in the first year after disturbance (2004). Ingress of deciduous vegetation increased in the clearcuts and high severity burns from 2004 to 2006, but it was purposefully removed from all of the experimental seedbeds. Removal of competing vegetation, particularly grasses, likely stimulated seedling survival and/or growth in all of the treatments. Regenerating grasses can often be N-limited after fire (Romme et al. 2009), and their absence or removal may allow conifer seedlings to take advantage of nutrient pulses.

The lack of vegetation competition in our seedbeds may have increased survival and growth of interior Douglas-fir relative to natural conditions, thus accelerating intraspecific competition among interior Douglas-fir seedlings. The intensity of intraspecific competition appeared to differ among treatments. Although germination and survival did not differ
significantly among disturbance treatments, seedlings in the high severity burn established at a lower density and in a patchier pattern than in the other treatments, likely associated with heterogeneity in seedbed conditions. This pattern suggests that individual seedlings in the high severity burn experienced more asymmetrical competition, where those near the middle of the clump experienced intense competition and those closer to the gaps experienced lower competition. This is evident in the increasing rate and variability of shoot growth in high severity burn compared with the other treatments over time (see Fig. 4). Intraspecific competition has been shown to affect growth of regenerating seedlings, even during the establishment period (Mendel et al. 1997). Regeneration clumps in the IDF forests commonly develop into dense thickets of saplings that grow very slowly in response to severe moisture limitations (Johnstone and van Thienen 2006).

2.4.4 Implications for natural regeneration

The results of this study suggest that high severity burns and clearcutting with screefing create the most favourable, albeit different conditions for natural regeneration. High severity fire created heterogeneous conditions for patchy regeneration of rapidly growing, nutrient-rich seedlings, while clearcutting with screefing created more uniform conditions for even stocking of relatively slower-growing seedlings. However, our study supplied seed to examine regeneration potential, while in nature, successful regeneration depends on provision of an adequate seed source. Large clearcuts with distant seed sources may naturally regenerate less readily than wildfires, where clumps of thick-barked Douglas-fir commonly escape mortality (Marshall and Wang 1996). In the mixed severity fire regime of interior Douglas-fir forests, fire severity and distribution varies greatly across landscape, with greater chance of a nearby seed source than in
the large clearcuts, which are becoming increasingly more common-place as a result of salvaging mountain pine beetle mortality (Klenner et al. 2008). Not only do wildfires leave seed sources, but they also create favourable seedbeds and open conditions that favour establishment and growth. Indeed, Stark et al. (2006) found significantly more conifer regeneration scattered on the severely burned sites than in the clearcuts in this study.

In the first growing season, regeneration potential in the low severity burn appeared comparable to the other disturbance treatments, particularly the clearcuts. In subsequent years, however, shoot growth in the low severity burn was poor due to low light and soil water conditions. In some environments the presence of an overstory of mature Douglas-fir can provide a seed source and a favourable environment for seedling establishment, provided that the density of mature trees allows for adequate light availability to seedlings (Burton et al. 2000). Our results agree with previous studies showing that without sufficient light, many establishing seedlings eventually die (Vyse et al. 2006). These results should caution against current restoration programs aiming to apply low severity fires across extensive areas to renew forests and reduce fuel loads in the interior Douglas-fir landscape (Baker et al. 2007). In contrast, our regeneration results support recent studies showing that interior Douglas-fir forests developed and prospered under a mixed severity disturbance regime (Marshall and Wang 1996, Klenner et al. 2008).

In a large landscape level study in southern Oregon, Shatford et al. (2007) found abundant natural regeneration 9 to 19 years after fires across a wide variety of conditions. Natural regeneration in the interior Douglas-fir forests of British Columbia is likewise abundant across a wide range of ecosystems, as evidenced by the old-growth forests that cover the current landscape (Kopra and Feller 2007). Where management objectives require rapid, homogenous
regeneration following clearcutting or wildfire, planting may still be necessary, particularly in large clearcuts where seed sources are sparse (Huggard et al. 2006). The rapid replacement of fire with harvesting as the primary natural-disturbance agent (Klenner et al. 2008) may require that planting dominate in some areas to reduce regeneration delay and prevent erosion, particularly on steep erodible sites or where there is insect damage followed by salvage logging. However, clearcutting and planting result in a different forested landscape than natural regeneration following fire or partial cutting. Plantations are not only spatially, structurally and compositionally more uniform, they may also be at greater risk of abiotic and biotic stresses (Woods et al. 2005), and be home to a lower diversity of birds and mammals (Huggard et al. 2006, Mahon et al. 2008).

2.6 Conclusion

The natural regeneration potential of interior Douglas-fir forests increased with disturbance severity, and was greatest in the high severity burn and screefed clearcut. Removal of canopy cover either through wildfire or clear-cutting relieved light limitations to seedling establishment, resulting in improved survival, growth and physiology. Regeneration patterns differed, however, between the two most severe treatments. High severity fire was associated with faster seedling growth and higher foliar nutrient contents, whereas screefing in clearcuts resulted in greater and more uniform germination, seedling survival and seedling density. High severity fire, therefore, appeared to relieve additional nutritional limitations to growth but also created a more heterogeneous seedbed because of the presence of ash and higher maximum daytime temperatures. The relative improvement of germination and growth in the low severity
burn treatment over the undisturbed provides further impetus to further examine the conditions under which partial canopy retention can facilitate Douglas-fir natural regeneration. Future studies on emulation silviculture in Interior Douglas-fir forests should therefore compare the effects of variable retention harvesting and mixed fire severity on the combined effects of seed source, substrate and resource availability on regeneration potential.
Table 2.1 Range of site properties in each disturbance treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Elevation range (m)</th>
<th>Slope range (%)</th>
<th>Aspect (# of sites)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High severity burn (HSB)</td>
<td>806-1179</td>
<td>0-6</td>
<td>NW (3), NE (1)</td>
</tr>
<tr>
<td>Low severity burn (LSB)</td>
<td>700-1176</td>
<td>2-24</td>
<td>NE (2), SE (1), SW (2)</td>
</tr>
<tr>
<td>Screefed clearcut (SCC)</td>
<td>1000-1268</td>
<td>8-20</td>
<td>NW (2), NE (1) SW (1)</td>
</tr>
<tr>
<td>Clearcut (CC)</td>
<td>1000-1268</td>
<td>8-20</td>
<td>NW (2), NE (1) SW (1)</td>
</tr>
<tr>
<td>Undisturbed forest (UF)</td>
<td>843-1176</td>
<td>0-11</td>
<td>NW (3), NW (1)</td>
</tr>
</tbody>
</table>
Table 2.2 Comparison of seedling growth and nutrient responses among disturbance treatments in September 2004. Standard errors are in brackets. Means with different letters are significantly different at $\alpha=0.05$. na=not applicable because foliar biomass in the undisturbed forest was too small to conduct foliar nutrient analyses. *n=18,**n=15.

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>HSB</th>
<th>LSB</th>
<th>SCC</th>
<th>CC</th>
<th>UF</th>
<th>F and P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>76$^a$ (3.3)</td>
<td>93$^a$ (3.3)</td>
<td>81$^a$ (3.0)</td>
<td>74$^a$ (3.0)</td>
<td>61$^b$ (3.0)</td>
<td>F=6.1, P=0.0048$^*$</td>
</tr>
<tr>
<td>Shoot biomass (g)</td>
<td>0.077$^a$ (0.01)</td>
<td>0.043$^b$ (0.01)</td>
<td>0.059$^a$ (0.01)</td>
<td>0.043$^b$ (0.01)</td>
<td>0.016$^c$ (0.003)</td>
<td>F=16, P&lt;0.0001$^*$</td>
</tr>
<tr>
<td>Root biomass (g)</td>
<td>0.076$^a$ (0.015)</td>
<td>0.043$^b$ (0.013)</td>
<td>0.058$^a$ (0.010)</td>
<td>0.042$^b$ (0.010)</td>
<td>0.017$^c$ (0.002)</td>
<td>F=13, P=0.0005$^*$</td>
</tr>
<tr>
<td>Shoot height (cm)</td>
<td>5.1$^a$ (0.30)</td>
<td>4.7$^b$ (0.27)</td>
<td>4.2$^{ab}$ (0.30)</td>
<td>4.8$^{ab}$ (0.30)</td>
<td>3.8$^b$ (0.50)</td>
<td>F=3.8, P=0.0068$^*$</td>
</tr>
<tr>
<td>Foliar N concentration (%)</td>
<td>2.6$^{ab}$ (0.12)</td>
<td>2.9$^a$ (0.11)</td>
<td>2.6$^{ab}$ (0.11)</td>
<td>2.2$^b$ (0.11)</td>
<td>na</td>
<td>F=4.8, P=0.0229$^{**}$</td>
</tr>
<tr>
<td>Foliar N content (mg)</td>
<td>0.016$^a$ (0.0011)</td>
<td>0.005$^b$ (0.0011)</td>
<td>0.008$^c$ (0.0011)</td>
<td>0.0053$^{bc}$ (0.0011)</td>
<td>na</td>
<td>F=23, P&lt;0.0001$^{**}$</td>
</tr>
<tr>
<td>Foliar P concentration (%)</td>
<td>0.29$^a$ (0.019)</td>
<td>0.30$^a$ (0.017)</td>
<td>0.31$^a$ (0.017)</td>
<td>0.28$^a$ (0.016)</td>
<td>na</td>
<td>F=0.76, P=0.522$^{**}$</td>
</tr>
<tr>
<td>Foliar P content (mg)</td>
<td>0.0018$^a$ (0.0015)</td>
<td>0.00053$^b$ (0.0013)</td>
<td>0.00010$^c$ (0.0013)</td>
<td>0.00062$^b$ (0.0013)</td>
<td>na</td>
<td>F=21, P&lt;0.0001$^{**}$</td>
</tr>
</tbody>
</table>

† Treatments: HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clearcut; UF=undisturbed
Table 2.3 Comparison of soil properties among disturbance treatments in 2004. Standard errors are in brackets. Means with different letters are significantly different at α=0.05. n=18.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HSB</th>
<th>LSB</th>
<th>SCC</th>
<th>CC</th>
<th>UF</th>
<th>F and P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total C (kg/ha)</td>
<td>54104&lt;sup&gt;a&lt;/sup&gt; (33580)</td>
<td>25720&lt;sup&gt;a&lt;/sup&gt; (15960)</td>
<td>15960&lt;sup&gt;a&lt;/sup&gt; (8081)</td>
<td>55860&lt;sup&gt;a&lt;/sup&gt; (28280)</td>
<td>57750&lt;sup&gt;a&lt;/sup&gt; (35840)</td>
<td>F=3.2, P=0.0543</td>
</tr>
<tr>
<td>Total N (kg/ha)</td>
<td>2160&lt;sup&gt;a&lt;/sup&gt; (1065)</td>
<td>1300&lt;sup&gt;a&lt;/sup&gt; (641)</td>
<td>665&lt;sup&gt;a&lt;/sup&gt; (270)</td>
<td>1710&lt;sup&gt;a&lt;/sup&gt; (694)</td>
<td>2270&lt;sup&gt;a&lt;/sup&gt; (1120)</td>
<td>F=3.2, P=0.0586</td>
</tr>
<tr>
<td>C:N</td>
<td>25&lt;sup&gt;a&lt;/sup&gt; (2.4)</td>
<td>20&lt;sup&gt;a&lt;/sup&gt; (2.4)</td>
<td>24&lt;sup&gt;a&lt;/sup&gt; (2.0)</td>
<td>33&lt;sup&gt;b&lt;/sup&gt; (2.0)</td>
<td>25&lt;sup&gt;a&lt;/sup&gt; (2.4)</td>
<td>F=5.0, P=0.0133</td>
</tr>
<tr>
<td>Mineralizable N (kg/ha)</td>
<td>37.5&lt;sup&gt;a&lt;/sup&gt; (16.2)</td>
<td>41.5&lt;sup&gt;a&lt;/sup&gt; (21.9)</td>
<td>23.1&lt;sup&gt;a&lt;/sup&gt; (8.60)</td>
<td>129&lt;sup&gt;b&lt;/sup&gt; (67.8)</td>
<td>217&lt;sup&gt;b&lt;/sup&gt; (93.6)</td>
<td>F=11, P=0.0008</td>
</tr>
<tr>
<td>Bray P (kg/ha)</td>
<td>256&lt;sup&gt;a&lt;/sup&gt; (37.8)</td>
<td>160&lt;sup&gt;a&lt;/sup&gt; (38.2)</td>
<td>157&lt;sup&gt;a&lt;/sup&gt; (43.2)</td>
<td>135&lt;sup&gt;a&lt;/sup&gt; (40.3)</td>
<td>139&lt;sup&gt;a&lt;/sup&gt; (37.3)</td>
<td>F=1.2, P=0.3399</td>
</tr>
<tr>
<td>pH</td>
<td>6.6&lt;sup&gt;a&lt;/sup&gt; (0.21)</td>
<td>6.9&lt;sup&gt;a&lt;/sup&gt; (0.21)</td>
<td>5.7&lt;sup&gt;b&lt;/sup&gt; (0.28)</td>
<td>5.5&lt;sup&gt;b&lt;/sup&gt; (0.26)</td>
<td>6.2&lt;sup&gt;a&lt;/sup&gt; (0.208)</td>
<td>F=7.04, P=0.0013</td>
</tr>
<tr>
<td>Bulk density (kg/ha)</td>
<td>810&lt;sup&gt;a&lt;/sup&gt; (40)</td>
<td>740&lt;sup&gt;a&lt;/sup&gt; (40)</td>
<td>750&lt;sup&gt;a&lt;/sup&gt; (70)</td>
<td>720&lt;sup&gt;a&lt;/sup&gt; (35)</td>
<td>750&lt;sup&gt;a&lt;/sup&gt; (36)</td>
<td>F=0.89, P=0.4766</td>
</tr>
</tbody>
</table>

<sup>1</sup> Treatments: HSB=high severity burn; LSB=low severity burn; SCC=screed clear-cut; CC=clearcut; UF=undisturbed forest.
Table 2.4 Comparison of average, maximum and minimum soil water content (%) among disturbance treatments from 2004-2006. Standard errors are in brackets. Means with different letters are significantly different at $\alpha=0.05$. $n=18$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>HSB</th>
<th>LSB</th>
<th>SCC</th>
<th>CC</th>
<th>UF</th>
<th>F and p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average (%)</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>$F=8.0$, $P&lt;0.0001$</td>
</tr>
<tr>
<td></td>
<td>(0.95)</td>
<td>(1.1)</td>
<td>(0.83)</td>
<td>(0.82)</td>
<td>(1.04)</td>
<td></td>
</tr>
<tr>
<td>Maximum (%)</td>
<td>17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>$F=7.0$, $P&lt;0.0001$</td>
</tr>
<tr>
<td></td>
<td>(1.3)</td>
<td>(1.5)</td>
<td>(1.1)</td>
<td>(1.1)</td>
<td>(1.4)</td>
<td></td>
</tr>
<tr>
<td>Minimum (%)</td>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>$F=7.3$, $P&lt;0.0001$</td>
</tr>
<tr>
<td></td>
<td>(0.80)</td>
<td>(0.93)</td>
<td>(0.70)</td>
<td>(0.69)</td>
<td>(0.88)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Treatments: HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clearcut; UF=undisturbed forest.
Table 2.5 Comparison of mean, maximum and minimum soil temperature (°C) by month and treatment from 2004-2006. Standard errors are in brackets. Means with different letters are significantly different at $\alpha=0.05$. n=13.

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
<th>F and P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (°C)</strong></td>
<td>9.83(^a)</td>
<td>13.0(^b)</td>
<td>16.1(^c)</td>
<td>16.0(^c)</td>
<td>10.1(^a)</td>
<td>F=140, P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(1.06)</td>
<td>(1.06)</td>
<td>(1.06)</td>
<td>(1.06)</td>
<td>(1.06)</td>
<td></td>
</tr>
<tr>
<td><strong>Maximum (°C)</strong></td>
<td>18.0(^a)</td>
<td>18.8(^b)</td>
<td>20.9(^c)</td>
<td>21.1(^c)</td>
<td>15.1(^a)</td>
<td>F=14, P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(1.07)</td>
<td>(1.07)</td>
<td>(1.07)</td>
<td>(1.07)</td>
<td>(1.07)</td>
<td></td>
</tr>
<tr>
<td><strong>Minimum (°C)</strong></td>
<td>3.88(^a)</td>
<td>7.65(^b)</td>
<td>11.3(^c)</td>
<td>11.0(^c)</td>
<td>5.17(^d)</td>
<td>F=94, P&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>(1.10)</td>
<td>(1.10)</td>
<td>(1.10)</td>
<td>(1.10)</td>
<td>(1.10)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>HSB</th>
<th>LSB</th>
<th>CC</th>
<th>UF</th>
<th>F and P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (°C)</strong></td>
<td>14.40(^a)</td>
<td>12.3(^a)</td>
<td>13.8(^a)</td>
<td>10.7(^a)</td>
<td>F=1.8, P=0.1546</td>
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<tr>
<td></td>
<td>(1.10)</td>
<td>(1.08)</td>
<td>(1.15)</td>
<td>(1.10)</td>
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<tr>
<td><strong>Maximum (°C)</strong></td>
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<td>17.04(^a)</td>
<td>21.05(^a)</td>
<td>14.80(^a)</td>
<td>F=2.8, P=0.0521</td>
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<tr>
<td></td>
<td>(1.12)</td>
<td>(1.10)</td>
<td>(1.18)</td>
<td>(1.12)</td>
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<tr>
<td><strong>Minimum (°C)</strong></td>
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<td>7.45(^a)</td>
<td>7.37(^a)</td>
<td>6.67(^a)</td>
<td>F=0.41, P=0.7453</td>
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<tr>
<td></td>
<td>(1.13)</td>
<td>(1.10)</td>
<td>(1.18)</td>
<td>(1.13)</td>
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</tr>
</tbody>
</table>

1 Treatments: HSB=high severity burn; LSB=low severity burn; CC=clearcut; UF=undisturbed forest.
Figure 2.1 Comparison of the number of germinants among treatments in 2004. Error bars represent $\pm 1$ s.e., $n=19$. Treatment means with different letters are significantly different at $\alpha=0.05$. HSB=high severity burn; LSB=low severity burn; SCC=scrreefed clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 2.2 Comparison of September shoot biomass among treatments from 2005 to 2006. Error bars = ± 1 s.e., n=11. Treatment means with different letters are significantly different within a year at α=0.05. Treatment means with different numbers of asterisks are significantly different between years at α=0.05. HSB=high severity burn; SCC=screefed clear-cut; CC=clear-cut.
Figure 2.3 Comparison of average September (2005/2006) shoot height among treatments. Error bars = ± 1 s.e., n=11. Treatment means with different letters are significantly different at α=0.05. HSB=high severity burn; SCC=screeded clear-cut; CC=clear-cut.
Figure 2.4 Comparison of $\delta^{13}$C values among treatments in 2004. Error bars = ±1 s.e., n=18. Treatment means with different letters are significantly different at $\alpha=0.05$. HSB=high severity burn; LSB=low severity burn; SCC=screeded clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 2.5 Comparison of average (a) and maximum (b) percent soil moisture among treatments. n=18. HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clear-cut; UF=undisturbed forest.
2.7 References


3. Recovery of ectomycorrhizal diversity of naturally regenerating interior Douglas-fir after wildfire and clearcut harvesting

3.1 Introduction

The EM symbiosis is an obligatory symbiosis that is horizontally transmitted, entailing de novo establishment with each germinating seed. Colonization of conifer seedlings by ectomycorrhizal (EM) fungi is essential for soil nutrient and water uptake and hence seedling survival (Kitajima and Fenner 2000). The life histories of interior Douglas-fir (*Pseudotsuga menziesii* var. *glauca*) and EM fungi that constitute the symbiosis are deeply influenced by soil organic matter, particularly the forest floor. Much of the benefit of the EM fungi to plants, and their influence on larger scale ecosystem processes, stem from their ability to release nutrients bound in organic matter (Schimel and Bennett 2004). Too much forest floor can hinder regeneration by creating unfavourable conditions for germination (Caccia and Ballare 1998, Bai et al. 2004), but EM fungi are vertically stratified in the soil profile, with some taxa strongly associated with certain levels, forms or distributions of organic matter (Dickie 2007). The forest floor contains a significant portion of the biomass (Wallander 2006) and species diversity of EM fungi, but a substantial portion occurs throughout the soil profile (Dickie et al. 2002, Rosling et al. 2003).

Wildfires have historically been a major avenue for natural regeneration in the dry interior forest of North America. Wildfire can facilitate Douglas-fir germination by disturbing the forest floor and reducing competing vegetation, both of which are primary substrates for EM

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inoculum. However, wildfire also has the potential to slow the onset of EM colonization, reduce the percentage of colonized roots, and alter the species diversity of EM fungi colonizing seedlings (Miller et al. 1998, Baar et al. 1999, Jones et al. 2002, Smith et al. 2005, Twieg et al. 2007). Studies that have sampled ecosystems before and after fire have found local reductions or temporary elimination of some EM species (Bruns et al., 2002; Dahlberg, 2002; Grogan et al., 2000; Stendell et al., 1999).

Disturbances that reduce EM fungal inoculum and/or cause EM host mortality could reduce mycorrhization of germinants and contribute to regeneration failure (Perry et al. 1989). Forms of EM inoculum available after fire include spores, sclerotia, recently killed root tips, and the living mycorrhizal root tips and mycelium of surviving EM trees or plants (Baar et al. 2002; Dahlberg 2002). Mycorrhizal networks of individual plants, or that link different plants together, may also be important in seedling colonization. Several studies have shown, for example, that establishing seedlings can become colonized by EM fungal species they share in common with residual mature trees, especially if they are of the same host species (Molina and Trappe 1982, Kennedy et al. 2003, Booth 2004, Cline et al. 2005, McGuire 2007, Teste et al. 2009). Residual trees can facilitate EM colonization either directly through physical root-to-root contact with seedling roots, thus forming continuous mycorrhizal networks, or indirectly through increasing levels of viable inoculum. Ectomycorrhizal fungal inoculum has been shown to decrease with increasing fire severity in northern forests (Bergner et al. 2004, Treseder et al. 2004).

Forest management practices that emulate natural disturbances such as wildfire may sustain biodiversity and forest productivity (McRae et al. 2001). A few studies have directly compared the effects of clear-cut harvesting and wildfire on EM fungal communities in mixed temperate forests of western Canada (Mah et al. 2001, Lazaruk et al. 2005, Twieg et al. 2007).
However, there have been no such studies in the Interior Douglas-fir (IDF) biogeoclimatic zone, where forest management practices vary widely, from low severity treatments such as single tree selection to high severity treatments such as clearcutting with screefing (Vyse et al. 1991). Moreover, the extent of severe fires in these forests is predicted to increase with climate change (Nitschke and Innes 2008).

The objective of this study was to determine the effects of disturbance severity from wildfire or clearcut harvesting on EM fungal colonization and diversity of naturally regenerating interior Douglas-fir seedlings during their first three years of establishment. We compared forest floor disturbance severity treatments within a mosaic of wildfire, clearcuts and undisturbed forest in the IDF zone: high severity burn (HSB), low severity burn (LSB), screefed clearcut (SCC), clearcut without screefing (CC), and undisturbed forest (UF). We tested three hypotheses regarding mechanisms by which disturbance affects EM colonization of seedlings. First, we hypothesized that increasing forest-floor disturbance severity would result in slower seedling EM fungal colonization, reduced percentage of colonized roots, and lower EM species richness and diversity. We expected reductions in forest floor by disturbance would reduce EM fungal inoculum potential. To test this hypothesis, we focused on a continuum of forest-floor disturbances: from intact forest floor (UF) to intermediate disturbance (CC, LSB) to near-complete removal of the forest floor (HSB, SCC). Second, we hypothesized that presence of mature hosts would increase seedling EM colonization and diversity by serving as an inoculum source. To accomplish this, we compared the HSB, CC and SCC treatments, where all EM hosts had been killed or removed, against the UF and LSB treatments, where the mature trees remained. Third, we hypothesized that wildfire would reduce EM fungal colonization and diversity more so than clear-cutting. We expected that the heat generated from burning would
destroy more fungal inoculum than the mechanical effects of clear-cutting. To test this hypothesis, we compared the wildfire (HSB, LSB) with clearcutting (CC, SSC) treatments. Overall, based on the presence of trees and forest floor, we predicted that EM colonization and diversity would increase in the order of HSB<SCC<CC<LSB<UF (Table 1).

3.2 Methods

3.2.1 Study area

The study was conducted at 700-1300 m elevation near Barriere, B.C. (51°00N, 120°00W). Most sites occurred in the Thompson Dry, Cool Interior Douglas-fir (IDFdk2) variant of the Interior Douglas-fir (IDF) biogeoclimatic zone, but some sites were transitional to the Thompson Dry, Mild Montane Spruce (MSdm2) variant of the Montane Spruce (MS) biogeoclimatic zone. The IDF zone typically has warm, dry summers and cool winters and the MS zone has short, warm summers and cold winters. The IDFdk2 variant occurs at 850-1130 m elevation and has a mean annual precipitation of 379 mm (range 295 to 452 mm) and mean annual temperature of 4.8 °C (range 3.4 to 6.6 °C) (Lloyd et al. 1990). The MS zone occurs at 1275-1530 m, has a mean annual precipitation of 606 mm (range 523 to 669 mm) and annual temperature of 2.8 °C (range 0.9 to 5.4 °C) (Lloyd et al. 1990). Soils at the study area were Brunisols and Luvisols of silty or sandy loam texture (Canadian System of Soil Classification 1998).

Prior to disturbance, the forests were dominated by mature interior Douglas-fir with increasing amounts of subordinate lodgepole pine (Pinus contorta var. latifolia (Engelm.) Critchfield) at higher elevations. Common understory species included Arctostaphlos uva-ursi (L.) Spreng., Linnaea borealis (L.), Lupinus arcticus S. Watson, and Calamagrostis rubescens
Buckl. (Lloyd et al. 1990). The most common disturbances in the IDFdk2 and MSdm2 variants are wildfire, harvesting, insect outbreaks, windthrow and livestock grazing. The IDF zone naturally has a mixed-severity fire regime, which includes patches of low- to high-severity disturbance and a variable fire return interval (Klenner et al. 2008).

3.2.2 Experimental design and treatments

In May 2004, 16 circum-mesic sites were located across a continuum of wildfire and clear-cut disturbance severities, ranging from undisturbed forests to high severity burns and screefed clearcuts. The sites included five treatments: HSB, LSB, SCC, CC and UF (Table 1). These treatments were replicated four times in a Completely Randomized Design. Replicate sites were at least 1 km apart, except for the CC and SCC treatments, which were located in the same replicate clear-cuts. In the burn treatments, burn severity was defined as the amount of organic material consumed by the fire (Johnstone and Chapin 2006). Burn severity was determined post-hoc based on depth of forest floor charring and exposure of mineral soil. At the HSB sites, the forest floor was completely consumed and mineral soil exposed (depth of forest floor 0.7±0.2 SD cm). Only charred boles of fire-killed trees remained of the canopy and understory trees, which were removed prior to study initiation using a mechanical harvester. At the LSB sites, at least 95% of the canopy had survived the fire but 100% of the understory was killed. Depth of forest floor averaged 1.8±0.5 SD cm. We required that the HSB and LSB conditions occur uniformly over an area at least 50m x 50m to qualify as a replicate site.

The undisturbed forest and clearcut sites were located either within the overall perimeter or at the immediate periphery (within 4 km) of the McLure fire. The undisturbed forests were dominated by mature Douglas-fir trees that were 100-200 years old. Forest-floor depth averaged 4.9±0.6 SD cm. The clear-cut sites were harvested in the winter of 1993-1994 and were cleared
of canopy cover over an area of at least 1.5 ha. In May 2004, two circular plots were located at least 20 m apart between the plot centers within each clear-cut and randomly assigned the CC or SCC treatment. Five seed beds (see below) were then randomly located within each of these treatments. In the SCC treatment, all vegetation and forest floor was removed (screefed) down to the mineral soil using shovels and rakes (0 cm forest-floor depth). In the CC treatment, the forest floor was left intact (3.8±0.4 SD cm forest-floor depth).

The original goal of the study was to compare EM colonization and diversity among all treatments for three years after disturbance (July 2004 to September 2006). However, the number of replicates in the UF and LSB treatments was reduced from four in August 2004 to one each by August 2006 due to logging (2 LSB sites), cattle grazing (1 UF site), and infection by a fungal root pathogen, *Rhizina* sp. (1 LSB site). One HSB site was excluded from this analysis because of germination failure.

Treatment effects were assessed within a single 15-m circular plot at each replicate site (or two circular plots at replicate clearcut sites, as explained above). Within each plot area, five seed beds (120 cm x 140 cm) were created at least 5 m apart. Wooden stakes were driven into the ground to mark the four corners and center of each seedbed. Seeds of interior Douglas-fir (seedlot 48523, Tree Seed Center, Ministry of Forests, Surrey, B.C., Canada) were stratified at 4 °C for 3 weeks and sown into the seedbeds during the first two weeks of May, 2004. At each seedbed, seven to eight rows were dug through the forest floor to expose bare mineral soil. The seeds were covered by a thin layer of mineral soil and organic matter, if present. To reduce seed predation, the seedbeds were covered with two types of mesh (2.5-cm-diameter mesh and a finer mesh). The mesh was supported by the stakes and its edges were buried in the ground.
All vegetation establishing within and adjacent to the seed beds was clipped at the root collar and removed from the plots. There was little vegetation, except for some pinegrass at the clearcut sites and fireweed (Epilobium latifolium L.) at the HSB sites. Natural interior Douglas-fir regeneration was absent from the clearcut, UF and HSB sites, but some occurred near the seedbeds in the LSB sites, and was removed.

3.2.3 Sampling of ectomycorrhizae

Seedling EM status was assessed twice a month from July until October, 2004, and May through September in 2005 and 2006. Seedlings were carefully removed from the soil using a pocketknife and the roots separated from the shoots at the root collar. The root samples were placed into plastic bags with a small amount of surrounding soil and stored at 4 °C until further processing. All samples were processed within 4 months of field sampling. Seedling root systems were washed under running tap water and subsequently cut into approximately 1- to 2-cm pieces. Root fragments were placed in a baking dish containing distilled water and thoroughly mixed. All collected root fragments were examined. Gross morphology of EM roots was described using a stereomicroscope, while the mantle, cystidia, emanating hyphae, and Hartig net were described using a compound microscope under 400 or 1000 X magnification. Morphological descriptions were based on Goodman et al. (1996) and Hagerman et al. (2001).

DNA extraction and PCR amplification of the ITS region of fungal nuclear rDNA was conducted on at least one subsample (up to five) per morphotype per treatment. Extraction and isolation of DNA, sequencing and nucleotide Basic Local Alignment Search Tool (BLAST) searches followed methods outlined in Twieg et al. (2007). Primer pairs used in PCR amplifications were: ITS1-F and ITS4; NSI1 and NLC2 (Martin & Rygiewicz, 2005). Samples that were not successfully
amplified or sequenced were given an EM taxon name based on morphotyping. Out of the 265 samples submitted for DNA analysis, 50% produced DNA suitable for BLAST searches.

3.2.4 Statistical analysis

Statistical analysis was performed using SAS version 9.1 (SAS institute Inc., Cary N.C.), except that non-metric scaling (NMS) ordinations and diversity calculations were performed with PC-ORD version 5 (see below). Ectomycorrhizal diversity was described by species richness and the Shannon-Weiner diversity index. Relative EM abundance was calculated as the number of root tips colonized by a given EM taxon in a treatment divided by the total number of EM tips at a site. Generalized linear models (GLMs) were used to detect treatment effects on percent colonization, species richness, Shannon-Weiner diversity index, and the relative abundance of individual EM taxa. Normal and Poisson distributions were used to model treatment effects using a log link. Akaike’s information criterion was used to select the best candidate from the set of models. When change over time was assessed, time was included in the model as a main factor.

Treatments effects on EM community structure (EM genera with relative abundance > 1%) was assessed visually with nonmetric multidimensional scaling (NMS) using PC-ORD’s (5.12) “autopilot mode” (McCune and Grace, 2002). Starting from random starting configuration and using the Sorensen distance measure, 40 runs were done on the real data and compared to 50 randomization runs. A Monte Carlo significance test was used to determine whether the axes extracted were stronger than by chance alone. The proportion of variation represented by each axis was assessed by calculating the coefficient of determination ($r^2$) between distances in the ordination space. Pearson correlations with soil property variables were assessed, with correlations higher than 0.2 overlaid as joint plot vectors on the NMS plot.
Multivariate statistical analysis of treatment effects was performed on the EM community data. Pairwise comparisons between treatment groups were tested with multi-response permutation procedures (MRPP) using the Sorensen distance measure.

3.3 Results

3.3.1 Initial EM colonization

Ectomycorrhizal colonization of seedlings was first detected in the first sampling, which was done in September, 2004. Colonization was significantly lower in the HSB and LSB than the CC and SCC treatments (F=2.49, P=0.0417) (Figure 3.1). Contrary to predictions, colonization of seedlings in the UF was lower than in the clear-cut treatments. The percentage of mycorrhizal seedlings averaged over all five treatments increased from 29% (se=0.05) in September 2004 to 47% (se=0.07) in October 2004 (F=4.74, P=0.0366). There was no interaction between month and treatment effect (F=1.27, P=0.2994). Starting in May 2005, all seedlings were mycorrhizal in all treatments.

3.3.2 Ectomycorrhizal diversity

Over 11,000 root tips were examined and 17 EM taxa were identified in this study (Table 3.2). Thirteen of the seventeen taxa were Basidiomycete fungi and the rest were Ascomycetes. The highest number of root tips counted on a seedling was 157. Although treatment effects were not significant, EM richness (averaged over 2004, 2005 and 2006) tended to be lowest in the burned treatments, especially the LSB (F=1.72, P=0.2160) (Figure 3.2a). The Shannon-Weiner diversity index was significantly lower in both burn treatments than the UF, and intermediate in the clear-cut treatments (F= 3.83, P=0.0348) (Figure 3.2b).
3.3.3 EM taxa relative abundance

Ascomycetes dominated the EM community, where Wilcoxina sp. was the most abundant EM taxon, especially in the burn treatments (Figs. 3.3 and 3.4). In 2004, Wilcoxina sp. was the only EM taxon with a relative abundance >5% in all five treatments. In 2005-2006, relative abundance of Rhizopogon also exceeded 5% in all five treatments, but all other taxa occurred in low numbers (Fig. 3.3). The UF and CC treatments were also associated with higher relative abundances of Basidiomycetes than the other treatments (Fig. 3.3).

In September 2004, Wilcoxina sp. relative abundance varied significantly by treatment, and was higher in the LSB and HSB (averaging over 85%) than CC treatment (36%) (F=3.47, P=0.0337) (Fig. 3.4). It was intermediate in the UF treatment (52%) (Fig. 3.4). Disturbance also significantly affected Wilcoxina sp. relative abundance in 2005/2006 (F=5.9, P=0.0073) (Figure 3.5). In the HSB and clear-cut treatments, Wilcoxina sp. relative abundance consistently exceeded 50% in 2005 and 2006, but there were no significant differences after P-value adjustment (Fig. 3.5). There was no evidence of a significant seasonal effect in 2005 or 2006 (F=0.18, P=0.8360). However, Wilcoxina sp. relative abundance significantly declined from 70% (se 8.0%) in 2005 to 65% (se 8.0%) in 2006 (F=4.7, P=0.0408).

3.3.4 Disturbance and host effects

MRPP revealed evidence of a disturbance treatment effect on the EM community found on the seedlings (A= 0.22, P=0.0034). The EM community in the UF treatment was significantly
different from the HSB (A=0.15, p=0.012), LSB (A=0.33, P=0.001), SCC(A=0.34,P=0.021) and CC (A=0.36,P=0.023) treatments (Fig. 3.6). The EM community in the wildfire treatments (HSB, LSB) had significantly different EM communities from the other treatments (SCC, CC, UF) (A=0.10, P=0.02). MRPP also revealed no evidence of host effect on the EM community (A=0.26, P=0.14). The NMS analysis shows an association between some EM taxa (e.g., *Russula*) and intact forest floor (correlation with Axis 1 - $r^2=0.43$) (Fig. 3.6). With the exception of *Wilcoxina* sp., the major EM taxa (> 5% relative abundance) tended to be associated with increasing forest-floor depth. Average growing season moisture was also correlated with the EM communities in disturbance treatments (correlation with Axis 1 - $r^2=0.24$; correlation with Axis 2 - $r^2=0.21$).

### 3.4 Discussion

#### 3.4.1 Effects of forest floor disturbance severity on EM fungi

Our first hypothesis, that increasing forest-floor disturbance severity would result in slower seedling EM fungal colonization, lower EM species richness and diversity, and simplified EM community composition, was only partially supported by our results. Contrary to our predictions, removal or disruption of the forest floor either through high severity fire or mechanical screefing did not limit EM colonization compared to the low severity fire or unprepared clearcuts where the forest floor was left relatively intact. While severe fire or screefing may reduce or eliminate EM biomass by removing the forest floor, EM propagules in the underlying mineral soil may be resistant to fire damage or be too deep in the soil profile to be directly harmed (Dahlberg 2002). Studies have shown that disturbance-resistant propagules
(spores, sclerotia) and excised mycorrhizal root tips can serve as inoculum sources for colonizing seedlings after wildfire (Baar et al. 1999, Stendell et al. 1999) and clear-cutting (Teste et al. 2009).

Disturbance severity effects on EM fungal diversity varied with the diversity measure and disturbance type. We expected that EM fungal diversity would decline and uniformity of species distributions would increase with disturbance severity (Baar et al. 1999, Horton and Bruns 2001). However, we found that EM fungal richness was not reduced by clear-cutting or wildfire, and that increasing severity of these treatments had no further effects (Figure 3.2a). The richness results suggest that sufficient mineral soil inoculum was available at depth to compensate for losses caused by forest floor disruption. Our results contrast with other studies showing decreasing EM fungal richness with clearcut harvesting (Byrd et al. 2000); moreover, our species richness was comparable to a nearby dry IDF forest studied by Teste et al. (2009), who found a total of 26 EM taxa colonizing interior Douglas-fir seedlings in clearcuts. In contrast to measures of richness, both clearcutting (but not significantly) and wildfire (significantly) reduced EM fungal diversity (measured by the Shannon-Weiner index) relative to the undisturbed forest (Figure 2b). It decreased further with screefing in clearcuts but not with high severity burns, contrasting with our expectation that heat from high severity burns would destroy fungal inoculum more so than mechanical disturbance from screefing. High severity fire appeared to have limited effects on inoculum deep in the mineral soil, which can contain a substantial proportion and richness of colonized root tips (Rosling et al. 2003). Our results suggest that EM propagules present in the mineral soil are more important determinants of EM richness and colonization than those present in the forest floor. In contrast to other ecosystems (e.g., boreal forests), fine-root density in dry interior forests may be greatest in the B and C horizons (Dumm
et al. 2008); thus, as seedlings grow larger root systems, EM diversity may continue to increase even in the absence of forest floor.

In contrast to EM species richness and diversity, species distribution patterns in 2005/06 changed with disturbance severity in congruence with our predictions (Figure 3.3). Our results indicate that increasing disturbance severity resulted in more uniform EM communities. Specifically, the number of dominant fungi (>5% frequency) decreased with increasing forest-floor disturbance severity, from the undisturbed forest to the high severity burns or screefed clearcut. We found that disturbing the forest floor via combustion altered the pattern of dominant fungi more so than mechanical removal. The pattern of a relatively few fungi dominating a disturbed community has been common in other studies where few EM species dominated (90% of the relative abundance) forests prior to disturbance (Horton and Bruns 2001, Pickles et al. 2010).

The greater magnitude of disturbance effects on EM community composition than richness suggests that seedlings are readily colonized by a few dominant fungi protected deep in the mineral soil following severe disturbance. Forest floor retained after disturbance is an important contributor of diverse viable EM inoculum, where excised roots remain active up to three growing seasons after clearcut harvesting (Hagerman et al. 1999). With increasing disturbance severity to forest floor, however, the dominant dispersal mechanism shifts away from mycelia on active roots to more disturbance-resistant propagules (e.g., spores) of only a few species (Baar et al. 1999, Smith et al. 2004). Retention of organic matter has the potential to enhance EM fungal diversity because EM fungi are vertically stratified, with many species restricted primarily or exclusively to the forest floor (Dickie et al. 2002, Rosling et al. 2003).
That we found similar patterns of EM community composition in the clearcut and undisturbed forest supports this theory.

Relative EM abundance of Basidiomycetes was greatest in the undisturbed forest, but it was also high in the clearcut. Some of the Basidiomycete species, such as *Russula* and *Inocybe*, which produce spores but rarely inoculate seedlings from spores (Peay et al. 2007), might also benefit from forest floor retention. Clear-cutting also increased abundance of the Ascomycete, *Cennococcum* sp., relative to the other disturbance treatments. *Cennococcum* is globally widespread and its presence is positively associated with organic matter content (Dickie 2007, Hryniewicz et al. 2009).

In contrast to the Basidiomycetes, Ascomycetes dominated the wildfire and screefed clearcuts. Dominance of Ascomycetes after disturbance, particularly wildfire, is consistent with a number of other studies (Wicklow 1975, Zak and Wicklow 1980, Cairney and Bastias 2007). We found that the Ascomycete, *Wilcoxina* sp., was the dominant EM fungus following both clear-cutting and wildfire, where it colonized over 50% of root tips. *Wilcoxina* sp. in particular has been found to increase in abundance following wildfire (Baar et al. 1999, Grogan et al. 2000) and harvesting (Hagerman et al. 2001, Teste et al. 2009). In their review of clearcutting effects on the EM fungal community, Jones et al. (2003) hypothesized that species adapted to disperse into disturbed environments are also more functionally adapted to post-disturbance environments. Researchers have also hypothesized that *Wilcoxina* sp. dominate post-disturbance environments, especially wildfire, through their adaptation to the environmental conditions of burned substrates (higher pH and lower moisture) and reduced competition from other EM taxa (Wicklow and Hirschfield 1979). The prevalence of *Wilcoxina* in all the treatments in the first year, not just following wildfire, supports the theory that *Wilcoxina* was released from
competition more so than the notion that it is better adapted to post-fire environments. *Wilcoxina* sp. also occurs in mature forests but in lower abundances than in post-disturbance environments (Smith et al. 2005). *Wilcoxina* sp. may be largely restricted to colonizing root tips in the mineral soil in some forest ecosystems (Rosling et al. 2003), but other studies have found an association of *Wilcoxina rehmii* with high levels of organic matter, such as in peat (Egger et al. 1991).

### 3.4.2 Effects of mature host presence

The presence of mature hosts in the undisturbed forest or low severity burn did not consistently result in greater EM colonization or diversity compared to the other treatments where mature trees had been killed by fire or logging, leading us to reject our second hypothesis. This suggests that presence of mature hosts was not the dominant determinant of EM colonization, but rather that the presence of inoculum in the soil was relatively more important. Despite presence of host trees in both treatments, the lower EM diversity in the low severity burn than undisturbed forest further indicates that the fire disturbance to the forest floor alone can reduce EM diversity levels. The tree crowns were largely intact in the low severity burn, but the fine roots in the forest floor were likely damaged, thus reducing the importance of the forest floor as a source of EM fungal inoculum. Studies of prescribed burns have shown that fine roots in the forest floor can be vulnerable to heat damage, but the extent of damage depends on a number of factors, such as soil moisture content (Smith et al. 2004).
3.4.3 Contrasting effects of wildfire and clearcut harvesting

We found that disturbing the forest floor via combustion altered abundance of the dominant fungi more so than mechanical removal, supporting our third hypothesis that wildfire has a greater impact on EM fungal communities than clear-cutting. Wildfire caused a short-lived reduction in EM colonization of new seedlings compared to clear-cutting but this effect was limited to the first growing season. Fire likely reduced EM colonization through direct mortality of EM propagules caused by heat damage to the forest floor and upper layer of mineral soil (Neary et al. 1999). Previous studies have shown that conifer seedlings can remain non-mycorrhizal for up to two years after wildfire (Miller et al. 1998, Purdy et al. 2002), suggesting that our wildfires were comparatively less severe and/or that they differed in nutrient availability (see Chapter 4). We also found that wildfire tended to reduce EM species richness and diversity more so than clear-cutting compared with the undisturbed forest over the three year study period, but not significantly. In spruce boreal forests of Canada, Lazaruk et al. (2005) also found that clearcutting and burning resulted in comparable reductions in EM diversity, but with somewhat greater reductions following burning. Similar to our study, they found that EM fungal communities on seedlings in wildfires and clearcuts clustered together relative to those in undisturbed stands.

Wildfire has been demonstrated to both alter EM fungal species diversity and retard symbiosis initiation on regenerating seedlings (Miller et al. 1998, Baar et al. 1999, Jones et al. 2002, Smith et al. 2005, Twieg et al. 2007). Some EM species have either declined or been temporarily eliminated by wildfire (Bruns et al., 2002; Dahlberg, 2002; Grogan et al., 2000; Stendell et al., 1999). Clearcutting has also been shown to alter EM species communities but not reduce colonization (Hagerman et al. 1999, Mah et al. 2001, Jones et al. 2003). Given the short-
lived effect of fire on EM fungal colonization, we suggest that wildfire is similar to clear-cutting in its greater effects on EM species composition than EM colonization, and that EM fungal communities recover from severe disturbances over time. In a long-term chronosequence study, Twieg et al. (2007) found that early EM diversity differences between clearcut and burned sites converged after crown closure (26 years), and were indistinguishable when the stands were 100 years old. Lazaruck (2005) argued that fire-related reductions in diversity may bring management goals to emulate natural disturbances and preserve biodiversity into conflict. However, the results of this study indicate that EM fungal diversity recovers quickly following wildfire, but elimination of the forest floor by severe fire does slow aspects of EM community recovery.

3.5 Conclusions

Wildfire resulted in slower early EM colonization than clearcutting, but the EM community recovered to undisturbed forest levels within one year of both clearcutting and wildfire. Hence, wildfire generally does not appear have a long-term negative effect on colonization by EM fungal communities and, conversely, clear-cutting appears to emulate well the effects of wildfire. However, increasing severity of wildfire disturbance to the forest floor led to slower de novo EM colonization and a shift in EM community composition for three years. The negative effects on the dominant fungi were more pronounced following severe wildfire than following clear-cutting with mechanical screefing, but the differences were not strong enough to be significant. In comparison to forest floor disturbance, death or removal of host trees had little effect on the EM community, suggesting that EM propagules in soils were more
important than presence of mature hosts. However, the presence of both living hosts and intact forest floor in the undisturbed forests resulted in the most diverse EM community comprised of later successional fungi. Any disturbance, by contrast, simplified the EM community to one dominated by *Wilcoxina* sp. These results indicate that EM communities of dry interior Douglas-fir forests are resilient to a range of disturbance agents and severities. Moreover, our results suggest that old forests can important reservoirs of diverse EM communities comprised of late successional EM fungi, and their presence in the landscape is likely important to the recovery of nearby forests disturbed by wildfire or clear-cutting.
Table 3.1 List of disturbance treatments with forest floor status, host availability, and predicted colonization and diversity.

<table>
<thead>
<tr>
<th>Disturbance</th>
<th>Forest Floor Status</th>
<th>Host</th>
<th>Predicted diversity ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Severity Burn (HSB)</td>
<td>Combusted</td>
<td>Provided by experimental seeding</td>
<td>1</td>
</tr>
<tr>
<td>Low Severity Burn (LSB)</td>
<td>Combusted</td>
<td>Provided by experimental seeding, Mature Douglas-fir</td>
<td>2</td>
</tr>
<tr>
<td>Screefed clearcut (SCC)</td>
<td>Manually removed</td>
<td>Provided by experimental seeding</td>
<td>3</td>
</tr>
<tr>
<td>Clearcut (CC)</td>
<td>Mixed during harvesting</td>
<td>Provided by experimental seeding</td>
<td>4</td>
</tr>
<tr>
<td>Undisturbed (UF)</td>
<td>Intact</td>
<td>Provided by experimental seeding, Mature Douglas-fir</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3.2 List of EM taxa with morphotype id number, closest blast match, number of base pairs, and similarity index percentage.

<table>
<thead>
<tr>
<th>Morphotype number</th>
<th>Blast match</th>
<th>Total base pairs aligned</th>
<th>NCBI% Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Amphinema byssoides</em></td>
<td>307</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td><em>Inocybe lacera</em></td>
<td>650</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td><em>Lactarius deliciosus</em></td>
<td>538</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td><em>Rhizopogon rogersii</em></td>
<td>530</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td><em>Rhizopogon rudus</em></td>
<td>568</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td><em>Rhizopogon vinicolor</em></td>
<td>525</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td><em>Suillus lakei</em></td>
<td>650</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td><em>Russula decolorans</em></td>
<td>616</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td><em>Russula acrifolia</em></td>
<td>520</td>
<td>99</td>
</tr>
<tr>
<td>6</td>
<td><em>Cenococcum geophilum</em></td>
<td>424</td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td><em>Heboloma</em></td>
<td>599</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td><em>Piloderma</em></td>
<td>595</td>
<td>96</td>
</tr>
<tr>
<td>9</td>
<td><em>Thelephora terrestris</em></td>
<td>589</td>
<td>99</td>
</tr>
<tr>
<td>10</td>
<td><em>Tomentella</em></td>
<td>561</td>
<td>99</td>
</tr>
<tr>
<td>11</td>
<td>MRA</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>12</td>
<td><em>Wilcoxina mikolae</em></td>
<td>479</td>
<td>97</td>
</tr>
<tr>
<td>12</td>
<td><em>Wilcoxina rehmii</em></td>
<td>489</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 3.1 Mean percentage of seedlings that were ectomycorrhizal averaged from September and October, 2004. Error bars = ±1 s.e. n=34. Treatment means with different letters are significantly different at α=0.05. HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 3.2 Comparison of (a) EM taxa richness, and (b) Shannon-Weiner diversity index among disturbance severity treatments averaged across 2004, 2005 and 2006. Error bars = ±1 se n=11. Treatment means with different letters are significantly different at α=0.05. HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 3.3 Relative abundance of dominant fungi (>5%) in each treatment in (a) 2004 and (b) 2005-2006.
Figure 3.4 Mean *Wilcoxina* relative abundance in the five treatments in 2004. Error bars = ±1 s.e. n=11. Treatment means with different letters are significantly different at α=0.05. HSB=high severity burn; LSB=low severity burn; SCC=screened clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 3.5 Mean *Wilcoxia* relative abundance in the HSB and harvesting treatments (CC, SCC) from 2005-2006. Error bars = ±1 s.e. n=11. Treatment means with different letters are significantly different at α=0.05. HSB=high severity burn; SCC=screefed clear-cut; CC=clear-cut.
Figure 3.6 NMS ordination of EM species with > 1% relative abundance in relation to treatment. The blue arrows represent the EM taxa in primary ordination space while the red arrows are environment variables ($r^2$ cutoff=0.2). The direction of the arrow indicates the direction of the strongest correlations while the length indicates the magnitude of the relationship. Monte Carlo test, P < 0.05 for both axes. The oval overlays represent the significant differences between the UF and disturbance treatments (P=0.033 for overall treatment effect). HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clear-cut; UF=undisturbed forest.
3.6 References


4. Through the non-mycorrhizal window: plant productivity and nutrient status influences seedling investment in ectomycorrhizal fungi

4.1 Introduction

“If something cannot go on forever, it will stop” (Herbert Stein's Law)

Establishment is a critical phase in the life history of plants. For species in the Pinaceae family, such as interior Douglas-fir, successful regeneration requires that seedlings form a putatively obligatory symbiotic relationship with ectomycorrhizal (EM) fungi (Smith and Read. 1997). The degree to which the symbiosis is mutualistic or parasitic depends on the nutritional, physiological, morphological and phenological status of the plant, fungus or soil environment (Johnson et al. 1997). Evidence points towards a largely mutualistic symbiosis (Karst et al. 2008), but the relationship is context-dependent, where the degree of mutualism, level of colonization and community diversity of EM fungi is determined by a complex interaction of factors, such a nutrient availability and plant productivity (Hoeksema et al. 2010).

Disturbances in forest ecosystems have profound effects on factors affecting seedling establishment and mycorrhizal formation, including substrate and resource availability (Pickett and White 1986). Therefore, understanding the effect of disturbance on the symbiosis formation is paramount to unravelling the dynamics of natural regeneration in forest ecosystems. Historically, species like interior Douglas-fir have naturally regenerated in a mixed-severity fire

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regime, which includes low to high severity fires. Within a single forest, seedlings can regenerate in a wide variety of post-fire conditions. Since the symbionts disperse independently (horizontal transmission) (Bruns et al. 2002), the pattern of disturbances can have a variety of outcomes on the symbiosis. In severe disturbances, for example, plant growth may be negatively affected through reduced inoculum levels and hence de novo colonization, or it may be increased through increases in resource availability. We have used the term “de novo colonization” for the very first formation of the EM symbiosis on seedlings following germination. The period prior to de novo colonization of regenerating seedlings we have termed the “non-mycorrhizal window”.

De novo mycorrhizal fungal colonization of germinants can occur in as little as one-month, but it can take up to two years following wildfire (Miller et al. 1998, Baar et al. 1999, Grogan et al. 2000). Even after colonization, disturbance can reduce the percentage of colonized roots (Miller et al. 1998, Baar et al. 1999, Jones et al. 2002, Smith et al. 2005, Twieg et al. 2007). Disturbances that facilitate natural regeneration can also reduce EM fungal community richness compared to undisturbed stands (Horton and Bruns 2001). Increased resource availability, a common outcome of disturbances, also reduces colonization and simplifies the EM fungal community (Jones 1990, Berch 2006), as shown in fertilization and nitrogen deposition studies (Treseder 2004, Hoeksema et al. 2010). For example, a nitrogen pulse typically follows wildfire, but it is short-lived and often subsides after one year (Wan et al. 2001). Nitrogen availability has been shown to increase initially after clearcut harvesting (Keenan and Kimmins 1993, Prescott 2002) but then decline once the early successional plant community establishes (Forge and Simard 2001). The effect of increased nutrient availability on regenerating seedlings may therefore be short-lived.
Increasing nutrient availability is thought to reduce host carbon investment in EM fungi (the host investment hypothesis), often through reduced belowground carbon allocation (Treseder 2004, Johnson et al. 2005, Druebert et al. 2009). EM fungal colonization levels decline as plants allocate less carbon belowground, especially under shaded conditions (Druebert et al. 2009). Douglas-fir can germinate in a range of light conditions given favourable moisture conditions, but in shade, it likely reduces carbon allocation to roots (Chen 1997). Even with low colonization levels in the shade, however, seedlings may support a greater diversity of EM fungi to help access scarce resources. The presence of overstory trees, for example, had a positive effect on mycorrhizal diversity of understory Douglas-fir seedlings (Simard et al. 1997b), with carbon subsidies to initiate and sustain colonization possibly provided by nearby mature trees (Simard et al. 1997a).

The objective of this study was to determine the effect of seedling growth and nutrient status on the EM fungal community of Douglas-fir seedlings establishing across a range of disturbance severities. Seeds were germinated in five disturbance treatments (high severity burn, low severity burn, clearcut, screefed clearcut, and undisturbed forest), and the seedlings were tracked for growth and mycorrhizal colonization for a three-year period. We tested two hypotheses regarding the relationship between seedling productivity, resource availability and EM colonization dynamics during the non-mycorrhizal window, and the time period shortly after its closure. First, we hypothesized that seedling de novo colonization and diversity of EM fungi declines with increased resource availability. We predicted that seedlings with high biomass accumulation enabled by favourable N and P availability would have the least EM colonization and diversity. Secondly, we hypothesized that seedlings become nitrogen-limited over time. We predicted that EM diversity and colonization increase as foliar nutrient concentrations decline.
with seedling age. We expected that seedlings in the high severity burn and both clearcut treatments would have the greatest initial growth and lowest EM fungal colonization and diversity, but that colonization and diversity of EM would increase over time to meet increasing seedling demands for soil resources. We expected the opposite trends in the light severity burn and undisturbed forest.

4.2 Methods

4.2.1 Study area

The study was conducted at 700-1300 m elevation near Barriere, B.C. (51°00N, 120°00W). Most sites occurred in the Thompson Dry, Cool Interior Douglas-fir (IDFdk2) variant of the Interior Douglas-fir (IDF) biogeoclimatic zone, but some sites were transitional to the Thompson Dry, Mild Montane Spruce (MSdm2) variant of the Montane Spruce (MS) biogeoclimatic zone. The IDF zone typically has warm, dry summers and cool winters and the MS zone has short, warm summers and cold winters. The IDFdk2 variant occurs at 850-1130 m elevation and has an annual precipitation of 379 mm (range 295 to 452 mm) and annual temperature of 4.8 °C (range 3.4 to 6.6 °C) (Lloyd et al. 1990). The MS zone occurs at 1275-1530 m, has an annual precipitation of 606 mm (range 523 to 669 mm) and annual temperature of 2.8 °C (range 0.9 to 5.4 °C) (Lloyd et al. 1990). Soils at the study area were Brunisols and Luvisols of silty or sandy loam texture (Soil Classification Working Group 1998).

Prior to disturbance, the forests were dominated by mature interior Douglas-fir with increasing amounts of subordinate lodgepole pine (*Pinus contorta* var. *latifolia* (Engelm.) Critchfield) at higher elevations. Common understory species included *Arctostaphlos uva-ursi* (L.) Spreng., *Linnaea borealis* L., *Lupinus arcticus* S. Watson, and *Calamagrostis rubescens*
The most common disturbances in the IDFdk2 and MSdm2 variants are wildfire, harvesting, insect outbreaks, windthrow and livestock grazing. The IDF zone naturally has a mixed severity fire regime, which includes patches of low to high severity disturbance and a variable fire return interval (Klenner et al. 2008).

4.2.2 Experimental design and treatments

In May 2004, 16 circum-mesic sites were located across a continuum of wildfire and clearcut disturbance severities, ranging from undisturbed forests to high severity burns and screefed clearcuts. The sites included five treatments: high severity burn (HSB), low severity burn (LSB), screefed clearcut (SCC), clearcut (CC) and undisturbed forest (UF). These treatments were replicated four times in a Completely Randomized Design. Replicate sites were at least 1 km apart, except for the CC and SCC treatments, which were located in the same replicate clearcuts. In the burn treatments, burn severity was defined as the amount of organic material consumed by the fire (Johnstone and Chapin 2006). Burn severity was determined post-hoc based on depth of forest floor charring and exposure of mineral soil. At the HSB sites, the forest floor was completely consumed and mineral soil exposed (depth of forest floor 0.7±0.2 SD cm). Only charred boles of fire-killed trees remained of the canopy and understory trees, which were removed prior to study initiation using a mechanical harvester. At the LSB sites, at least 95% of the canopy had survived the fire but 100% of the understory was killed. Depth of forest floor averaged 1.8±0.5 SD cm. We required that the HSB and LSB conditions occur uniformly over an area at least 50m x 50m to qualify as a replicate site.

The undisturbed forest and clearcut sites were located either within the overall perimeter or at the immediate periphery (within 4 km) of the McLure fire. The undisturbed forests were dominated by mature Douglas-fir trees that were 100-200 years old. Forest-floor depth averaged
4.9±0.6 SD cm. The clearcut sites were harvested in the winter of 1993-1994 and were cleared of canopy cover over an area of at least 1.5 ha. In May 2004, two 15-m circular plots were located with at least 5 m between plot centers within each clearcut and randomly assigned the CC or SCC treatment. Five seed beds (see below) were then randomly located within each of these treatments. In the SCC treatment, all vegetation and forest floor was removed (screefed) down to the mineral soil using shovels and rakes (0-cm forest floor depth). In the CC treatment, the forest floor was left intact (3.8±0.4 SD cm forest-floor depth).

Treatment effects were assessed within a single 15-m circular plot at each replicate site (or two circular plots at replicate clearcut sites, as explained above). Within each plot area, five seed beds (120 cm x 140 cm) were created at least 5 m apart. Wooden stakes were driven into the ground to mark the four corners and center of each seedbed. Seeds of interior Douglas-fir (seedlot 48523, Tree Seed Center, Ministry of Forests, Surrey, B.C., Canada) were stratified at 4 °C for 3 weeks and sown into the seedbeds during the first two weeks of May, 2004. At each seedbed, seven to eight rows were dug through the forest floor to expose bare mineral soil. The seeds were covered by a thin layer of mineral soil and organic matter, if present. To reduce seed predation, the seedbeds were covered with two types of mesh (2.5-cm diameter mesh and a finer mesh). The mesh was supported by the stakes and its edges were buried in the ground.

All vegetation establishing within and adjacent to the seed beds was clipped at the root collar and removed from the plots. There was little vegetation, except for some pinegrass at the clearcut sites and fireweed (*Epilobium latifolium* L.) at the HSB sites. Natural interior Douglas-fir regeneration was absent from the clearcut, UF and HSB sites, but some occurred near the seedbeds in the LSB sites, and was removed.
4.2.3 Growth and mycorrhizal sampling

Methods for seedling growth and soil measurements are described in chapter 2. Methods for EM mycorrhizal colonization and diversity measurements are described in chapter 3.

4.2.4 Statistical analysis

Principle components analysis (PCA) was used to examine the relationship between growth, resource availability, and mycorrhizal colonization and diversity using PC-ORD, version 5.12 (McCune and Grace 2002). Two ordinations were created using plant growth responses to the treatments as the main matrices. In the first ordination, we related seedling growth (main matrix) to mycorrhizal colonization and diversity (secondary matrix) using average growth responses in September 2004 only as well as edaphic factors, soil moisture and soil nutrient status (see Chapter 2). In the main matrix, growth variables included were shoot and root biomass, shoot/root biomass ratio, shoot height, and root length. In secondary matrix, the mycorrhizal variables were percentage of mycorrhizal seedlings, EM richness, and Shannon-Wiener diversity index. The soil nutrient variables were total C, total N, C:N ratio, mineralizable N, Bray P, pH, and bulk density in 2004. In the second ordination, we examined changes in the relationship between seedling growth (main matrix) relative to EM diversity (secondary matrix) over time using data collected in September of 2004, 2005 and 2006 in the three no-canopy treatments (SCC, CC and HSB). The growth variables were shoot and root biomass, shoot height, foliar N and P concentrations, foliar N and P content, and foliar N:P ratio. The EM variables were the Shannon-Wiener diversity index, species richness, and average relative abundance of Wilcoxina from the September sampling period, which was chosen because of its dominant position in the EM community throughout the study period. We used the PC-ORD
default cut-off ($r^2=0.2$) to determine whether joint plot vectors should be included in the ordination space in both ordinations.

Multivariate statistical analysis of treatment effects on seedling growth was performed on the EM community data. Pairwise comparisons between treatment groups were tested with multi-response permutation procedures (MRPP) using the Euclidian distance measure.

Linear regression was used to examine relationships between mycorrhizal status and growth measures using SAS version 9.2 (SAS institute Inc., Cary N.C.). To develop these relationships, we used a combined AIC and stepwise regression approach (PROC GLMSELECT) to select explanatory variables, but only shoot biomass was significant ($P<0.05$). Therefore, we only included the regression between mycorrhizal status and shoot biomass.

4.3 Results

4.3.1 Ordination of EM colonization, diversity and seedling growth in 2004

In the first PCA ordination relating mycorrhizal status to seedling growth in 2004, 60% of the variation was explained by axis 1 and 16% of the variation was explained by axis 2 (Figure 4.1). There was no evidence of a general disturbance treatment ($A=0.065$, $P=0.19$), wildfire ($A=0.041$, $P=0.12$), or canopy effect ($A=-0.026$, $P=0.77$). Though not significant, the ordination does show some separation between the treatments along axis 1, with the UF and HSB treatments separated at opposite ends and the remaining treatments dispersed along axes 1 and 2. Overall, the burn treatments formed the most coherent clusters. On axis 1, the joint plots revealed a opposition trend between some of the environmental factors and EM colonization. Percent EM seedlings ($r^2=0.30$) along with forest floor depth ($r^2=0.21$) trended towards some of the UF and CC sites, while was average % growing season moisture ($r^2=0.40$) and C:N ratio ($r^2=0.31$)
trended towards the high severity burn sites. There was a trend of lower EM colonization and forest floor depth associated with higher biomass accumulation, seedling height and root length along axis 1 (Fig 4.1). In contrast, shoot: root ratios showed a high correlation with both axes. A separate regression analysis provided evidence for a correlation between shoot biomass and % EM seedlings (Figure 4.2).

4.3.2 Ordination of EM diversity and seedling growth in the no-canopy treatments from 2004 to 2006

In the second PCA ordination relating EM diversity and seedling growth over time, 56% of the variation was explained by axis 1 and 38% of the variation was explained by axis 2 (Figure 4.2). There was no evidence of a significant treatment effect (A= -0.022, P=0.67) but there was a significant time effect (A=0.59, P<0.0001). In the ordination, each year was significantly different from each other (P<0.0001 in all cases), with the greatest separation between the 2004 growing season and subsequent years. Seedling biomass, height and foliar N and P contents showed a strong trend towards the 2006 data in both axes, while foliar nutrient concentrations and N:P ratio trended most strongly along axis 1 towards 2004. Of the joint plot measures, only Wilcoxina relative abundance had a correlation (r² = -0.20) with axis 1, and trending towards 2005. Both diversity and richness did not meet the criteria for inclusion with correlations with both axes having r² values > 0.05.
4.4 Discussion

4.4.1 Host investment in EM de novo colonization and diversity in 2004

Our study provides evidence that favourable environments for seedling establishment and growth can be associated with reduced investment of host seedlings in de novo EM colonization, supporting our first hypothesis. The greatest early seedling growth occurred in the no-canopy treatments, especially the high severity burns, where there were the highest soil nutrient and soil water levels (see chapter 2). Reduced de novo colonization in these treatments was likely to be determined more by seedling productivity than inoculum availability given that the most abundant EM taxon, Wilcoxina, produces widely dispersed spores (Egger 1996). Lack of inoculum is primarily limited to areas where the vegetation is dominated by non-EM hosts, such as grasslands, meadows, bogs or forest plantations in exotic locations (i.e., outside of the tree species native ranges) (Nunez et al. 2009), which was not the situation in our study. Inoculum of specific species may have been locally restricted by forest floor disturbance, however, since some EM taxa are strongly associated with the presence of forest floor (Chapter 3). Alternatively, post-fire soil conditions may have also contributed to the low levels of de novo colonization, especially in the HSB treatment. Peay et al. (2009) found that soil heating effects and selective mortality by influenced community shifts, but whether these shifts would be enough to suppress de novo colonization should be investigated further.

Regardless of the mechanism, we observed that the large seedlings with low initial EM colonization in the resource rich treatments had root systems dominated by a taproot with root
hairs. The roots largely lacked lateral root tips, which are stimulated by plant hormones elicited by ectomycorrhizal colonization (Niemi et al. 2002). Root hairs are capable of sufficient soil nutrient and water uptake to meet growth and survival demands under favourable conditions, but they are typically not as carbon-efficient as fungal hyphae in acquiring soil resources under resource-limited conditions unless specially modified (e.g., cluster roots).

Our results provide partial support of our prediction that the presence of mature host trees could provide subsidies to slow growing seedlings for mycorrhizal colonization. As predicted, EM colonization and diversity were high in the undisturbed forest even though the seedlings were growing very slowly in the deep shade. The seedlings had little needle development beyond the cotyledon stage and it is unlikely they had sufficient photosynthetic capacity to initiate and sustain colonization without an external carbon source. We speculate that the mature trees not only provided a rich fungal inoculum source but that they also provided an external source of carbon. This external supply of carbon may have been necessary to initiate fungal colonization given that the host’s ability to supply sufficient carbon is the limiting step to initiation of colonization (Corrêa et al. 2010). The EM fungi may have obtained the additional carbon from excised root tips (Hagerman et al. 1999), directly via mycorrhizal networks (Simard et al. 1997a, Teste et al. 2009), or from saprotrophic abilities (Talbot et al. 2008, Cullings and Courty 2009). Detached root tips remain active for up to 9 months after excision (Ferrier and Alexander 1985), and so could supply EM with non-structural carbohydrates or provide a carbon source via enzymatic decomposition. The strongest circumstantial evidence for a direct role of mature trees in assisting de novo colonization comes from the high abundance of *Rhizopogon* colonizing the struggling seedlings in the undisturbed forest (Chapter 3). *Rhizopogon* has an extensive mantle and extra-radical mycelia (Massicotte et al. 1994), and it is unlikely that the deeply shaded
seedlings could have met their carbon demands. Moreover, *Rhizopogon* spp. have been shown to form networks connecting old trees with young trees in interior Douglas-fir forests (Beiler et al. 2010) and to facilitate colonization on establishing seedlings (Teste et al. 2010).

In contrast to our predictions, initial seedling growth and mycorrhizal colonization patterns in the light severity burn followed those of the open canopy treatments more closely than those of the undisturbed forest, despite the presence of mature hosts in both treatments. These results suggest that even moderate seedling productivity can reduce the need to invest in EM fungi and thereby extend the non-mycorrhizal window. Alternatively, poor root growth in the LSB may have contributed to low de novo colonization in the LSB by reducing the probability of initial contact with, especially since EM inoculum of some species may be patchily distributed in the soil (Pickles et al. 2010). Additionally, the fire damage may have reduced the available inoculum by damaging roots and eliminating forest floor inoculum. However, as Figure 4.1 indicates, high levels of de novo colonization were associated with relatively poor root growth. The UF treatment had the third highest level of de novo colonization despite very poor root growth (see Chapter 3). Given that seedling productivity was enhanced by the high soil nutrient concentrations (Chapter 2) in the light severity burn, lower investment in mycorrhizas may have played a role in allowing seedlings in the light severity burn to invest more carbon into height growth for light interception.

### 4.4.2 Nutrient status and plant EM investment

Since the end of the de novo colonization period were associated with falling nitrogen concentrations, our results suggest that foliar nutrient dynamics may have been a key driver in determining when the non-mycorrhizal window closed. Our results agree with Correa et al. (2008), who found that colonization declined as N concentrations increased under N-limited
conditions in the lab. In our study, we found that seedlings remained non-mycorrhizal under favourable N and P conditions in the no-canopy treatments, but that the non-mycorrhizal window closed with declining N and P concentrations from 2004 to 2005. Plant productivity and nutrient status are linked so that it is not possible to separate the effects of these two factors on de novo colonization. However, it is possible that high initial growth rates delayed colonization until falling nutrient concentrations triggered de novo colonization. Evidence for this shift is the closer match between EM fungal colonization and foliar nutrient patterns than biomass accumulation patterns. Specifically, the decline in foliar nutrient concentrations from 2004 to 2005 mirrored the increases in EM colonization and diversity from 2004 to 2005. In contrast, biomass continued to increase each year of the study. These results provide support for our second hypothesis that EM colonization and diversity increase as foliar nutrient concentrations decline with seedling age.

After the first growing season, the root tips of all seedlings were almost completely colonized (>80% root tip colonization), reflecting substantial investment in EM colonization. We expected that the increased investment in EM colonization would be accompanied by an increase in EM diversity to meet increasing nutrient demands. No relationship between diversity and growth emerged, however, from the ordinations. This is consistent with other studies showing minor effects of plant N status on mycorrhizal diversity (Baxter and Dighton 2001, Karst et al. 2008). Our findings agree with the meta-analysis of EM inoculation studies by Karst et al. (2008), which showed that increasing EM diversity does not necessarily promote a mutualistic outcome in seedlings. One reason that EM diversity may not affect growth is that many EM species may be functionally equivalent over a broad range of conditions. On the same study site as ours, Jones et al. (2010) found functional equivalence among mycorrhizal communities in the
enzymatic activity for accessing P. Additionally, intraspecific EM variation may provide adaptations to local conditions that may mask diversity effects using standard taxonomic diversity measures (Cairney 1999).

The limited spatial and temporal scale of our study may have obscured relationships between EM diversity and host productivity. For example, our study period may have been too short for trees to reach sufficient size to require the services of a diverse taxonomic group of fungi for meeting their growing nutrient demands. Additionally, the relationship between productivity and diversity may change over time. Other studies showing that EM diversity increases as stands develop have led researchers to hypothesize that increases in productivity drive EM diversity (Kernaghan 2005). In a study of two-year-old *Fagus sylvatica*, Druebert et al. (2009) found strong evidence that plant productivity drives EM diversity and not the converse. In their study, the difference between high and low diversity seedlings was approximately 100 grams total biomass. In our study, seedlings were much smaller (Chapter 2) than those in the low diversity treatment of Druebert et al. (2009). Moreover, EM fungi differ in their carbon demands and thus the ability of seedlings to host them (Gibson and Deacon 1990). More investigation is needed to determine the relationship between plant productivity, EM carbon demands, and their ability to access different forms of nutrients. These studies are needed over the full life-span of trees, not just during the establishment phase.

### 4.4.4 Anthropogenic ecosystem changes and context dependency

Wildfires are expected to increase in extent and severity in the interior forests of western North America with climate change (Krawchuk et al. 2009). As noted above, wildfire appears to have a short-lived “fertilization effect” that benefits natural regeneration, but at the same time can simplify mycorrhizal communities. Field fertilization studies showing that increased N
availability is related to reduced mycorrhizal colonization (Treseder 2004). Lab studies have also shown that favourable nutrient availability can reduce EM colonization (Correa et al. 2008, Hobbie et al. 2009). The decline in colonization with increased nutrient availability has been associated with reduced belowground allocation (Lilleskov et al. 2002), but this was not the case in our study. Where EM fungi are nitrogen limited, by contrast, colonization has been shown to increase in response to N additions (Treseder 2004). Overall, we found that shoot:root biomass allocation was similar regardless of colonization levels in the no canopy treatments. Additionally, in 2004, we found that seedlings in the undisturbed forest and clearcut treatments had low belowground investment but very high colonization levels.

More frequent and severe droughts are projected for the interior Douglas-fir forests of Western Canada with climate change (Spittlehouse 2008). Evidence suggests that that EM colonization is greatest at intermediate levels of plant stress (intermediate stress hypothesis) (Zhou et al. 1997, Swaty et al. 2004). We found that low colonization was associated with favourable growth and moisture levels, but poorly performing seedlings growing under drought conditions may also be associated with low colonization (Swaty et al. 2004). Drought can negatively affect the ability of the fungus to provide soil resources due to physiological constraints (Kennedy and Peay 2007). At the same time, low soil moisture can restrict the ability of the host to provide the fungus with carbon (Dosskey et al. 1991). Hence, future drought conditions may shift the status of the fungal and plant symbiosis.

4.5 Conclusion

This study supports our hypothesis that initial investment in EM declines with increasing seedling productivity and nutrient availability in a natural regeneration setting. We found that
seedlings with the highest productivity on the most resource replete sites invested the least in de novo colonization. As seedlings grew from 2004 to 2006, they increased investment in mycorrhizas in correspondence with falling N and P concentrations. Contrary to our expectations, however, we did not find that EM diversity increased as seedlings grew. In low-light environments, increased nutrient availability with low-severity fire disturbance resulted in colonization patterns that more closely matched stand replacing disturbances than undisturbed forests. Here, the effects of high nutrient availability overwhelmed the influences of mature trees observed in undisturbed forests. In the undisturbed forests, we found that low productivity was associated with high levels of EM fungal colonization and diversity, suggesting that the colonization of EM fungi on seedlings benefited from an external carbon source. In summary, we found that seedlings growing in environments enriched by stand replacing disturbance do not suffer from reduced or delayed EM colonization, nor is their growth suppressed where colonized by a uniform EM community dominated by the ascomycete, Wilcoxina. Based on earlier studies in these forests, colonization and diversity are expected to increase as seedling resource demands increase with crown closure (Twieg et al. 2007).
Figure 4.1 PCA ordination of seedling growth responses (averages) and disturbance severity treatments, in September 2004. The blue arrows represent seedling growth responses from the main matrix. The red arrows indicate vectors for the environmental variables and seedling the EM community 2nd matrix ($r^2>0.2$). The direction of the arrow indicates the direction of the strongest correlations while the length indicates the magnitude of the relationship. $n=18$. HSB=high severity burn; LSB=low severity burn; SCC=screefed clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 4.2 Linear regression showing the correlation between shoot biomass and ratio of non-mycorrhizal seedlings to the total number of seedlings in September, 2004. n=18. HSB=high severity burn; LSB=low severity burn; SCC=screeded clear-cut; CC=clear-cut; UF=undisturbed forest.
Figure 4.3 PCA ordination of seedling growth responses (averages) to disturbance severity treatments, from no canopy treatments in September 2004 to 2006. The blue arrows represent seedling growth responses from the main matrix. The red arrows indicate vectors seedling EM community 2nd matrix ($r^2>0.2$). The direction of the arrow indicates the direction of the strongest correlations while the length indicates the magnitude of the relationship. n=18. HSB=high severity burn; SCC=screfed clear-cut; CC=clear-cut.
4.6 References


5 Conclusions

5.1 Research Context

Despite the advent of industrial-scale planting, natural regeneration has remained a dominant pathway in forest renewal. We undertook this study to better understand the potential of major disturbances common in the dry interior of B.C. to affect natural regeneration: wildfire and clearcut harvesting. Interest in using fire as a restoration tool has brought the issue of fire severity to the forefront (Brown et al. 2004). In recent years, interest has developed putting into practice “emulation silviculture” which attempts to recreate historical disturbance regime (Long 2009). In this study, we focused our investigation on how disturbance severity affects natural regeneration potential, with an emphasis on the role of the EM symbiosis on natural regeneration.

5.2 Main research findings

5.2.1 Increasing disturbance severity can affect natural regeneration potential

In general, removal of canopy cover either through wildfire or clear-cutting relieved light limitations to seedling establishment, resulting in improved survival, and growth (Chapter 2) and mycorrhizal colonization (Chapter 3) in the first three growing seasons. In these treatments, 30% of the 2004 germinants survived to the end of the study. Seedlings biomass increased each year and foliar N and P levels were above deficiency levels throughout the study (Chapter 2). In all treatments, the non-mycorrhizal window closed by the start of the second growing season.

The growth trends suggested that increasing disturbance severity by burning or removing the forest floor could lead to improvements in aspects of regeneration potential (Chapter 2). The high severity fire treatment was associated with faster seedling growth that was likely enabled by
increased nutrient availability resulting from the forest floor combustion. However, fire also appeared to create a more heterogeneous seedbed, which was likely due to the presence of ash and higher maximum daytime temperatures. In contrast, screeing in clearcuts resulted in greater and more uniform germination, seedling survival and seedling density. The growth trends in the no-canopy treatments were converging by the end of the study (Chapters 2 and 3).

5.2.2 Plant productivity can affect seedling investment in EM colonization

Initial investment in EM declined with increasing seedling productivity and nutrient availability in regenerating seedlings, resulting in a longer non-mycorrhizal window for the seedlings with the highest growth rates (Chapter 4). Any negative consequences of delaying the obligatory colonization were offset by favourable growing conditions. We found indications that changes in foliar nutrients status may influence the degree of host investment in EM fungi. Full seedling colonization corresponded with falling N and P concentrations. In contrast, EM diversity was not affected by enhanced by increasing in productivity as the open-canopy treatments were colonized by a uniform EM community dominated by the Ascomycete, *Wilcoxina*.

5.2.3 Retention of forest floor can enhance post-disturbance EM diversity

The EM community results indicated that disturbance resistant propagules in the mineral soil provided a basis for the de novo colonization and diversity of seedlings, with the EM community dominated by *Wilcoxina*, which forms spores (Chapter 2). On the whole, the results point toward a role of the forest floor in increasing viable inoculum, with the presence of mature hosts further enhancing diversity. In particular, forest floor retention can increase the abundance of EM species, such as *Cennococcum*, that are associated with organic matter and/or late successional stands.
5.2.4 Douglas-fir regeneration potential can be high under closed canopies

The relative improvement of germination and growth in the low severity burn treatment over the undisturbed provides further impetus to further examine the conditions under which partial canopy retention can facilitate Douglas-fir natural regeneration. In low light environments, increased nutrient availability with low severity fire disturbance resulted in colonization patterns that more closely matched stand-replacing disturbances than undisturbed forests.

5.2.5 Douglas-fir contribution to resilience adaptive capacity

Successful natural regeneration is a key determinant of ecosystem resilience after high-severity disturbances. Resilience is defined as capacity of an ecosystem to absorb change without changing state (Gunderson 2000). In dry interior forests of B.C., a key management objective is to ensure or preserve the ability of ecosystems to recover to a forested state after stand replacing disturbance to prevent shifts to alternate states, such as shrubs or grassland dominated ecosystems. Our study provided evidence that clearcuts and wildfires have comparable abilities to contribute in terms of growth, survival, and mycorrhizal colonization of natural regenerating Douglas-fir.

Species that can germinate after high-severity disturbances, like Douglas-fir, provide adaptive capacity after stand-replacing disturbances. Adaptive capacity is defined as the ability of an ecosystem to return to a stable state after a disturbance (Le Goff et al. 2005). The results of this study clearly demonstrate that Douglas-fir can contribute to the adaptive capacity of an ecosystem after both high-severity and low-severity disturbances, provided that the forest floor is sufficiently disturbed to allow for successful regeneration.
5.3 Study limitations and future directions

5.3.1 Seeds

Perhaps the most important limitation of this study stemmed from our use of experimental seeding, which was done to ensure sufficient seedlings to track over a three-year period. The first and most pressing issue in natural regeneration, of course, is whether sufficient seed fall occurs to facilitate natural regeneration. Notwithstanding localized regeneration problems, research and forest inventory statistics indicate that sufficient seedfall is occurring to facilitate conifer natural regeneration (Natural Resources Canada 2007, Shatford et al. 2007). We took steps to reduce seed predation by the protecting the seedbeds before germination. Seed consumption by small mammals can limit natural regeneration of interior conifers (Stoehr 2000, Huggard and Arsenault 2009). Exposed mineral seedbeds can increase seed predation by small mammals in interior forests (Huggard and Arsenault 2009). Huggard and Arsenault (2009) hypothesized that mast years may crucial factor interior in successful Douglas-fir regeneration because the large volume of seed overwhelms the ability of small mammals to consume the seeds.

5.3.2 Longer-term trade-offs

The forest floor is pivotal in natural regeneration because it effects everything from seed banks to forest productivity to mycorrhizal community dynamics. Though small in terms of overall C pool sizes, the activity, such as decomposition, that takes place in the forest floor make it a key component of forest ecosystems (Tiedemann et al. 2000). Our focus in this study was focused on seedling productivity in response to forest-floor disturbances in a three-year time frame, but there is potential for longer-term (decadal and beyond) trade-offs. Researcher have raised questions about the long-term effects of both wildfire (Tiedemann et al. 2000, Meigs et al. 2009) and mechanical site-preparation techniques (Simard et al. 2003, Hope 2007) on forest
productivity. Concerns have been raised that site-preparation treatments may have negative consequences which outweigh the short-term benefits (Simard et al. 2003). Post-harvest reduction of the forest floor may reduce nutrient storage and availability, but that these changes do not necessarily negatively affect productivity (Hope 2007). Thus long-term reductions in productivity may be minimized if the forest floor recovers quickly enough.

The longer-term effects of fire on productivity is more difficult to assess since the impact of fire in large part depends on the frequency and severity of the fire, with uncertainties about fire effects on ecosystem processes further hindering our ability to predict consequences (Bormann et al. 2008). Even if disturbances promote successful establishment by new seedlings, there is evidence that wildfires of all severity types can reduce productivity by more mature trees (Meigs et al. 2009, Keyser et al. 2010). Positive growth responses observed in the short term (under 3 years) can decline in the longer term, due to factors such as drought, damage to trees, changes in nutrient availability and mycorrhizal community (Tiedemann et al. 2000).

As noted above, changes in mycorrhizal communities has been suggested as contributing factor in productivity declines following wildfire (Tiedemann et al. 2000). Since wildfire alters the factors, such as nutrient availability and plant productivity, that determine EM community dynamics, untangling the role of mycorrhizae is a difficult task under field conditions. If productivity is a primary driver of mycorrhizal diversity (Druebert et al. 2009) then productivity losses related to fire may be more the result of direct damage to the host (reduced photosynthetic capacity e.g.) by fire than by changes in the ability of mycorrhizae to supply nutrients. The results of this study suggest that after disturbances mycorrhizal colonization and diversity does not fall below a minimum threshold to negatively affect plant productivity and seedling regeneration. The same might also apply to mature trees which have less capacity to invest in
mycorrhizae. It should be noted that there is evidence that the effects of EM diversity on plant productivity is context dependent, without outcomes changing with nutrient availability (Jonsson et al. 2001). Therefore, in some post-fire environments, declines in host productivity may be linked to alterations in the ability of the mycorrhhizal community to access nutrients. More research is needed to understand the connections between productivity and EM community dynamics in a broad array of contexts. In a natural regeneration setting, inclusion of other EM hosts commonly found in early successional environments would be necessary to take into account factors that might affect plant investment in EM fungi, such as host specificity.

### 5.3.3 Stochastic factors

Observed patterns in ecosystems are composites of stochastic and mechanistic processes (Pickett et al. 2007). The community dynamics of plants and mycorrhizae are shaped by the interaction between stochastic factors, such as dispersal, and deterministic factors, such as niche partitioning (Tilman 2004). In this study, we treated natural regeneration as a linear mechanistic process with environmental conditions and mycorrhizal colonization hypothesized as major drivers. However, successful natural regeneration requires a sequential alignment of factors, from seed production onwards to establishment, that are affected by processes that display some degree of stochastic behaviour, such as climate and wildfire behaviour. This situation contributes to a sense that conditions that allow for successful natural regeneration are so difficult to meet that it should be a very rare occurrence, but as noted previously, studies confirm that forests naturally regenerate after disturbances when examined over a timeframe of several decades. However, variability associated with natural regeneration and very long regeneration times in drier ecosystems (a century or more) have led some to continue to advocate outplanting (Sessions et al. 2004, Newton et al. 2006). Managers can use the results of this study and
landscape studies to assess the viability of using natural regeneration to meet management objectives (Shatford et al. 2007, Astrup et al. 2008), but predicting future responses of ecosystems to disturbances is still hampered by a lack of long-term data and uncertainties about ecosystem responses (Bormann et al. 2008). One clear trend that emerges from the research: if global climate brings drier conditions to interior forests (as predicted by (Hamann and Wang 2006) then conifer natural regeneration has the potential become an even slower and rarer phenomenon.

5.3.4 Directions for future studies

This study has raised a number of areas that warrant further investigation. Here I mention three possibilities that integrate the three data chapters. First, the reduction of competing vegetation to improve growth and survival is one of the primary rationales given for disturbing the forest floor (Simard et al. 2003). Aerial post-fire seeding with grass species is common in response to wildfires and clearcutting in interior British Columbia to prevent erosion and provide cattle forage (Thompson et al. 2000). We avoided aerial seeded areas in this study, but incorporation of experimental seeding of grasses experiment at varying densities would increase the applicability of this study design. It would also allow for testing how host investment in EM fungi is affected by competition from plants with AM fungi.

Secondly, this study did find evidence that Douglas-fir can establish successfully under closed canopies, but the long-term growth prospective was poor. The experimental seeding approach could be incorporated into variable-retention harvesting and mixed fire severity on the combined effects of seed source, substrate and resource availability on regeneration potential. The field work could also be paired with lab experiments that allow for manipulation of light levels and allow for an accurate C budget for the seedlings using stable isotope labelling.
Finally, in the area of statistical analysis, structural equation modeling (SEM) could be used to help resolve a number of issues that arose during the course of this research. First, SEM can allow interdependencies among sets of correlated variables to better address issues related to cause and effect (Arhonditsis et al. 2006), such as sorting the role of mycorrhizal communities in plant productivity in relation to changes in the soil environment. Secondly, SEM allows for the testing of qualitative concepts that are not directly measured (latent variables) (Grace and Pugesek 1997). This study tested or touched upon latent variables, such as plant investment or resilience, which could be tested with SEM. Finally, SEM also allows for accounting of stochastic factors in assessing post-disturbance diversity patterns (Grace and Kelley 2006). I plan to pursue SEM analysis to address the above mentioned issues with the data contained in Chapter 4.
5.4 References


