Cascading effects of insect outbreak on plant and fungal community structure and function

by

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Abstract

Western North American landscapes are rapidly being transformed by forest die- off caused by mountain pine beetle with implications for plant and soil communities. The mechanisms that drive changes in plant and soil community structure and function, particularly for understory vegetation and the highly prevalent ectomycorrhizal fungi in pine forests, are complex and intertwined. In this thesis, I use a recent bark beetle outbreak in lodgepole pine (*Pinus contorta*) forests of western Canada to disentangle the relative importance of beetle-induced tree mortality from changes in environmental conditions following tree death, and in turn, its effects on: (1) understory plant community diversity and productivity, (2) the richness and composition of soil fungal communities, (3) the spatial structuring of ectomycorrhizal and saprotrophic fungal communities, and (4) the functional importance of ectomycorrhizal fungal networks on tree seedling establishment. My results indicate that both deterministic and stochastic processes structure plant and soil fungal communities following landscape-level insect outbreak and reflect both the independent and shared roles tree mortality, soil chemistry, and spatial distance play in regulating both these communities. My results also demonstrate that ectomycorrhizal fungal networks seemed to not be degraded with stand level tree mortality, with access to these networks improving both the growth and nutrition of tree seedlings. Taken together, this thesis demonstrates the far-reaching effects of biotic disturbance and emphasizes the interconnectedness between understory vegetation, trees, soils, and soil fungi.

Preface

A version of Chapter 2 of this thesis has been published as Pec G.J., J. Karst, A.N. Sywenky, P.W. Cigan, N. Erbilgin, S.W. Simard, and J.F. Cahill, Jr., "Rapid increases in forest understory diversity and productivity following a mountain pine beetle (*Dendroctonus ponderosae*) outbreak in pine forests," *PLoS ONE* 10(4): e0124691. G.J.P., J.K., N.E., S.W.S., and J.F.C. conceived and designed the research. G.J.P., A.N.S., and P.W.C. conducted the fieldwork. G.J.P. analyzed the data. G.J.P. prepared the manuscript. G.J.P., J.K., N.E., S.W.S., and J.F.C. contributed to manuscript edits.

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Chapter 1: General Introduction

Community assembly is driven by many ecological processes occurring across many spatial and temporal scales (Levin 1992, HilleRisLambers 2012). Environmental conditions, dispersal limitation, and competition can all contribute to the structuring of these communities (Morin 2011). Identifying the determinants that structure communities is thus a central theme in community ecology (HilleRisLambers 2012). It has been suggested that a combination of both deterministic and stochastic processes occurring over both spatial and temporal scales are needed for a complete understanding of community assembly and functioning (Gotzenberger et al. 2012, HilleRisLambers 2012). It has also become increasingly clear that above- and belowground communities are intimately linked, where neither can be fully understood without considering the complexity of interactions among them (Wardle 2006). Concepts of community structure have been widely applied to plant communities; however, the ecological mechanisms that govern the structure of belowground communities, in particular fungi, have largely been overlooked due to their cryptic and semi-quiescent nature (Peay et al. 2008). Recent adoptions of molecular tools have greatly reduced this barrier to determine what structures belowground communities (Peay et al. 2008). However, basic questions remain unanswered about the composition and diversity of belowground communities following disturbance (Courty et al. 2010, Stursova et al. 2014), their spatial structuring and turnover across environmental gradients (Pickles et al. 2010), and the relationships with plant community structure (Peay et al. 2013) and functioning (Fellbaum et al. 2014).

The goal of this thesis is to explore the biotic and abiotic factors that influence community assembly of both above- and belowground communities. Specifically, I use the recent outbreak of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) in lodgepole pine forests (*Pinus contorta* Dougl. Ex. Loud.) of west central Alberta as a model system to examine the effects of insect-induced tree mortality on (1) the diversity and productivity of aboveground understory communities, (2) the diversity of the belowground soil fungal communities, (3) the spatial structuring of belowground ectomycorrhizal and saprotrophic fungal communities, and (4) the functioning of belowground fungal networks on tree seedling regeneration. I first introduce the study system with a focus on lodgepole pine dominated forests. I continue with a brief discussion on disturbance and an emphasis on the recent mountain pine beetle outbreak and forest regeneration. I further introduce soil fungi and, in particular, the role of mycorrhizal fungi and fungal networks as an integral part in the functioning of the forest system. I develop the literature for the importance of using spatial scale when investigating the structure and function of communities with an emphasis on belowground communities. Finally, I provide an overview of research objectives for my thesis.

1.1 The Boreal Forest

Over one billion hectares of the northern hemisphere is occupied by boreal forest with roughly one-third located in Canada (Brandt 2009). The boreal forest experiences harsh winters and a short growing season. It has been subjected to several continental glacier advances and retreats. Forest cover has only reestablished in the current boreal regions between 8,000 to 12,000 years ago, making today's boreal forests relatively young (Brandt 2009, Burton 2014). The boreal forest varies greatly in its vegetative composition throughout

its circumpolar range. Although, the boreal forest has the fewest vascular plant species than any other forested system, this system is a complex and dynamic network of components acting directly and indirectly with each other and their environment (Burton 2014). For instance, many boreal plant species have broad distributions, indicating a wide tolerance to changes in seasonal temperatures, precipitation, and preferences for shade, nutrients and soils (Hart and Chen 2008, Burton 2014). In Canada, trees comprise a relatively small portion of the total plant diversity present in these forests, with herbaceous taxa making up the vast majority of the diversity (Hart and Chen 2006). Forests are comprised of either closed-canopy coniferous stands, pure deciduous stands, or interspersed with mixed conifer-deciduous stands leading to high spatiotemporal complexity among these forests (Burton 2014).

In boreal forests, the majority of plant diversity is located in the understory (Hart and Chen 2006). Understories are comprised of an assemblage of vascular plants, mosses and lichen. Understory composition, diversity, and productivity are influenced by a number of environmental factors including: light availability, temperature, soil moisture, and soil nutrient availability (Hart and Chen 2006, Edwards et al. 2015). These factors are driven primarily by overstory tree density and diversity, although factors such as soil type and moisture regime, herbivory and soil disturbance can play a significant role in understory community composition and diversity (Nilsson and Wardle 2005, Perry et al. 2008).

1.1.1 Lodgepole Pine Dominant Forests

Lodgepole pine (*Pinus contorta* Dougl. Ex. Loud.) has a wide ecological range in North America, from the Pacific coast to the Rocky Mountain range and from Alaska to Baja

California (Ying and Liang 1994). Lodgepole pine is present as a dominant species throughout both British Columbia and Alberta where it occurs in both dry or wet areas, in various soil types, and can grow rapidly at a young age successfully competing for light availability and soil resources (Dhar and Hawkins 2011). It is an early-successional, shade-intolerant species that colonizes areas following disturbance such as stand-replacing wildfire. Reproduction of lodgepole pine is cued to fire disturbance, where a proportion of its cones release seeds in the presence of intense levels of heat (Teste et al. 2011). Seedling establishment is also linked to conditions following fire disturbance, particularly to exposed mineral soil, decayed wood and organic material for increased germination success (Nyland 1998, McIntosh and Macdonald 2013). In the absence of disturbance, lodgepole pine is eventually replaced by more shade-tolerant conifer species (Dhar and Hawkins 2011). Similarly, successful infestation of lodgepole pine by insect outbreak can also promote growth of shade-tolerant conifer species, which may lead to a non-pine dominated system (Nigh et al. 2008). In either scenario, tree loss may lead to complex effects on the structure and function of the forest system, both above- and belowground.

1.2 Disturbance

Disturbance has been recognized as strongly influencing the structure and function of various ecosystems, in particular forests (Pickett and White 1985, Rydgren et al. 2004). Most of the attention has focused on the size and frequency of disturbance, with less consideration on severity (Rydgren et al. 2004). Disturbance severity is defined as the amount of forest overstory and understory removed and the amount of forest floor and soil destroyed (Gilliam 2014). Low severity disturbances essentially leave an intact forest with few gaps (e.g. treefall in old-growth forest), whereas moderate to high severity disturbances kill most, if not all, of

the overstory and understory, disrupting and potentially removing the forest floor and the top soil layers (Perry et al. 2008).

The boreal forest of western Canada is considered a disturbance prone system, historically shaped by fire and insect outbreaks (Bergeron and Fenton 2012). These disturbances result in a landscape characterized by both spatial and temporal variability in forest structure and functioning (Axelson et al. 2009, Amoroso et al. 2013). The severity and frequency of forest mortality has increased over the past several decades resulting from decreased winter temperatures and altered precipitation patterns (McKenzie et al. 2004, Volney and Fleming 2007). Changes in the natural disturbance regime (e.g. severity, agent) can dramatically alter aboveground community structure (Sousa 1984) and stability (Halpern 1988), however less is known about the reciprocal effects on belowground community composition and stability (Simard 2009b).

Diversity is thought to increase the stability of the system and the communities within that system (McCann 2000). Maintaining more diverse communities may provide functional redundancy and complementarity (Naeem 1998) under uncertain disturbance events and environmental conditions which might enhance the resistance and resilience of the community (Elmqvist et al. 2003, Perry 2008). Resistance and resilience are two aspects of stability that are most commonly studied (Sousa 1980). Resistance is the degree to which a system can absorb small disturbing forces, preventing them from escalating into larger disturbances (Connell and Sousa 1983). Whereas, resilience is the ability of a system to return to its original conditions, its structure and function, following disturbance (Holling 1973). The stability of a system depends on the characteristics of the disturbance (Perry

2008). However when the disturbance event is beyond the historical extreme, communities might exhibit a positive feedback where the overall system may move toward a new state comprised of different assemblages of species (Sutherland 1981, Elmqvist et al. 2003).

1.2.1 Mountain Pine Beetle Outbreak

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is a native insect of forest systems in western North America. The forests of western North America have co-evolved with the mountain pine beetle disturbance, creating a natural cycle of forest growth and regeneration (Safranyik 2006). However, in the past several decades, insect outbreaks have occurred at greater intensity and in areas not previously subject to occurrence (Dordel et al. 2008, Safranyik et al. 2010, Diskin et al. 2011). Currently, mountain pine beetle has significantly expanded its range in western Canada, invading novel lodgepole pine habitat east of the Rocky Mountains into northwestern Alberta (de la Giroday et al. 2012). Greater than 18 million hectares of lodgepole pine forest has already been attacked in western Canada, with more than 1.3 million hectares in Alberta (Natural Resources Canada 2014). The scale of the current mountain pine beetle outbreak is having cascading effects on the structure and function of the forest system, including changes to the hydrology, carbon storage, and nutrient cycling of the system (Kurz et al. 2008, Coates 2009, Cigan et al. 2015).

Mountain pine beetle differs from other disturbances, such as wildfire or harvesting, by affecting the overstory structure without direct disruption to the forest floor, soil, or understory vegetation (Burton 2008). Large populations of mountain pine beetle can kill host trees through tree girdling and gallery excavation. Host death is accompanied by transmission of a mutualistic fungi (*Ceratocystis* spp.) that disrupts water transport in the tree

(Safranyik 2006). Although the current mountain pine beetle outbreak is unprecedented based on historic records, the effects of such large insect outbreaks on lodgepole pine dominated forest systems is not unnatural (Dhar and Hawkins 2011). Historical evidence of large outbreaks exists and dates back to the 1890s (Wood 1996). Mountain pine beetle outbreaks were historically spatially concentrated with varying levels of attack severity. The severity of the current outbreak may be likely due to recent warming events and changes in precipitation patterns (Wood 1996; Chapman et al. 2012; Chen 2014). However, the historically large outbreaks suggest that insect induced tree mortality is a significant driver of forest structure in lodgepole pine systems of western Canada. The extent of mountain pine beetles' effects on above- and belowground community structure will depend on the severity of the attack and the overstory-understory tree species composition prior to infestation (Chan-McLeod 2006).

1.2.2 Forest Regeneration

The initial response of the understory following disturbance can influence overstory canopy development, future community composition and ecosystem functioning (Nilsson and Wardle 2005, Royo and Carson 2006). Natural regeneration requires successful seed source, suitable seedbed, and microclimatic conditions for germination and establishment (McIntosh and Macdonald 2013). Natural regeneration following a disturbance event can range from immediately following that disturbance up to fifty years post-disturbance with the possibility of establishment failure due to competition with vegetation or unfavorable site conditions (Shatford et al. 2007, Halpern and Lutz 2013). Forest litter can also inhibit seed germination and establishment by either creating a mechanical barrier or through inhibitory allelochemicals (Mallik 2003).

Following disturbance, light levels are generally elevated as a result of overstory tree loss, which subsequently influences rates of nutrient cycling and mineralization in forest soils (Perry et al. 2008, Edburg et al. 2012). Seedling survival post-germination is vulnerable to limited carbohydrate reserves and small root systems (Canham et al. 1999). By ten to fifteen years post-disturbance, many remaining snags (i.e. dead standing trees) fall and start decaying as downed logs on the forest floor. These downed logs can provide a significant and stable source of nutrients as well as suitable regeneration substrates (Greene et al. 1999, Perry et al. 2008). Further, disturbance events that reduce or remove the forest floor can create favorable growing conditions for tree germination by reducing competition from surrounding vegetation, exposing mineral soil for suitable seedbed, and improving soil moisture and temperature conditions (Greene et al. 1999, Herr et al. 1999).

Advanced regeneration (i.e. secondary structure, residual vegetation) is also an important component of post-disturbance forest development and trajectory (Dhar and Hawkins 2011, Halpern and Lutz 2013). Advanced regeneration can accelerate the recovery of disturbed areas by functioning as refugia in the recolonization of the understory, forest floor, and soil fungal communities (Turner et al. 1997, Hagerman et al. 2001). Compensatory responses of advanced regeneration can also account for nutrients retained in forests following disturbance (Rhoades et al. 2013). However, overstory tree loss can place stress on advanced regeneration and raise overall natural regeneration failure by increasing the potential for frost injury and creating anaerobic soil conditions through changes in hydrological cycling (Ruel et al. 2000, Rhoades et al. 2013).

Furthermore, the regeneration of host species and survival of advanced regeneration is a major determinant of soil fungal community composition and diversity (Smith 2008, Fichtner et al. 2014, Lewandowski et al. 2015). The aboveground community can significantly alter not only the composition but also the functioning of the belowground community through changes in water use, litter chemistry, and changes to soil properties (Birkhofer et al. 2012, Wu et al. 2012). In turn, the recovery of the belowground communities to pre-disturbance conditions will have a significant role on the functioning of the system through processes such as decomposition and nutrient cycling (Fichtner et al. 2014) as well as providing aid for regeneration of host species (Simard and Durall 2004, Simard et al. 2012).

1.3 Soil Fungi

The diversity of soil fungi is immense, projected at over 1.5 million species (David and Gregory 2005). Fungi play pivotal roles in many ecological processes such as in the decomposition and cycling of organic matter as well as in the acquisition of mineral nutrients (David and Gregory 2005, Smith 2008, Clemmensen et al. 2013). Fungi are heterotrophs, requiring external sources of carbon for energy. They acquire carbon via different strategies: as saprotrophs, necrotrophs, and biotrophs (Smith 2008). As saprotrophs, they control the rates at which organic matter is returned in inorganic nutrient form for plant uptake. As necrotrophs, they cause mortality and affect system turnover and as biotrophs can provide nutrients to their hosts (David and Gregory 2005, Smith 2008). Fungi are thus intimately involved with energy acquisition and distribution, playing an essential role in various aspects of forest system development, stability and function (Smith 2008, Simard 2009a, Courty et al. 2010, Clemmensen et al. 2013).

1.3.1 Mycorrhizal fungi

Of the three carbon acquiring strategies, biotrophs often form mutualistic or symbiotic associations, which include mycorrhizal fungi (Smith 2008, Lukac 2011). Mycorrhizas are symbiotic relationships between fungi and plant roots. These associations have evolved independently several times within fungal and plant lineages (Brundrett 2002). Mycorrhizal fungi thus have an array of morphological, physiological and ecological traits that range from mutualistic to parasitic (Neuhauser and Fargione 2004, Karst et al. 2008, Smith 2008). In general, mycorrhizal fungi enhance the uptake of soil nutrients (i.e. nitrogen and phosphorus) and water for their host plant in exchange for carbon (Smith 2008). Besides the nutrient relations, there is increased evidence that mycorrhizal fungi can protect host plant roots against soil pathogens (Morin et al. 1999), improve soil aggregate stability (Smith 2008), protect against heavy metal toxicity (Jones and Hutchinson 1988), and increase weathering of soil minerals (van Breemen et al. 2000). Mycorrhizal fungi are also an important component of the overall diversity and biomass in soil microbial communities (David and Gregory 2005, Baldrian et al. 2013).

There are several major types of mycorrhizal fungi, of which the most ubiquitous to the majority of North American tree species are ectomycorrhizal fungi (Molina and Trappe 1982, David and Gregory 2005). Ectomycorrhizal fungi are predominantly from the Basidiomycota and Ascomycota (Smith 2008) forming associations with multiples host species (Nara 2006). Ectomycorrhizal fungi vary in their abundance, spatial and temporal distribution, and function within soils (Lilleskov et al. 2004, Izzo et al. 2005, Koide et al. 2005, Pickles et al. 2012). Ectomycorrhizal fungi differ in their functional contributions for carbon demand (Bidartondo et al. 2001), nutrient uptake (Read and Perez-Moreno 2003), and

drought tolerance (Parke et al. 1983) as well as in their sensitivity to changes in nitrogen and phosphorus (Treseder 2004). Ectomycorrhizal fungi not only influence the growth of their host plants but also are of particular benefit to the establishment of seedlings (Nara and Hogetsu 2004, Nara 2006, Booth and Hoeksema 2009). Although some conifer species have been shown to grow without ectomycorrhizal fungi during their first year of growth (Christy et al. 1982), colonization by ectomycorrhizal fungi is essential for overcoming nutrient and water limitations and overall seedling survival (Parke et al. 1983, Teste et al. 2009, Courty et al. 2010).

1.3.2 Mycorrhizal networks

Mycorrhizal networks are fungal hyphae that connect roots of the same or different host species (Selosse et al. 2006). These hyphal networks function as bridges for carbon, nutrients, and water sharing among host species and sources of fungal inoculum for seedlings (Simard and Durall 2004, Simard et al. 2012). Mycorrhizal networks have been shown to facilitate establishment of seedlings, particularly when resources or fungal propagules were deficient in soils (McGuire 2007, Teste and Simard 2008). Evidence also suggests that mycorrhizal networks have the potential to mitigate effects of overstory competition on seedling regeneration (Booth and Hoeksema 2009). For example, in interior Douglas-fir forests of southern British Columbia, mycorrhizal networks appeared to facilitate natural regeneration of seedlings by the transfer of nitrogen from mature trees (Teste et al. 2009).

However, little is known about how these existing links between host species and their fungal partners may be vulnerable to biotic and abiotic stresses associated with disturbance (Simard 2009b). Mature stands may persist, to some degree, following

disturbance (e.g. insect outbreak), but dramatic changes in seedling regeneration may take place due to newly created microclimates or competition from advanced regeneration and recently established understory vegetation. This can also be accelerated by a shift in the fungal community composition following disturbance, where a loss of fungal propagules may lead to decreased or delayed seedling recruitment or overall seedling regeneration failure (Simard 2009a).

1.4 Spatial Scale

Over the next several decades, a greater portion of western Canada's boreal forest will be threatened by increased disturbances, such as fire and insect outbreaks (Williamson 2009). Recent evidence suggests that a better understanding of spatial scale is important when investigating the structure of communities (Turnbull et al. 2007) and overall ecosystem function (Maestre et al. 2005) in relation to disturbance events (Seidl et al. 2014). The structure of a community is inevitably linked to its function, such that the composition and diversity of species within a community can affect certain ecosystem components such as the resistance and resilience to disturbance (Cardinale et al. 2006).

In particular, it is critical to identify these structure-function relationships for belowground fungal communities as they are vital to system processes such as (1) nutrient cycling and (2) and can directly affect aboveground community structure through pathogenic or mutualistic interactions (Strickland et al. 2009). Understanding fungal systems requires incorporating a community-environment-function approach at multiple spatial scales (Talbot et al. 2014). Certain processes are of particular importance, but only at a particular scale. A number of biotic and abiotic factors could influence the composition and diversity of fungal

communities at larger, landscape scales. For example, the type of plant community can result in different distributions of fungi across sites (Peay et al. 2013). Large-scale disturbances also play a role across landscapes where ectomycorrhizal fungal composition shifts and diversity decreases following fire (Barker et al. 2013) or logging activities (Jones et al. 2003).

Nested within the landscape scale is variation within plant communities. The local diversity of plant communities may thus influence the composition of the fungal communities. For example, a number of studies have shown host-specificity and host-preference for different plant species (Molina and Trappe 1982, Bever et al. 1996, Massicotte et al. 1999). As the structure of the aboveground community changes, the variation in aboveground distribution could lead to belowground fungal structural changes (Pickles et al. 2010, Pickles et al. 2012, Peay et al. 2013). Further, abiotic conditions such as temperature, soil moisture, pH, and soil nutrients have revealed, positive or negative, changes in the composition and diversity of fungal communities (Egerton-Warburton and Jumpponen 2005, Toljander et al. 2006, Rincón et al. 2014, Treseder et al. 2014). Finally, at the scale of the root systems of individual plants, there can also be spatial variation in the composition of fungal communities (Kennedy et al. 2007, Pickles et al. 2012). This can occur in the rhizosphere (where spores and hyphae extend from roots in the soil) or where fungi colonize the roots of host plants (Anderson et al. 2013). It could be expected that a major driver of spatial distribution at individual roots would be the co-occurrence within and among fungal species; interactions that are either competitive and/or facultative between species (Kennedy 2010, Bever et al. 2012). Thus understanding the importance of spatial scale when investigating the role of fungi in ecological systems is necessary for proper evaluation of

their roles in the functioning of the ecosystem. How this structure-function relationship changes in response to disturbance, particularly insect outbreak, and its subsequent feedbacks on forest regeneration and recovery is unclear.

1.5 Overview of Thesis

This thesis explores the linkages among overstory tree mortality, understory vegetation, soil fungi, and tree seedling regeneration; allowing me to disentangle the complexity of biotic and abiotic interactions that are occurring at various spatial and functional scales as a function of biotic disturbance (i.e. insect outbreak), and in turn, its effect on: (1) plant and fungal community structure, and (2) the functional importance of fungal networks in forest regeneration (Fig. 1.1). In Chapter 2, I investigate the immediate effects of overstory tree mortality caused by mountain pine beetle on the diversity and productivity of understory plant communities. In Chapter 3, I characterize the structure of soil fungal communities across a gradient of mountain pine beetle-induced tree mortality using next-generation DNA sequencing. I also determine the effects of environmental factors (i.e., soil nutrients, moisture, and phenolics) and geographical location, both of which can influence the structure of soil fungi. In Chapter 4, I investigate what environmental factors determine the spatial structure of ectomycorrhizal and saprotrophic fungal communities and how these spatial patterns may vary following a large-scale biotic disturbance. In Chapter 5, I experimentally test the importance of access to ectomycorrhizal fungal networks for the establishment (survival, growth, and nutrition) of lodgepole pine and white spruce seedlings along a gradient of beetle-induced tree mortality. Finally, in Chapter 6, I end my thesis with a general discussion and conclusion of my research.

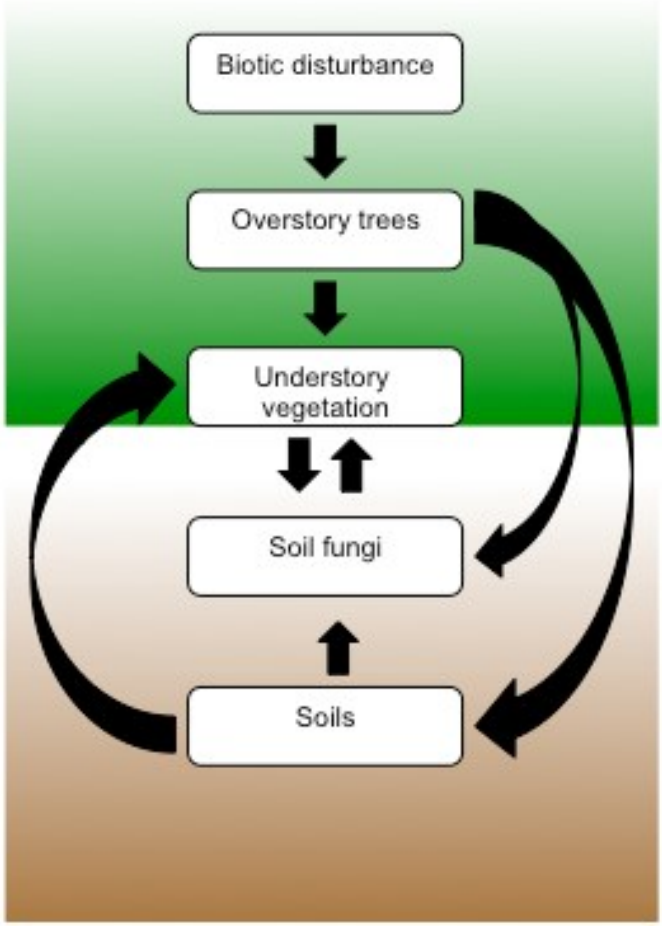

Figure 1.1. Conceptual diagram of the linkages among overstory trees, understory vegetation, soil fungi, and soils following biotic disturbance.

Chapter 2: Rapid increases in forest understory diversity and productivity following a mountain beetle (*Dendroctonus ponderosae*) outbreak in pine forests

2.1 Introduction

Over the past century, forests in North America have experienced increased disturbances from insect outbreaks, wildfire, and harvesting (Turner 2010; Gauthier et al. 2015; Trumbore et al. 2015; Johnstone et al. 2016; McKenzie and Littell 2016). Disturbances, such as wildfire and harvesting, affect resource availability, remove dominant tree species from the landscape and can increase vegetative heterogeneity in the forest system (Chipman and Johnson 2002, Hart and Chen 2006). Though wildfire has historically been the primary agent of sudden tree mortality in western boreal forests (Greene et al. 1999, Chipman and Johnson 2002) insect outbreaks have increased both in frequency and spatial scale (Collins et al. 2011, Kayes and Tinker 2012, Amoroso et al. 2013). Of the current outbreaks, that resulting from the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) has emerged as the largest recorded in western Canada (Axelson et al. 2009, Safranyik et al. 2010). *Dendroctonus ponderosae* plays an important role in maintaining structural diversity in forest ecosystems (Dordel et al. 2008, Diskin et al. 2011). It typically attacks stressed trees (Amman 1977, Safranyik et al. 2010) when beetle populations are low; however, under epidemic conditions mass mortality of healthy overstory trees frequently occurs (Kayes and Tinker 2012). Unlike wildfire and harvesting, *D. ponderosae* leaves the dead overstory and residual understory vegetation intact (Burton 2008, Edburg et al. 2012).

Though there exists information on changes to overstory structure following infestation by *D. ponderosae* (Astrup et al. 2008, Dordel et al. 2008, Coates 2009), response

of understory plant species has received less attention. In particular we lack information on immediate responses of understories to *D. ponderosae*-induced tree mortality (but see (Kovacic et al. 1985, Stone and Wolfe 1996)). This information is important for two reasons. First, understory plant communities can act as a strong filter on tree seedling regeneration and future forest trajectories by modifying microclimatic conditions such as light availability and soil moisture status (Stuart et al. 1989, Cater and Chapin 2000, Royo and Carson 2006). Non-tree understory plants may also compete with tree seedlings for resources (Putz and Canham 1992, Messier 1993). Second, compensatory responses by residual understory vegetation are likely to be an important component for predicting resources available following *D. ponderosae*-induced tree mortality (Rhoades et al. 2013). For example, compensatory responses accounted for about half of the nitrate retained in forests by surviving residual vegetation following *D. ponderosae* outbreak in lodgepole pine forests in Colorado (Rhoades et al. 2013). Productivity in residual vegetation will be important to mitigate changes in soil nutrients resulting from post-mountain pine beetle harvest practices aimed at stimulating tree seedling regeneration (Griffin et al. 2013) and decreasing nitrate release from watersheds (Rhoades et al. 2013).

The objective of this study was to investigate the immediate effects of tree mortality caused by *D. ponderosae* on: (a) the overall understory plant community and (b) herbaceous and woody perennials individually. Specifically, the following three questions were addressed: (1) Is there a relationship between aboveground understory biomass and tree mortality? (2) Can increases in tree mortality explain changes in species richness and evenness? (3) Do changes in resource availability result in increased productivity and diversity?

2.2 Methods

2.2.1 Study Area

The study area is approximately 70 km south of Grande Prairie within the Lower Foothills natural subregion of west central Alberta (54°39'N, 118°59'W; 950-1150 m) (Beckingham 1996). Soils are classified as Orthic Gray Luvisols that are moderately well drained over glacial till. Forests are dominated by mature, even-aged *Pinus contorta* Douglas ex Loudon interspersed with *Picea glauca* (Moench) Voss, *Abies balsamea* (L.) Mill., *Picea mariana* Mill. Britton, Sterns, & Poggenb., *Betula papyrifera* Marshall and *Populus tremuloides* Michx. in the subcanopy. We located eleven sites within a 625-km^2 region of recent *D. ponderosae* activity (since 2009) that bordered provincial permanent sampling plots (see Treu et al. (2014) for details on site selection and stand composition and Randall et al. (2014) for details on site locations).

2.2.2 Plant Survey

In May 2012, we used a 1600-m^2 (40 m x 40 m) area at each of the eleven sites to establish ten evenly distributed 9 m x 9 m plots for a total of 110 plots (Appendix 1.1). In June 2012, we recorded species identity, diameter at breast height (≥ 1.37 m), and health status of all mature trees and saplings within each plot. Attack by *D. ponderosae* on mature trees was confirmed by the presence of pitch tubes, boring dust, exit holes, and subcortical galleries (Safranyik 2006). We revisited these trees in June 2013 to document current beetle-induced tree mortality. Basal area by species was calculated for each plot and tree mortality was calculated as beetle-killed *P. contorta* basal area over total basal area expressed as a percentage. We established a 1 m x 1 m subplot in a random cardinal direction next to the

center of each 9 m x 9 m plot across all 110 plots in May 2012 (Appendix 1.1). During three sampling periods (June, July, August) in 2012 and 2013, we identified individual herbaceous and woody perennials by species within all subplots (see Appendix 1.2 for detailed list). To determine biomass, we harvested all aboveground parts of the understory vegetation by species from each subplot in August 2012 and 2013. Harvested plants were dried at 70 °C for 48 hours, weighed, and pooled per individual subplot. To account for any direct effects of the 2012 harvest on understory productivity and sampling in 2013, a 1 m x 1 m subplot was established and sampled adjacent to the original subplot in May 2013.

During the three sampling periods, in 2012 and 2013, all subplots were assessed for resources likely limiting plant growth in these forests, i.e., light, water and nutrients (Barbier et al. 2008, Hart and Chen 2008). Light transmission readings were taken within two hours of solar noon on overcast days using a linear photosynthetic active radiation (PAR) sensor (Decagon Devices Inc., Pullman, WA, USA) above the shrub layer (~1 m). Light transmission was recorded as the ratio of PAR above the shrub layer (L_1) compared with PAR above the forest canopy (L_2) transformed into % PAR by the following equation: % PAR = (L_1/ L_2) x 100. Using a Theta Probe soil moisture sensor (Delta-T Devices, Cambridge, UK), volumetric soil moisture content was measured at the four cardinal directions in each subplot from the upper 10 cm of the soil column and pooled. Soils were sampled for macronutrients using Plant Root Simulator (PRS^{TM}) probes (Western Ag Innovations, Inc., SK, Canada). In June 2012 and 2013, PRS probes were inserted 10 cm into mineral soils of the A-horizon at a sampling intensity of four cation and anion probe pairs per subplot. PRS probes were removed from the soil in August 2012 and 2013, cleaned, and shipped to Western Ag Laboratories for macronutrient analysis. Soil nitrogen concentrations

were determined using an autoanalyzer, while potassium, calcium, magnesium, phosphorus, and sulfur concentrations of soils were measured by inductive-coupled plasma spectrometry.

Air temperature and relative humidity at the forest floor were measured across all sites and precipitation across the study area was taken during the three sampling periods in 2012 and 2013. In May 2012, air temperature and relative humidity sensors (HOBO U23 Pro V2 Temperature/Relative, Onset Computer Corp., Bourne, MA, USA) were housed in PVC pipe and mounted to the forest floor across seven random locations at a minimum distance of 7 m apart within each of our eleven sites. Readings were taken at thirty minute intervals from June through August 2012 and 2013. Precipitation data was taken at daily intervals for June through August 2012 and 2013; data was obtained from the Pinto Lookout meteorological station (54°78'N, 119°39'W) located within ~30 km of field sites in west central Alberta. Data was provided by the Alberta Agriculture and Rural Development, AgroClimatic Information Service (ACIS) http://agriculture.alberta.ca/acis/ (data retrieved, May 2014). Data from this study are made available at Dryad (doi: 10.5061/dryad.g23f6).

2.2.3 Data Analysis

Understory species richness was calculated at the community and functional group level for each subplot. At the functional group level, we categorized species according to growth form (herbaceous and woody perennials) and calculated species richness as the number of species in a functional group. Evenness was also calculated at both the community and functional group level as: $J = H'/\log(S)$, where H' is the Shannon diversity index and S is the total number of species. Individual species biomass was used to determine the Shannon diversity index.

We used linear mixed effects models to test for variation in productivity, species richness, and evenness across the gradient of *D. ponderosae*-induced tree mortality at both the community and functional group level for each year separately. Linear mixed effects models were also used to determine if variation in resource availability were associated with increased productivity and diversity of understory vegetation at both the community and functional group level for each year separately. Tree mortality, light, soil moisture and nutrients were included as fixed factors. Site was used as a random factor to account for potential spatial autocorrelation due to the clustering of subplots into sites. Since macronutrients were highly correlated to one another ($r = 0.92$), a principal components analysis (PCA) using a variance-covariance matrix (McCune and Grace 2002) was conducted on the following macronutrients: nitrogen, potassium, calcium, magnesium, phosphorus, and sulfur. PCA axis 1 scores were used for nutrients on all subsequent analyses. All variables included had low levels of colinearity (i.e. $r < 0.50$). All models were analyzed using the R-package *nlme* (Pinheiro et al. 2012). All model assumptions were checked by visual inspection of residual patterns (Zuur et al. 2009) .

Using these linear mixed effects models, we developed candidate models that included all combinations of the explanatory variables and their interactions (i.e., *D. ponderosae*-induced tree mortality, soil moisture, light, and nutrients) and used information-criteria to rank the relative importance of those variables in the models. We used the dredge function in the R-package *MuMln* (Barton 2009) for model selection, recording and ranking all statistical models using Akaike's Information Criterion corrected for small sample sizes (AIC_C). We also calculated an Akaike weight (w_i) for each model (Burnham and Anderson 2002). We then defined a 95% confidence set of models for inference, summing w_i from best

to worst model until the sum of w_i exceeded 95%. Models not meeting a 95% confidence set were excluded. When there was no clear parsimonious model (w_i<0.90), we used the model.avg function in the R-package *MuMln* (Barton 2009) for model averaging to determine the direction and magnitude of the effect of each explanatory variable (i.e., *D. ponderosae*-induced tree mortality, soil moisture, light, and nutrients) (Burnham and Anderson 2002). Instead of relying on the estimates of the best candidate model, we computed a weighted average of the estimate for a given parameter based on the Akaike weights. We further calculated the unconditional standard error (or precision; SE not restricted to the single "best" model) of the model-averaged estimate. We also computed the relative importance of the fixed factors by summing the w_i of the models that contained each factor. When an explanatory variable was not strongly ranked (Σw_i<0.50), we considered it important when the associated 95% confidence interval of the model-average estimate did not overlap with zero (i.e., there was an effect). All data analyses were run using R 3.0.1 (Team 2013).

2.3 Results

All environmental conditions varied between years. Air temperature at the forest floor in 2012 (mean=14.07 ± 0.009 °C (SE)) was higher than in 2013 (12.99 ± 0.009 °C) (t=83.64, P<0.0001). Relative humidity measured at the forest floor was lower in 2012 (84.6 ± 0.03%) than in 2013 (90.92 ± 0.02%) (t=159.3, P<0.0001). Precipitation across the study area was greater in 2013 (2.64 ± 0.32 mm) than in 2012 (1.84 ± 0.44 mm) (GLM; z=3.01, P=0.003). These results show that 2012 was a warmer and drier year than 2013.

Model selection resulted in two models for 2012 and four models for 2013 used for inference on the factors underlying variation in total aboveground biomass (Table 2.1). Soil moisture and tree mortality were found to be the most important in explaining total aboveground biomass of the understory plants in 2012 (Table 2.1 and 2.2; Fig. 2.1). Total aboveground biomass increased with *D. ponderosae*-induced tree mortality and greater soil moisture levels in 2012, but not in 2013 (Appendix 1.3). The ranked relative importance was low for tree mortality ($\Sigma w_i = 0.07$) and soil moisture ($\Sigma w_i = 0.17$) with model-averaged coefficients for tree mortality and soil moisture also being small and uncertain in explaining total aboveground biomass in 2013 (Table 2.2; Fig. 2.1). The effects of soil nutrients were found to be a strong predictor of total aboveground biomass only in 2013 (Table 2.2; Fig. 2.1; Appendix 1.3). Ranked relative importance for light levels above the understory (but below the canopy) in explaining understory productivity along the *D. ponderosae*-induced tree mortality gradient were low in 2012 ($\Sigma w_i = 0.08$) and 2013 ($\Sigma w_i = 0.26$) with the model-averaged coefficient being small and showing no effect on understory productivity (Table 2.1 and 2.2; Fig. 2.1).

Model selection resulted in six models for 2012 and two models for 2013 used for inference on the factors underlying richness of the understory community. Tree mortality ($\Sigma w_i = 0.94$) was found to be an important predictor of understory community richness only in 2013 (Table 2.1 and 2.2; Fig. 2.2; Appendix 1.4). Soil moisture (2012; $\Sigma w_i = 0.56$, 2013; $\Sigma w_i = 1.00$) and soil nutrients (2012; $\Sigma w_i = 0.67$, 2013; $\Sigma w_i = 1.00$) were most important in both years (Table 2.1 and 2.2, Fig. 2.2). Understory community richness increased with soil moisture and soil nutrients in both years, and with tree mortality only in 2013 (Appendix 1.4). Light availability was relatively unimportant to either understory community richness or

evenness (Table 2.1 and 2.2; Fig. 2.2). In addition, tree mortality (2012; $\Sigma w_i = 0.05$, 2013; $\Sigma w_i = 0.04$), soil moisture (2012; $\Sigma w_i = 0.03$, 2013; $\Sigma w_i = 0.01$), and nutrient levels (2012; $\Sigma w_i = 0.01$, 2013; $\Sigma w_i = 0.01$) were not related to understory community evenness (Table 2.1 and 2.2).

Underlying the effects of *D. ponderosae*-induced tree mortality on the understory community as a whole, were pronounced differences in responses among herbaceous and woody perennials. Model selection resulted in five models for 2012 and one model for 2013 for inference on the factors underlying richness of herbaceous perennials. Four models for both 2012 and 2013 resulted for model selection on the factors underlying richness of woody perennials. Richness of herbaceous perennials was best explained by soil nutrients in 2012 (evidence ratio = 1.00; $\Sigma w_i = 0.71$) and 2013 (evidence ratio = 1.00; $\Sigma w_i = 1.00$) (Table 2.3 and 2.4), while tree mortality and soil moisture were found to be predictors of richness of herbaceous species in 2013, but not in 2012 (Table 2.3 and 2.4). Richness of herbaceous perennials increased with soil nutrients in both years and with increased *D. ponderosae*-induced tree mortality and soil moisture levels in 2013 (Appendix 1.5). In contrast, richness of woody species was not strongly linked to increased tree mortality in both years (2012; $\Sigma w_i = 0.25$, 2013; $\Sigma w_i = 0.43$) (Table 2.3 and 2.4; Appendix 1.5). Soil moisture, soil nutrients and light were also relatively unimportant ($\Sigma w_i < 0.50$) as predictors of the richness of woody species in either year (Table 2.3 and 2.4) with model-average coefficients being small and showing no effect on the richness of woody species (Fig. 2.3).

Model selection resulted in five models for 2012 and two models for 2013 for inference on the factors underlying evenness of herbaceous species, while model selection

resulted in two models for both years on the factors underlying evenness of woody species. Tree mortality was the most important predictor of evenness in herbaceous perennials in 2013 (evidence ratio = 1.00; $\Sigma w_i = 0.99$), but not 2012 (evidence ratio = 89.0; $\Sigma w_i = 0.01$) (Table 2.5 and 2.6). In contrast, evenness of woody perennials was not strongly linked to tree mortality across years (2012; $\Sigma w_i = 0.02$, 2013; $\Sigma w_i = 0.21$) (Table 2.5 and 2.6), while both the evenness of herbaceous and woody perennials were unresponsive to levels of light ($\Sigma w_i < 0.10$) and soil resource availability ($\Sigma w_i < 0.05$) (Table 2.5 and 2.6).

2.4 Discussion

Within four years following beetle outbreak, productivity of understory plant communities increased along a gradient of beetle-induced tree mortality. Richness and evenness of herbaceous species also increased along the gradient of tree mortality while woody species richness and evenness did not. Our results reveal the complex drivers of understory diversity and productivity in which the magnitude of these shifts depended on the severity of beetle attack. Insect induced tree mortality is a dynamic process in which site conditions change as a function of tree death and time since infestation. The response of soils, for example, is not static with soil moisture and nutrient concentrations shifting over time (Cigan et al. 2015). Further, inter-annual changes in precipitation and temperature will have an effect on understory plants. In our study, understory diversity and productivity were also contingent upon current year microclimatic conditions. Below we discuss the relative importance of these findings.

2.4.1 Effects of tree mortality and resource availability on understory plant communities

In our study, understory species responded to immediate changes in increased soil moisture and soil nutrients with *D. ponderosae*-induced tree mortality; aboveground biomass nearly doubled across the attack gradient, while the diversity of herbaceous species increased with the rapid availability of soil nutrients and nearly doubled in the following year as soil moisture levels rose. Previous studies testing the effects of *D. ponderosae*-induced tree mortality on understory diversity and productivity have shown similar results. For instance, Kovacic et al. (1985) reported that understory biomass increased by 50 % five years following attack by *D. ponderosae* in ponderosa pine (*Pinus ponderosa*) stands in eastern Colorado. Herbaceous species richness increased in attacked stands while increases in soil moisture increased plant biomass production. Likewise, Stone and Wolfe (1996) examined the response of understory vegetation to increased *D. ponderosae*-induced tree mortality in lodgepole pine stands of northern Utah. They found that understory diversity peaked at the highest levels of tree mortality, while understory biomass increased (>100 g m^{-2}) exponentially in stands ranging from 14 to 95% tree mortality. However, another study of *D. ponderosae*-induced tree mortality in Colorado found no difference in understory vegetative cover during the first three years of post outbreak (Klutsch et al. 2009).

In addition to soil moisture and nutrients, light is also considered another major limiting factor in understory plant communities (Barbier et al. 2008, Hart and Chen 2008). Most foliage, twigs and branches fall from attacked trees within the first eight to ten years of beetle attacks (Mitchell and Preisler 1998, Lewis and Thompson 2011), with associated increases in light availability to the forest floor (Axelson et al. 2009). In our study, light levels were similar across the *D. ponderosae*-induced tree mortality gradient (Linear Mixed

Model; t=0.688, P=0.492) and subsequently did not explain changes in understory diversity or productivity along this gradient. Across our sites, dead lodgepole pine trees remain in the overstory, many with needles, twigs and branches intact even in the most heavily attacked stands. In addition, advanced regeneration of shade-intolerant woody tree species (*A. balsamea, P. glauca,* and *P. mariana)* in the subcanopy established prior to the beetle outbreak provides shade that is partially characteristic of an intact overstory. For example, Ehrenfeld (1980) investigated understory species composition seven years after gypsy moth (*Lymantria dispar (L.)*) infestation in a mature oak forest in eastern North America and found no differences between understory species in open gap and closed gap sites following disturbance. The study further suggested that the response of understory species to insect outbreak was dependent on the relative densities of the subcanopy (Ehrenfeld 1980).

2.4.2 Mountain pine beetle outbreaks versus fire and harvesting

In our study, herbaceous richness increased one-fold while herbaceous perennials became more evenly distributed across the gradient of tree mortality, but only after soil moisture and nutrients increased across years. Increases in understory species richness and productivity seen here are similar to observations during the first three years post-fire of forest understory vegetation in mixed conifer forests of northwestern Arizona (Huisinga et al. 2005) and in *P. ponderosa-Pseudotsuga menziesii* forests of central Colorado (Fornwalt et al. 2014). However, the possible mechanisms underlying each disturbance agent are quite different. Changes in resource availability and growing conditions for residual vegetation shortly after *D. ponderosae*-induced tree mortality is not as severe as those observed after stand-replacing fires which often eliminate most of the existing understory vegetation (Wang and Kemball 2005, Hart and Chen 2008) allowing for increases in light availability, soil

moisture, soil nutrients and favorable seedbed conditions for regenerating shade-intolerant tree species such as lodgepole pine (Chen and Popadiouk 2002, Axelson et al. 2009). In contrast, relatively slow changes in beetle-killed stands may prevent colonization by early-successional species and reduce tree seedling recruitment (Axelson et al. 2009, McIntosh and Macdonald 2013).

Similarly, studies on forest harvesting also find support for increases in understory richness and productivity following removal of overstory (Haeussler and Bergeron 2004, Hart and Chen 2008). Increases in understory diversity in many sites are driven by increases in herbaceous species due to reduced disturbance to the organic layer and greater light availability reaching the forest floor (Bradbury 2004, Hart and Chen 2008). Understory productivity can remain high following logging due to the presence of many residual late-successional herbaceous and woody species (Bock and Van Rees 2002). In our study, we found that understory diversity of herbaceous perennials increased and understory biomass nearly doubled across the gradient of *D. ponderosae*-induced tree mortality driven by initial increases in soil nutrients followed by increased soil moisture in the following year. Not surprisingly, richness and evenness of woody perennials did not change across the tree mortality gradient. Across our sites, many well established residual species (e.g. *Linnaea borealis, Vaccinium* sp.) are shade-tolerant, late-successional, and may be physiologically limited to rapidly increase growth rates rapidly in response to increased resource availability (Hart and Chen 2006).

Griffin et al. (2013) reported a decrease in residual herbaceous cover and advanced regeneration following post-*D. ponderosae* salvage harvest in lodgepole pine forests of

northeastern Wyoming. In addition, soil nitrogen availability increased following harvest. This increase in nutrients may have been due to a decrease in herbaceous cover from these older harvested stands (Griffin et al. 2013). This is contrary to our study in which understory productivity, particularly of herbaceous perennials, increased due to an increase in soil nutrients following *D. ponderosae*-induced tree mortality. In general, immediately following disturbance, there is potential for rapid loss of nutrients stored in soils due to decreased rates of biomass uptake (Vitousek 1975). However, the continued gain in understory productivity might allow the forest system to retain rather than lose nutrients following *D. ponderosae*-induced tree mortality.

2.5 Conclusion

Understory vegetation showed an immediate response to *D. ponderosae*-induced tree mortality. The increase in abundance of herbaceous perennials may be attributed to a potential release from belowground competition of beetle-killed trees, similar to stand-replacing fires and harvesting. The decrease in belowground competition from the surrounding neighborhood could have allowed for rapid growth and a strong pulse of recruitment for some herbaceous species following disturbance. Similar observations of an increased response in advanced tree regeneration following *D. ponderosae*-induced tree mortality have been reported (Romme et al. 1986, Amoroso et al. 2013) and are worth further investigation for understory vegetative establishment and dispersal strategies. Understory diversity and productivity also increased as a result of increased soil moisture and nutrients, which were contingent upon current year growing conditions. Between years there was a lack of effect from light although there was a constant effect of soil moisture on forest understory vegetation. When soil nutrients became more abundant, soil moisture became increasingly

important for continued and rapid increase in understory diversity. Further, our findings suggest that in sites with increased vegetation following *D. ponderosae*-induced tree mortality, tree seedling recruitment and forest recovery may be delayed. Measuring the pulse of tree seedling recruitment and possible continued increase and nutrient retention in residual understory vegetation will be important next steps toward assessing future trajectories of forest structure and composition.

Table 2.1. Candidate models used for inference on the productivity, richness and evenness of understory plant community responses to Dendroctonus ponderosae-induced tree mortality, light, soil moisture, and nutrients.

Productivity

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	Soil moisture	478.2	0.00	0.56	1.00
2	Tree mortality, soil moisture	478.6	0.48	0.44	1.27

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	Nutrients	546.3	0.00	0.40	1.00
2	Light	546.9	0.62	0.29	1.37
3	Null model (intercept only)	547.8	1.57	0.18	2.22
4	Soil moisture, nutrients, light	549.7	3.43	0.07	5.71

Richness

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	Soil moisture, nutrients	443.7	0.00	0.35	1.00
2	Nutrients	444.4	0.66	0.25	1.40
3	Soil moisture	445.5	1.79	0.14	2.50
4	Light	445.6	1.87	0.14	2.50
5	Tree mortality, soil moisture, nutrients	446.8	3.01	0.08	4.37
6	Null model (intercept only)	447.6	3.85	0.05	7.00

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Tree mortality, soil moisture, nutrients**	514.6	0.00	0.94	1.00
2	Soil moisture, nutrients	520.3	5.66	0.06	15.6

Evenness

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Null model (intercept only)**	-122.1	0.00	0.95	1.00
2	Tree mortality	-116.1	6.03	0.05	19.0

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Null model (intercept only)**	-166.2	0.00	0.95	1.00
2	Tree mortality	-159.6	6.61	0.03	31.6
3	Nutrients	-157.0	9.13	0.01	95.0
4	Soil moisture	-156.7	9.44	0.01	95.0

Notes: The most likely models (w_i>0.90; difference in evidence ratio>2.7) are shown in **bold**. AIC_C = Akaike's Information Criterion corrected, ΔAIC_C = difference between AIC_{Ci} and $AIC_{C\ best\ model}$, w_i = Akaike weight, and evidence ratio = $w_{j\ best\ model} / w_i$.

Table 2.2. Ranked relative importance of variables associated with the productivity, richness, and evenness of the understory plant community showing model estimates of slope and variance. The most likely explanatory variables are shown in bold (based on model-average estimate being different from zero when the confidence interval excludes zero).

Productivity

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Soil moisture	0.99	1.146	0.326	**Nutrients**	0.47	0.565	0.274
Tree mortality	0.44	1.131	0.325	Light	0.26	0.140	0.273
Nutrients	0.08	0.020	0.111	Soil moisture	0.17	0.021	0.128
Light	0.08	0.007	0.088	Tree mortality	0.07	0.001	0.077

Richness

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Nutrients	0.67	0.580	0.252	**Soil Moisture**	1.00	0.567	0.247
Soil moisture	0.56	0.498	0.254	**Nutrients**	1.00	0.937	0.234
Light	0.14	0.077	0.216	**Tree mortality**	0.94	0.716	0.237
Tree mortality	0.08	0.004	0.077	Light	0.01	0.000	0.009

Evenness

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Tree mortality	0.05	0.021	0.012	Tree mortality	0.04	0.017	0.010
Soil moisture	0.03	0.019	0.012	Nutrients	0.01	-0.005	0.010
Nutrients	0.01	-0.004	0.012	Soil moisture	0.01	-0.001	0.010
Light	0.01	0.002	0.012	Light	0.01	-0.000	0.002

Table 2.3. Candidate models used for inference on the richness of herbaceous and woody understory response to *Dendroctonus ponderosae*-induced tree mortality, light, soil moisture, and nutrients.

Richness of herbaceous perennials

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Nutrients**	352.3	0.00	0.68	1.00
2	Soil moisture	355.9	3.63	0.11	6.18
3	Tree mortality	356.1	3.82	0.10	6.80
4	Light	356.6	4.29	0.08	8.50
5	Soil moisture, nutrients, light	358.3	5.99	0.03	22.6

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Tree mortality, soil moisture, nutrients**	424.7	0.00	1.00	1.00

Richness of woody perennials

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	Null model (intercept only)	230.2	0.00	0.59	1.00
2	Tree mortality, soil moisture	232.0	1.71	0.25	2.36
3	Light	234.0	3.74	0.09	6.55
4	Nutrients	234.5	4.27	0.07	8.42

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	Tree mortality, soil moisture	356.9	0.00	0.45	1.00
2	Null model (intercept only)	357.0	0.10	0.43	1.04
3	Light	360.7	3.85	0.07	6.42
4	Soil moisture	361.0	4.08	0.06	7.50

Notes: The most likely models (w_i>0.90; difference in evidence ratio>2.7) are shown in **bold**. AIC_C = Akaike's Information Criterion corrected, ΔAIC_C = difference between AIC_{Ci} and $AIC_{C\ best\ model}$, w_i = Akaike weight, and evidence ratio = $w_{j\ best\ model} / w_i$.

Table 2.4. Ranked relative importance of variables associated with richness of herbaceous and woody understory showing model estimates of slope and variance. The most likely explanatory variables are shown in bold (based on model-average estimate being different from zero when the confidence interval excludes zero).

Richness of herbaceous perennials

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Nutrients	0.71	0.416	0.183	**Tree mortality**	1.00	0.745	0.184
Soil moisture	0.14	0.202	0.189	**Soil moisture**	1.00	0.348	0.187
Light	0.11	0.162	0.188	**Nutrients**	1.00	0.714	0.183
Tree mortality	0.10	0.201	0.188	Light	0.01	0.175	0.201

Richness of woody perennials

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Tree mortality	0.25	-0.082	0.154	Soil moisture	0.49	0.090	0.131
Soil moisture	0.25	0.030	0.078	Tree mortality	0.43	-0.162	0.204
Light	0.09	0.010	0.046	Light	0.07	0.007	0.043
Nutrients	0.07	-0.005	0.036	Nutrients	0.06	0.001	0.031

Table 2.5. Candidate models used for inference on the evenness of herbaceous and woody understory response to *Dendroctonus ponderosae*-induced tree mortality, light, soil moisture, and nutrients.

Evenness of herbaceous perennials

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Null model (intercept only)**	-96.1	0.00	0.89	1.00
2	Light	-91.0	5.02	0.07	12.7
3	Nutrients	-87.7	8.40	0.01	89.0
4	Tree mortality	-87.3	8.81	0.01	89.0
5	Soil moisture	-87.3	8.82	0.01	89.0

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Tree mortality**	-71.8	0.00	0.99	1.00
2	Null model (intercept only)	-61.3	10.55	0.01	99.0

Evenness of woody perennials

2012

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Null model (intercept only)**	-31.0	0.00	0.98	1.00
2	Tree mortality	-23.4	7.64	0.02	49.0

2013

	Candidate models	AIC_C	ΔAIC_C	w_i	Evidence ratio
1	**Null model (intercept only)**	-89.2	0.00	0.78	1.00
2	Tree mortality	-86.6	2.55	0.22	3.54

Notes: The most likely models (w_i>0.90; difference in evidence ratio>2.7) are shown in **bold**. AIC_C = Akaike's Information Criterion corrected, ΔAIC_C = difference between AIC_{Ci} and $AIC_{C\ best\ model}$, w_i = Akaike weight, and evidence ratio = $w_{j\ best\ model} / w_i$.

Table 2.6. Ranked relative importance of variables associated with evenness of herbaceous and woody understory showing model estimates of slope and variance. The most likely explanatory variables are shown in bold (based on model-average estimate being different from zero when the confidence interval excludes zero).

Evenness of herbaceous perennials

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Light	0.07	-0.027	0.014	**Tree mortality**	0.99	0.070	0.015
Nutrients	0.01	0.009	0.014	Soil moisture	0.01	-0.003	0.016
Tree mortality	0.01	0.003	0.014	Nutrients	0.01	0.009	0.016
Soil moisture	0.01	0.003	0.014	Light	0.01	-0.041	0.016

Evenness of woody perennials

2012				2013			
Explanatory variable	Σw_i	Model-average estimate	Unconditional SE	Explanatory variable	Σw_i	Model-average estimate	Unconditional SE
Tree mortality	0.02	-0.014	0.021	Tree mortality	0.21	0.007	0.014
Soil moisture	0.02	-0.012	0.021	Soil moisture	0.03	0.021	0.015
Nutrients	0.02	0.002	0.021	Nutrients	0.01	0.004	0.014
Light	0.02	-0.005	0.021	Light	0.01	-0.013	0.014

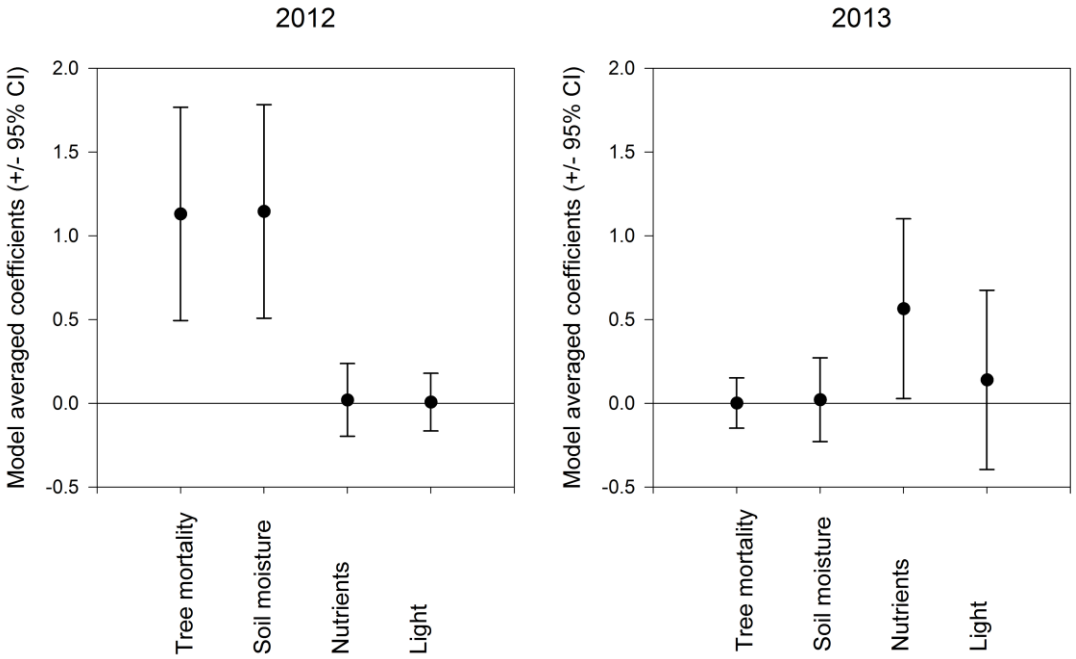

Figure 2.1. Model-averaged coefficients and 95% confidence intervals of *Dendroctonus ponderosae*-induced tree mortality, percent soil moisture, soil nutrients and light predicting understory plant community biomass in 2012 and 2013.

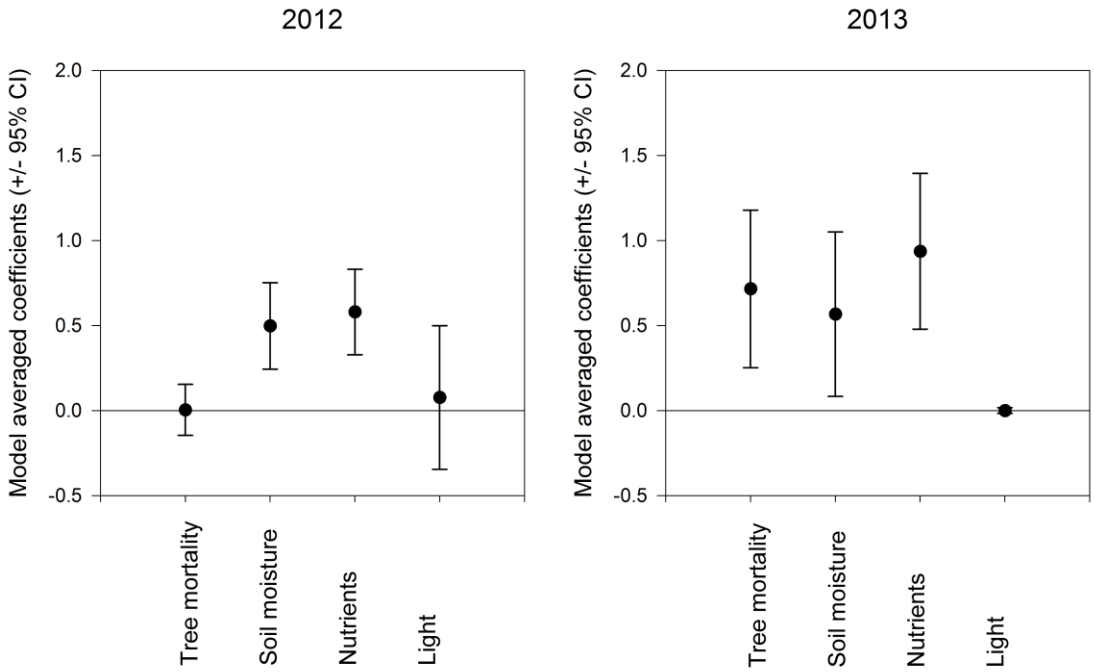

Figure 2.2. Model-averaged coefficients and 95% confidence intervals of *Dendroctonus ponderosae*-induced tree mortality, percent soil moisture, soil nutrients and light predicting understory plant community richness in 2012 and 2013.

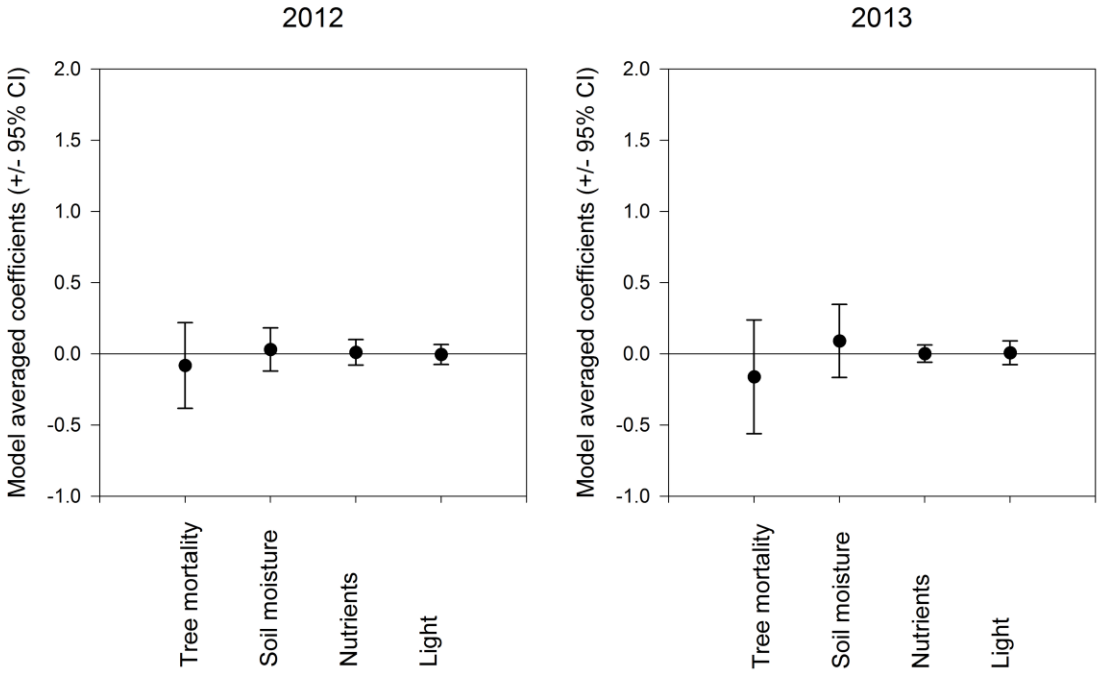

Figure 2.3. Model-averaged coefficients and 95% confidence intervals of *Dendroctonus ponderosae*-induced tree mortality, percent soil moisture, soil nutrients and light predicting evenness of understory woody perennial species in 2012 and 2013.

Chapter 3: Change in soil fungal community structure driven by a decline in ectomycorrhizal fungi following a mountain pine beetle outbreak

3.1 Introduction

Soil fungi form diverse and spatially variable communities (Talbot et al. 2014, Tedersoo et al. 2014), where mycorrhizal and saprotrophic fungi play fundamental roles in the cycling of nutrients (Cairney and Meharg 2002, Lindahl et al. 2007). Mycorrhizal fungi acquire carbon from and enhance the water and nutrient uptake of living host plants (Smith and Read 2008) and decompose soil organic material (Shah et al. 2016). Saprotrophic fungi decompose plant litter and wood to acquire carbon and other nutrients, as well as mobilize nutrients from soil organic material (Cairney and Meharg 2002, Floudas et al. 2012). The mechanisms by which different groups of soil fungal communities are assembled may vary owing to differences in their trophic lifestyle (Peay et al. 2013, Kivlin et al. 2014, Peay et al. 2015). Disturbances that result in the death or removal of host trees such as wildfire (Dahlberg 2002), harvesting (Barker et al. 2013) and insect caused-mortality (Del Vecchio et al. 1993, Saravesi et al. 2015) may affect ectomycorrhizal and saprotrophic fungi differently, as they rely on living and dead plants, respectively. For instance, tree death typically changes the community structure of ectomycorrhizal fungi through reductions in richness and abundance, while the richness of saprotrophic fungi has been shown to increase with a shift in composition following tree mortality (Stursova et al. 2014, Treu et al. 2014). Environmental conditions such as soil chemistry, soil moisture, soil nutrients, and substrate quality can alter community composition and diversity of both groups of fungi (Egerton-Warburton and Jumpponen 2005, Toljander et al. 2006, Prescott and Grayston 2013, Ottosson et al. 2014, Treseder et al. 2014). The death or removal of a host tree often

coincides with changes in soil environmental conditions, thus disentangling the relative importance of tree mortality from changes to soils following tree death is difficult, particularly with regard to ectomycorrhizal fungi which functionally connect tree and soils.

In addition to differences in the relative importance of deterministic factors governing fungal community assembly, the mechanism(s) of assembly by which these communities respond can also differ based on stochastic processes such as random fluctuations in species abundance or dispersal limitation (Cline and Zak 2014, Crowther et al. 2014, Bahram et al. 2016). Assembly of fungal communities, in general, has been observed to be stochastic, particularly in the upper surface layers of soil (10-30 cm) (Powell et al. 2015) as well as on roots of host plants (Beck et al. 2015). Specifically, spatial distance has predicted community composition of saprotrophic fungi (Feinstein and Blackwood 2013, Tedersoo et al. 2014). However, recent evidence suggests that both deterministic and stochastic processes work in tandem to regulate the assembly of ecological communities (Chase and Myers 2011, Hanson et al. 2012, Feinstein and Blackwood 2013). Disentangling these effects is difficult as biotic and abiotic factors vary with distance at multiple levels of organization (community versus functional group) (Miyamoto et al. 2015, Tedersoo et al. 2016). For example, Dumbrell *et al.* (2010) found that although soil abiotic factors, particularly soil pH, were strong predictors of mycorrhizal fungal community composition and diversity, these communities were also influenced by dispersal limitation (Dumbrell et al. 2009). Shifts in saprotrophic fungal composition and diversity has also been observed to be governed by both deterministic and stochastic processes, for example, as shown on decomposing leaves from the O horizon of upland and riparian forests (Feinstein and Blackwood 2013) and from remaining tropical forests on former agricultural and logged areas of Puerto Rico (Bachelot et al. 2016).

Nonetheless, it is critical to determine the extent to which soil fungi are constrained by deterministic or stochastic processes as these taxa are pivotal to ecosystem functions such as carbon and nutrient cycling as well as forest regeneration and succession (Jones et al. 2003, Read and Perez-Moreno 2003, Clemmensen et al. 2013).

In western Canada, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), a native to temperate conifer forests in western North America, has expanded east of the Rocky Mountains into pine habitats reaching as far as the boreal forest (Cullingham et al. 2011). Our previous research in this region has shown that compared to undisturbed stands, beetle-killed lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* Engelm.) stands have higher soil moisture content, higher soil nutrient availability and decreased levels of soil phenolics (Cigan et al. 2015). Soil phenolics, a broad class of carbon-rich plant secondary compounds, are known to influence nutrient cycling, particularly the availability of N (Northup et al. 1995, Hättenschwiler and Vitousek 2000). Increased levels of soil phenolics have lead to decreased colonization rates in ectomycorrhizal fungi (Siqueira et al. 1991), shifts in the community composition of fungi on the roots of their hosts (Krupa and Fries 1971), with positive to negative responses in spore germination and hyphal growth for soil fungi (Kuiters 1990, Siqueira et al. 1991, Bárta et al. 2010). Our previous work has demonstrated a decrease in the richness of fruiting bodies of ectomycorrhizal fungi in beetle-killed compared to undisturbed pine stands (Treu et al. 2014) as well as compositional differences in fungal communities on lodgepole pine seedlings that were inoculated with soil fungi from undisturbed and beetle-killed stands in a glasshouse experiment (Karst et al. 2015).

In this study, we build on this previous research (Treu et al. 2014, Karst et al. 2015) to report changes in the richness and community composition of soil fungi following a landscape-scale beetle outbreak. We used next-generation sequencing of DNA from soil fungi together with measurements of tree mortality, soil abiotic factors (i.e., nutrients, moisture, and phenolics) and spatial distance. We used this information to answer the following questions at two levels of organization: the overall soil fungal community and the functional group, i.e. ectomycorrhizal or saprotrophic fungi:

(1) Do increases in tree mortality result in decreases in richness of soil fungal communities with subsequent effects on community composition?

(2) How does variation in soil nutrients, moisture, and phenolics influence the richness and composition of soil fungi?

(3) What is the relative importance of tree mortality, soil abiotic factors, and spatial distance in determining the composition of soil fungi?

3.2 Materials and Methods

3.2.1 Study area

Eleven forest stands were located within a 625-km^2 region experiencing mountain pine beetle activity since 2009 and bordering provincial permanent sampling plots within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta (54°39'N, 118°59’W; 950-1150 m above sea level). Canopies were dominated (≥ 80%) by even-aged (120 ± 0.4 SE years old) lodgepole pine and across stands, a gradient of beetle-induced tree mortality was captured (0 to 82% lodgepole pine basal area killed) (Cigan et al. 2015). Although trees were

not explicitly tested, we infer that they are lodgepole pine and not lodgepole pine x jack pine (*Pinus banksiana* Lamb) hybrids based on genetic ancestry maps (Cullingham et al. 2012). Within stands, *Abies balsamea* (L.) Mill, *Betula papyrifera* Marshall, *Picea glauca* (Moench) Voss, *Picea mariana* Mill. Britton, Sterns, & Pogenb., and *Populus tremuloides* Michx. were interspersed in the subcanopy (0 to 14% of total basal area) along with a mixture of mostly herbaceous (e.g. *Chamerion angustifolium* (L.)) and to a lesser extent woody (e.g. *Vaccinium* spp.) vegetation in the understory (Pec et al. 2015). Soils were classified as Orthic Gray Luvisols derived from imperfectly drained glacial tills (Soil Classification Working Group, 1998). The stands were typical of lodgepole pine forests found within this region of Alberta (Beckingham 1996), exhibiting similar initial stand productivity (Treu et al. 2014) and edaphic characteristics while showing no relationship to any topographic characteristics such as elevation, slope or aspect (Cigan et al. 2015). Detailed information on stand selection and description, including stand locations and structure, is presented in Treu et al. (2014) and Cigan et al. (2015).

In May 2012, we installed a 1600-m^2 (40 m x 40 m) plot within each of the eleven stands. Within each plot in June 2012, ten soil cores (5 cm diameter, 20 cm deep) were sampled for fungi found on fine roots and in soil at a distance of 1 m from the nearest mature pine tree. A minimum distance of 7 m was maintained between sampled soil cores to increase sample independence (Pickles et al. 2012). Geographical coordinates (Garmin GPSmap 60Cx; Garmin International, Olathe, KS, USA) were also recorded at each sampling point. To determine the effect of tree mortality on fungal richness and composition, we recorded diameter at breast height (≥ 1.3 m), species identity, and health status (i.e., alive or dead, crown color and years dead) as described by (Klutsch et al. 2009) of all mature pine trees

within a 3 m radius of each sampled soil core in June 2012. We also measured the diameter at breast height and species identity of all subordinate tree species within this same 3 m radius. Attack by mountain pine beetle on mature lodgepole pine trees was confirmed by the presence of pitch tubes, boring dust, exit holes, and subcortical galleries (Safranyik 2006). Tree mortality was calculated as lodgepole pine basal area killed over total basal area of all trees expressed as a percentage.

To determine whether variation in soil moisture, nutrients, or phenolics were associated with changes in fungal richness and composition, these factors were measured within 20 cm of each sampled soil core. In brief, we sampled supply rates of macronutrients (i.e., Ca, K, Mg, N, P and S) using Plant Root Simulator (PRS^{TM}) probes (Western Ag Innovations, Inc., SK, Canada). Probes were inserted 10 cm (length of probe) into mineral soils of the A-horizon in June 2012, removed from the soil in August 2012, cleaned, and shipped to Western Ag Laboratories for macronutrient analysis. Volumetric soil moisture content was measured from the upper 10 cm of the soil column using a Theta Probe soil moisture sensor (Delta-T Devices, Cambridge, UK) during June 2012. To determine phenolics, soil samples from the top of the A-horizon were taken within two weeks of soil core sampling in early July 2012. Samples were air-dried for 48 hours and a quantitative assay for phenolic acids and compounds was used (Tel and Covert 1992). We added Folin-Ciocalteau phenol reagent (Sigma-Aldrich, Oakville, ON, Canada) to soil water extracts followed by spectroscopy (Enesys 10S UV-vis Spectrophotometer, Thermo, Fisher Scientific, Madison, WI, USA) to determine absorbance (750 nm). A more detailed description on the sampling methods is available in Cigan et al. (2014) and Pec et al. (2015). These measures were part of a more detailed field survey on nutrient cycling dynamics

following beetle outbreak and thus measures were taken to be representative of key biogeochemical periods (see Cigan et al., 2015). In the present study, we use these measures to understand the broader indirect impacts of beetle outbreak on soil fungal community structure. Although our soil cores were sampled to a greater depth than those for the soil abiotic factors measured, this depth best represents the location of soil fungi in the soil profile, particularly ectomycorrhizal fungi, and is comparable to sampling depths in other field studies from boreal systems (Pickles and Pither 2014).

3.2.2 Sampling and molecular characterization of fungi

Fungi occurring in soils and on roots were sampled from the soil cores. In total, 110 samples (10 soil cores x 11 plots) were transported on ice and frozen at -20 ^{o}C until processed. Soil samples were thawed and fine roots (< 2 mm) as well as any higher order roots were washed and separated using a #14 (1.4 mm opening) sieve stacked over a #80 (180 micron opening) sieve. Subsamples of 125 mg of roots as well as 125 mg of previously subsampled soil (from same soil core) were placed in a pre-chilled freeze-dryer (VirTis Freezermobile FM25XL; SP Scientific, Warminster, Pennsylvania, USA) at -45 ^{o}C and lyophilized for 24 hours. Freeze-dried roots were combined with subsampled soil, twice ground and homogenized to a fine powder using a mixer mill (Retsch Type MM 301; Retsch GmbH, Haan, Germany) for 1 minute at 25.0 Hertz. Genomic DNA was isolated from 250 mg of ground roots and soil using a CTAB protocol according to (Roe et al. 2010) with one modification: pellets were resuspended in 50 µl of nuclease-free water (Life Technologies, Carlsbad, CA, USA).

A two-step PCR amplification was performed to amplify the internal transcribed spacer (ITS) 1 region of nuclear rDNA using primers ITS1F and ITS2 and sequenced on an Ion Torrent™ PGM 400 Sequencing Kit and Ion 316™ Chips (Life Technologies, Carlsbad, California, USA) at the Molecular Biological Sciences Facility, University of Alberta (see Appendix 2.1). Quality filtering, clustering of sequences, and taxonomic identities of representative sequences were processed through bioinformatic analysis of Ion Torrent™ data using the QIIME pipeline v.1.8 (Caporaso et al. 2010)(see Appendix 2.1). Representative sequences of fungal OTUs are deposited in GenBank under accession numbers (KR584666-KR584685; KX497205-KX498025).

3.2.3 Statistical analysis

All statistical analyses were carried out using R v.3.1.2 (R Development Core Team 2015). Sequence data were first rarefied to account for uneven sequence depths, which is a suitable approach used with next-generation sequencing data (Gihring et al. 2012). We rarified the number of sequences in each soil core to the minimum number of sequences observed across all soil cores (305 sequences) using 1000 iterations with the *rarefy* function in the package *vegan* (Oksanen et al. 2013)(Appendix 2.2). Richness and composition of soil fungi were calculated at both the community and functional group level (see Appendix 2.1 and 2.3 for placement of fungi into functional groups).

To test for changes in the richness of soil fungi as a result of beetle-induced tree mortality at both the community and functional group level, we used linear mixed effects models using the R package *nlme* (Pinheiro et al. 2015). Linear mixed effects models were also used to determine if variation in soil abiotic factors were associated with changes in

fungal richness at both the community and fungal group level. Tree mortality, soil nutrients, moisture, and phenolics were included as fixed factors and had low levels of colinearity (Appendix 2.4). Site was used as a random factor to account for potential spatial autocorrelation due to the clustering of samples into sites. All macronutrients except for magnesium were correlated with one another (Appendix 2.4). Magnesium was not associated with changes in the richness or composition of soil fungi and was dropped from further analyses. A principal components analysis (PCA) using a correlation matrix was conducted on the following macronutrients: nitrogen, potassium, calcium, phosphorus, and sulfur. PCA 1 axis scores, which explained 75% of the variation in the data structure, were used to describe soil nutrients in all subsequent analyses. All model assumptions were checked by visual inspection of residual diagnostic plots (Zuur et al. 2009).

Indicator species analysis was performed to identify strongly responding fungal OTUs found across the tree mortality gradient using the *multipatt* function in the R package *indicspecies* (Cáceres and Legendre 2009). To determine if particular groups of taxa were gained or lost across the tree mortality gradient, we calculated a ratio reflecting the number of OTUs gained or lost for a particular phylum or order by dividing the total number of gains by the total number of losses. Values greater than one indicated that gains exceeded losses and values less than one indicated that losses exceeded gains. Nonparametric *t*-tests with 999 Monte Carlo permutations were used to statistically evaluate the gain and loss of fungal phyla or orders present in soil cores from undisturbed (n = 23) compared to severely beetled-killed (n = 30) (>60% *Pinus contorta* killed basal area) stands.

To determine the relative importance of beetle-induced tree mortality, variation in soil abiotic factors (see Appendix 2.5 for a detailed summary), and spatial distance on fungal composition, we first calculated Principal Coordinates of Neighbour Matrices (PCNM) spatial eigenvectors based on geographical coordinates of sampled soil cores using the *pcnm* function in the R package *vegan* (Oksanen et al. 2013). These PCNM vectors represent spatial variation among sampled soil cores across the study area and are used as explanatory variables suitable for constrained ordination analyses (Dray et al. 2006), with the first PCNM vectors showing large scale variation and latter PCNM vectors showing finer scale spatial variation (Borcard et al. 1992). Spatial distance represents species patterns emerging from dispersal-limitation and/or unmeasured ecological factors. Variance partitioning analysis (Borcard et al. 1992) was conducted to estimate the relative contribution of component sources of variation (i.e., tree mortality, soil abiotic factors, spatial distance) in shaping fungal composition at both the community and functional group levels using the *varpart* function in the R package *vegan* (Oksanen et al. 2013). Variables for this analysis were first forward selected based on adjusted coefficients of determination using the *forward.sel* function in the R package *packfor* (Dray et al. 2009). Redundancy analysis (RDA) was performed to interpret the importance of each selected variable in shaping the observed structure in fungal composition.

3.3 Results

3.3.1 General characterization of fungal community

A total of 31,542,423 sequences were obtained across all cores. Quality filtering reduced this value to 15,988,949 sequences (mean = 24,1597; SE = 7,011 across all soil

cores), which were clustered into 865 fungal OTUs at a 97% similarity level. There were 168 OTUs assigned to ectomycorrhizal fungi, 96 OTUs assigned to saprotrophic fungi, 16 OTUs assigned to arbuscular mycorrhizal fungi and 6 OTUs assigned to pathogenic fungi. In addition, there were 550 (64%) unidentified fungal OTUs with most not being assigned to a phylum and 29 unresolved fungal OTUs in which OTUs were assigned a genus affiliation though their functional grouping was uncertain. Of the 411 fungal OTUs (all identified to phylum) across all sampled soil cores, Basidiomycota was the most dominant phylum and accounted for 24.96% of all OTUs, followed by Ascomycota (19.12%), basal clades of the former Zygomycota (2.42%), and Glomeromycota (1.36%)(Table 3.1). In Basidiomycota, there were several taxonomic orders representing the majority of OTUs, which included Agaricales, Atheliales, Russulales, and Thelephorales, while in Ascomycota the order Helotiales was the most abundant (Table 3.1).

3.3.2 Fungal OTU richness

Mean fungal OTU richness across all cores was 85 OTUs (SE = 1.73, $n = 96$). Fungal OTU richness declined across the tree mortality gradient (undisturbed: 105 ± 5 SE; >80% attacked: 83 ± 5 SE) driven by a decline in ectomycorrhizal fungi (undisturbed: 18 ± 1 SE; >80% attacked: 13 ± 1 SE) and saprotrophic fungi (undisturbed: 26 ± 2 SE; >80% attacked: 19 ± 2 SE) (Table 3.2; Fig. 3.1). In particular, there was a greater loss of Agaricales (17%), Atheliales (16%), Hypocreales (20%), Mucorales (20%) and Glomerales (14%) across the tree mortality gradient (Appendix 2.6). In contrast, Pezizales and Saccharomycetales increased in frequency along the tree mortality gradient. There was a total of 24 indicator OTUs identified across the tree mortality gradient (Appendix 2.7). Undisturbed forests harbored a mixture of ectomycorrhizal (e.g. *Cortinarius* spp., *Suillus* sp.), arbuscular

mycorrhizal (e.g. *Glomus* sp.) and dark septate endophyte (e.g. *Phialocephala* sp.) fungi as indicators. In contrast, in forests with high tree mortality, substantially fewer ectomycorrhizal fungi were present, while saprotrophic (e.g. Saccharomycetales), pathogenic (e.g. *Verticillium* sp.), and parasitic (e.g. *Tremella* sp.) fungi increased in abundance (Appendix 2.7). There were also positive relationships between total fungal OTU richness and soil phenolics and ectomycorrhizal fungal OTU richness and soil phenolics (Table 3.2). However, total fungal OTU richness as well as ectomycorrhizal and saprotrophic fungal OTU richness did not vary with any other soil abiotic factor (Table 3.2).

3.3.3 Fungal composition

Overall, shifts in fungal community composition were driven by tree mortality ($F_{1,87}$=2.20, P=0.016), soil nutrients ($F_{1,87}$=2.44, P=0.007) and spatial distance within plots (PCNM3: $F_{1,87}$=4.91, P=0.038) and among plots (PCNM1: $F_{1,87}$=71.9, P=0.008) (Fig. 3.2a). The community composition of ectomycorrhizal fungi was mostly affected by tree mortality ($F_{1,87}$=2.22, P=0.023), soil phenolics ($F_{1,87}$=2.09, P=0.034) and spatial distance within plots (PCNM3: $F_{1,87}$=4.19, P=0.044) (Fig. 3.2b), while the community composition of saprotrophic fungi was driven by spatial distance among plots (PCNM1: $F_{1,87}$=4.29, P=0.041) (Fig. 3.2c). Variance partitioning analyses revealed that the independent effects of tree mortality (12%), spatial distance (15%), and soil abiotic factors (14%) accounted for 41% of the total variation in fungal community composition, while only 9% of the total variation was explained by the shared effect of tree mortality and spatial distance (Fig. 3.3a). The independent effects of tree mortality (12%) and soil abiotic factors (11%) contributed to most of the variation explained in ectomycorrhizal fungal composition (Fig. 3.3b), while the

independent effect of spatial distance accounted for only a small portion (1%) of the total variation in the composition of saprotrophic fungi (Fig. 3.3c).

3.4 Discussion

Widespread tree mortality caused by mountain pine beetle led to changes in belowground fungal richness and community composition, driven primarily by shifts in ectomycorrhizal fungi. Soil nutrients, soil phenolics and spatial distance also influenced the overall fungal community; however, the relative importance of these factors differed between communities of ectomycorrhizal and saprotrophic fungi. Previous studies have also demonstrated both environmental filtering and spatial distance to be important regulators of fungal communities (Lekberg et al. 2007, Talbot et al. 2014, Taylor et al. 2014). Although the individual components of variation were significant in our study, a majority of the variation in fungal community composition was still unexplained by the factors that were investigated. This was particularly the case for saprotrophic fungi. Factors that may have contributed to the variation in the distribution of these fungi could include environmental conditions not considered here, or possibly stochastic factors such as annual variation in precipitation and temperature (Peay and Bruns 2014).

In our study, there was an overall decline in belowground fungal community richness and a shift in composition with tree mortality. Ectomycorrhizal fungi depend on their hosts for carbon (Smith and Read 2008). A direct consequence of tree mortality is presumed to be a severe reduction in carbon flow from these hosts. Our previous work found a similar trend where the richness of ectomycorrhizal fruiting bodies declined and ectomycorrhizal fungal community composition shifted between undisturbed and beetle-killed stands driven by a

loss in the dominant tree species, lodgepole pine (Treu et al. 2014). Similarly, a decline in the richness of ectomycorrhizal fungi was detected from soil 2-3 years following a European spruce bark beetle (*Ips typographus*) outbreak in a Norway spruce (*Picea abies*) forest in Central Europe (Stursova et al. 2014), and ectomycorrhizal fungal richness and abundance within soils declined by 70-80% following an insect defoliation of mountain birch (*Betula pubescens* ssp. *czerepanovii*) in northern Finland (Saravesi et al. 2015).

Similar to a decline in the richness of ectomycorrhizal fungi, the richness of saprotrophic fungi declined across the gradient of tree mortality. This differs from our previous work where saprotrophic sporocarp richness remained invariant across the gradient of tree mortality (Treu et al. 2014). A possible explanation for this disparity is that saprotrophic fungi in this study were sampled from soil, which represented mostly humus-degrading fungi. The decline in both richness (this study) and abundance (Treu et al. 2014) of ectomycorrhizal fungi in soils may have limited the substrate available for decomposition by saprotrophic fungi. In addition to the direct effects of the loss ectomycorrhizal fungi on saprotrophic fungi, our sampling strategy may also have excluded wood-decaying fungi that colonize dying or dead trees, as well as litter- decaying fungi found on the soil surface. There is a pulse of needle deposition following tree mortality in these stands (Cigan et al. 2015), however dead trees may remain standing for at least a decade post-attack (Lewis and Thompson 2011). Thus, saprotrophic fungi occurring at the soil surface may not have been limited by substrates to the same extent as fungi occurring belowground. Although the richness of saprotrophic fungi declined with tree mortality, saprotrophic fungal composition was largely unexplained along the same gradient. Though there was a lack of a deterministic effect of tree mortality in our study, saprotrophic community composition was driven by

spatial distance among plots. This might suggest that larger-scale forces, either deterministic and/or stochastic, underlie assembly of saprotrophic fungi (Hiscox et al. 2015, Peay et al. 2016).

Composition of the overall fungal community was also influenced, in part, by variation in soil nutrient availability, soil phenolics, and spatial distance. Variation in features of the soil environment, such as those measured in our study can have a strong influence on soil fungal communities due to spatiotemporal variability within the soil (Tedersoo et al. 2012, Treseder et al. 2014). Although ectomycorrhizal fungi are directly dependent on their hosts for carbon, they are also sensitive to variation in soil conditions, which vary greatly in forested systems (Hättenschwiler and Vitousek 2000, Ettema and Wardle 2002, Bahram et al. 2015). Interestingly, the composition of ectomycorrhizal fungi, but not saprotrophic fungi, shifted as soil phenolics decreased with tree mortality (Supplementary Table S2). Ectomycorrhizal fungi may be sensitive to certain soil phenolic compounds such as benzoic acid and gallic acid, which have been shown to both induce and inhibit ectomycorrhizal growth (Rose et al. 1983, Cot et al. 1988, Kuiters 1990). Earlier studies have also demonstrated that the community composition of ectomycorrhizal fungi can be altered by the increased production and concentration of phenolic compounds in tree roots (Krupa and Fries 1971, Napierała-Filipiak et al. 2002). For example, ectomycorrhizal fungi, depending on the stage of colonization, may be inhibited (pre-colonization) or have a neutral response (colonized) to increased levels of soil phenolics (Kuiters 1990). An indirect effect of soil phenolics is their interference with N mineralization (Hättenschwiler and Vitousek 2000). In our study, the decline of ectomycorrhizal fungi across the tree mortality gradient might partly be explained by the decrease in soil phenolics levels and increased soil N across this same

gradient. Ectomycorrhizal fungi therefore might be responding to the soil phenolics directly or indirectly through increased N in the soil. Saprotrophic fungi may be less sensitive to the inhibiting properties of soil phenolic compounds, for example, by producing a greater diversity of laccases, which may be involved in the detoxification of phenolic compounds (Kuiters 1990, Thurston 1994). However, saprotrophic fungi can be influenced by different soil abiotic factors, such as variation in soil moisture levels and soil nutrient availability (Taylor et al. 2014, Tedersoo et al. 2016), though the influence of resource composition (e.g. dead wood) as substrate may make them especially vulnerable to changes in forest structure (Hottola et al. 2009). Thus, the unexplained variation in saprotrophic fungal composition during the first few years following mountain pine beetle outbreak might be due to a lag effect in resource composition. As it takes a decade or more for >90% of attacked trees to fall following mountain pine beetle outbreak (Mitchell and Preisler 1998), stochastic processes might govern saprotrophic fungi in soil in the first few years following disturbance, while changes to resources and niche space that potentially occur beyond the timeframe of this study, might elicit compositional shifts in saprotrophic fungi.

3.5 Conclusion

Our results provide novel insight into the underlying mechanisms and outcomes of widespread tree mortality on soil fungi. Collectively, our results suggest that both environmental and stochastic factors determine the community assembly of soil fungi; however, there were stronger unique than shared effects of tree mortality, soil abiotic factors, and spatial distance on the overall community composition of soil fungi. This suggests that tree mortality, soil chemistry, and spatial distance independently play important roles in structuring the community composition of soil fungi. Though tree death by bark beetles

triggers a chain of events connected by the plant-soil continuum, these events do not act in unison on soil fungi; rather they appear to act in isolation. Tree species diversity in the boreal forest is relatively low (Perry 2008), and the widespread death of a single common species affects the distribution of hundreds of fungal species directly or indirectly dependent on pine for resources.

Table 3.1. Taxonomic distribution of operational taxonomic units (OTUs) making up phyla and orders of soil fungi in lodgepole pine (*Pinus contorta*) forests in Alberta, Canada.

Phylum	**Order**	**% OTU**
Basidiomycota		24.96
	Agaricales	6.57
	Atheliales	4.01
	Russulales	3.77
	Thelephorales	3.28
	Sebacinales	0.97
	Boletales	0.85
	Other Basidiomycota[1]	1.46
	Unidentified	4.05
Ascomycota		19.12
	Helotiales	6.33
	Pezizales	1.00
	Saccharomycetales	1.00
	Hypocreales	0.73
	Other Ascomycota[2]	3.37
	Unidentified	6.69
Basal lineages		2.42
	Mortierellales	1.94
	Mucorales	0.48
Glomeromycota		1.36
	Glomerales	1.00
	Diversisporales	0.24
	Archaeosporales	0.12
Unidentified		52.14

Notes: [1] Includes orders Polyporales, Tremellales, Auriculariales, Cantharellales, and Filobasidiales of the phylum Basidiomycota.

[2] Includes orders Magnaporthales, Chaetothyriales, Eurotiales, Rhytismatales, Capnodiales, Pleosporales, Venturiales, Geoglossales, Peltigerales, Orbiliales, and Archaeorhizomycetales of the phylum Ascomycota.

Table 3.2. Models used to test the effects of *Dendroctonus ponderosae*-induced tree mortality, soil nutrients, moisture, and phenolics on the richness of the total soil, ectomycorrhizal and saprotrophic fungi.

Predictor	**Total soil fungi**		**Ectomycorrhizal fungi**		**Saprotrophic fungi**	
	$F_{1,84}$	P	$F_{1,84}$	P	$F_{1,84}$	P
Tree mortality	4.93	**0.028**	11.88	**0.009**	11.69	**0.001**
Soil nutrients	0.04	0.830	1.97	0.164	0.21	0.646
Soil moisture	1.79	0.183	2.01	0.159	0.25	0.612
Soil phenolics	5.93	**0.017**	6.73	**0.011**	0.03	0.843

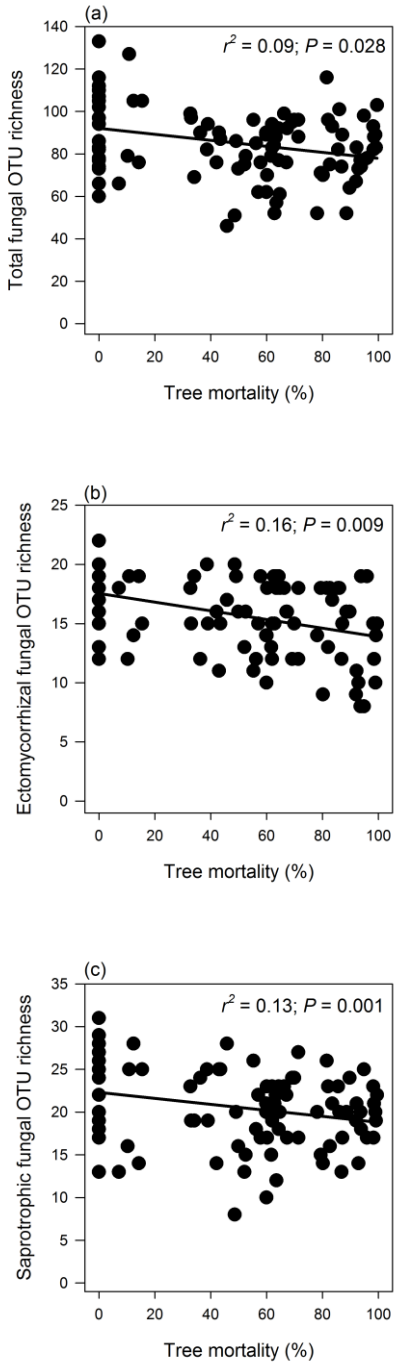

Figure 3.1. Response of OTU richness of (a) total soil fungi, (b) ectomycorrhizal fungi, and (c) saprotrophic fungi to *Dendroctonus ponderosae*-induced tree mortality.

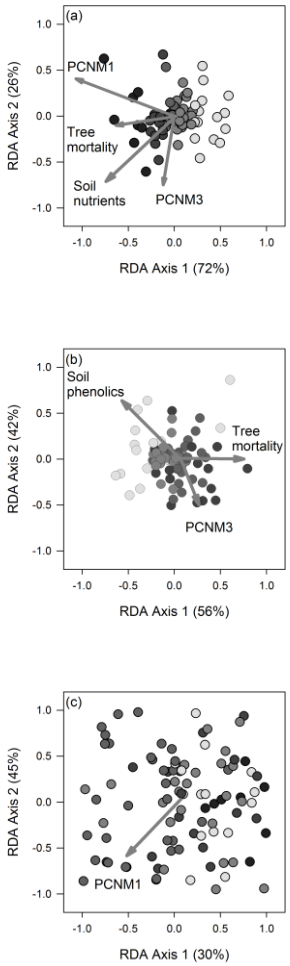

Figure 3.2. Redundancy analysis (RDA) ordinations of soil fungal communities: (a) total soil fungal composition, (b) ectomycorrhizal fungal composition, and (c) saprotrophic fungal composition. Total variance explained by each constrained axis is represented. Only significant ($P<0.05$) predictors are indicated. PCNM1 and PCNM3 predictors represent spatial eigenvectors based on geographical coordinates of sampled soil cores. PCNM represents across site variation while PCNM3 represents within-site variation. Colored dots indicate extent of tree mortality, with lighter colors representing soil cores taken from undisturbed plots and darker colors representing soil cores taken from beetle-killed sites.

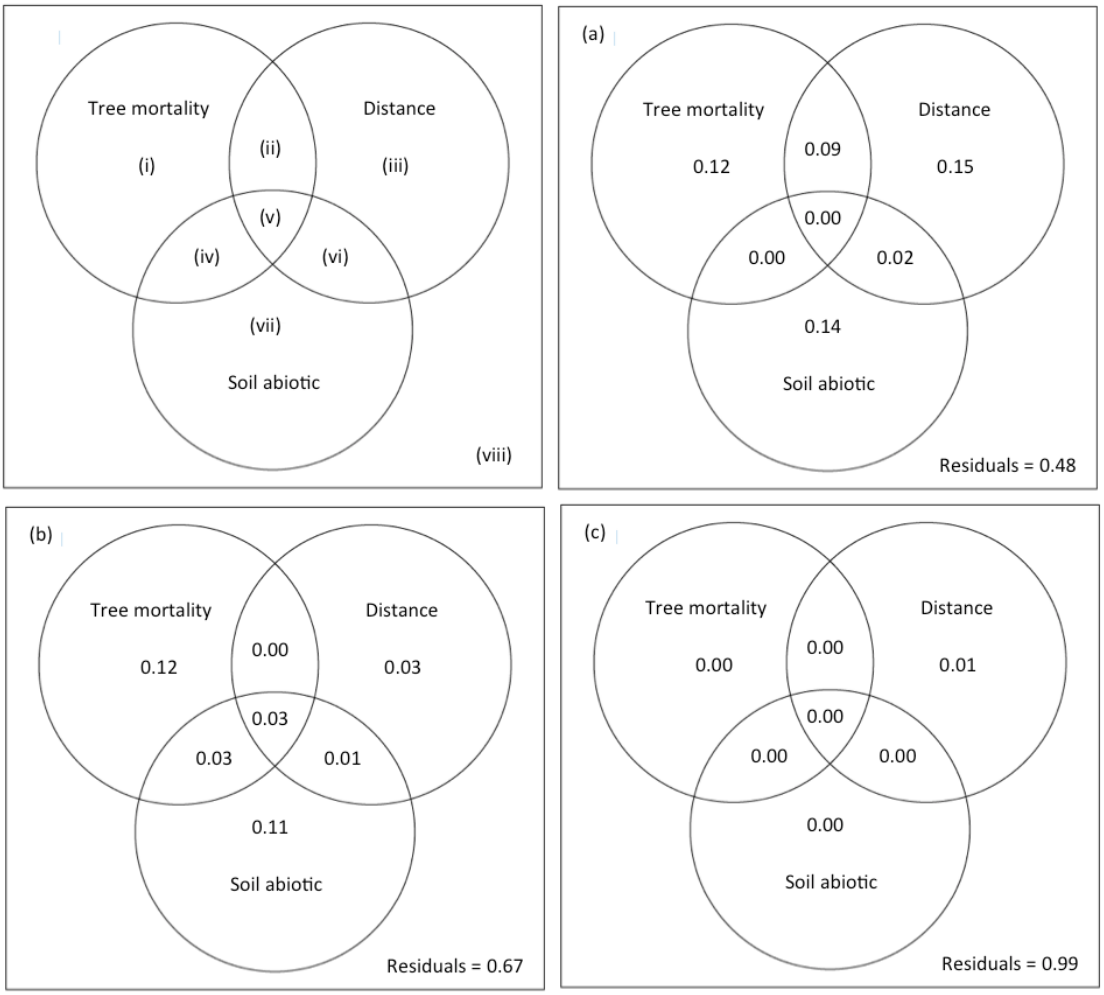

Figure 3.3. Variance partitioning of the effects of tree mortality, spatial distance and soil abiotic factors on the community structure of: (a) total soil fungi, (b) ectomycorrhizal fungi, and (c) saprotrophic fungi. Values show the percentage of variation explained by (i) tree mortality, (ii) shared contribution of tree mortality and spatial distance, (iii) spatial distance, (iv) shared contribution of spatial distance and soil abiotic factors, (v) shared contribution of tree mortality, spatial distance, and soil abiotic factors, (vi) shared contribution of spatial distance and soil abiotic factors, (vii) soil abiotic factors, and (viii) the residual unexplained variation.

Chapter 4: Large-scale insect outbreak homogenizes the spatial structure of soil fungal communities

4.1 Introduction

The spatial structure of ecological communities can arise from both niche-related and neutral processes (Peres-Neto and Legendre 2010). For example, differences in the distribution of ecological communities can derive from differences in niche requirements, where certain ecological taxa occur or thrive under particular environmental conditions and are sorted according to those requirements (Cottenie 2005). In contrast, effects on the spatial patterning of ecological communities may also reflect the importance of neutral processes such as dispersal limitation (Lekberg et al. 2007), priority effects or chance events (Fukami 2015). The roles of both these processes individually or in combination can influence the strength of the spatial structuring of ecological communities (Soininen et al. 2007, Chase and Myers 2011). Despite an increased awareness of the relative influence of the biological processes in shaping the spatial structure of animal and plant communities (Nekola and White 1999, Condit et al. 2002, Poulin 2003), the underlying ecological mechanisms on the spatial structuring of microbial communities, particularly soil fungi, remains poorly understood (Green et al. 2004, Green and Bohannan 2006).

Soil fungi, primary plant symbionts and decomposers in terrestrial ecosystems (Smith and Read 2008), play vital roles in ecosystem functions such as carbon flow and nutrient cycling as well as in forest regeneration and succession (Read and Perez-Moreno 2003, Smith and Read 2008, Clemmensen et al. 2013). The distribution of soil fungi is often spatially clustered, particularly for ectomycorrhizal fungi (Izzo et al. 2005, Peay et al. 2010,

Bahram et al. 2013). Ectomycorrhizal fungi, which acquire carbon from and enhance the nutrient uptake of living host plants (Smith and Read 2008), vary in their spatial distribution at smaller scales (cm-m) (Lilleskov et al. 2004, Pickles et al. 2010, Pickles et al. 2012). Many ectomycorrhizal fungal spores, however, are dispersal limited (Peay et al. 2007) and fall within a meter of their fruiting bodies (Li 2005). In contrast, saprotrophic fungi, decomposers of plant litter and wood for the acquisition of carbon and other nutrients (Cairney and Meharg 2002), may have the potential for long-distance dispersal as a result of their spore productivity (Hallenberg and Kúffer 2001). Furthermore, environmental factors such as host species presence and diversity (Tedersoo et al. 2016), variation in soil moisture (Erlandson et al. 2016), soil nutrient availability (Toljander et al. 2006), soil pH (Dumbrell et al. 2009), and soil phenolics (Pec et al. 2016) can all alter the composition and diversity of both groups of fungi. While, disturbances that result in the death or removal of host trees such as from wildfire and harvesting (Barker et al. 2013) and insect caused-mortality (Stursova et al. 2014, Saravesi et al. 2015, Pec et al. 2016), typically change the composition of soil and root associated fungal communities. However, the extent to which the spatial structuring of soil fungi is influenced by changes in the biotic and abiotic environment remains unclear.

In recent decades, insect outbreaks have occurred with greater intensity and in areas not previously subject to occurrence (Diskin et al. 2011; Bentz et al. 2010; Weed et al. 2013; Ramsfield et al. 2016). In western Canada, the mountain pine beetle (*Dendroctonus ponderosae* Hopkins) has expanded east of the Rocky Mountains into novel pine habitats (Cullingham et al. 2011, Cigan et al. 2015). Our previous research in this region has shown that compared to undisturbed stands, beetle-killed lodgepole pine (*Pinus contorta* Dougl. ex. Loud. var. *latifolia* Engelm.) stands have altered forest structure due to the death of the

dominant tree species (i.e, lodgepole pine), an increase in subordinate tree species in the subcanopy, a decline in overall root biomass (Cigan et al. 2015), and an increase in productivity and diversity of understory vegetation (Pec et al. 2015). The death of trees also often coincides with changes in soil environmental conditions, as in the case of our previous work in this region, where stands with widespread mortality of pine had higher soil moisture content, higher soil nutrient availability and decreased levels of soil phenolics (Cigan et al. 2015). As soil fungi as sensitive to variation in both above- and belowground environmental conditions (Treseder 2004, Tedersoo et al. 2016, Pec et al. 2016), the relative importance of beetle-induced tree mortality, and variation in vegetative productivity, vegetative diversity, and soil abiotic conditions may lead to differences in the spatial structuring of these belowground communities.

In this study, we build on our previous research (Treu et al. 2014, Karst et al. 2015, Pec et al. 2016), applying spatially explicit sampling and geostatistics to investigate what factors determine the spatial structure of soil fungal communities following large scale insect outbreak. Specifically, we asked the following questions: (1) Is the distance at which the community composition of soil fungi is no longer spatially autocorrelated attributed to variation in tree mortality, vegetative diversity and productivity, and soil abiotic conditions? (2) Does the variation attributed to tree mortality, vegetation, and soil abiotic conditions disrupt the spatial structure of soil fungi?

4.2 Materials and methods

4.2.1 Study area

Eleven forest plots were located within a 625-km^2 region experiencing mountain pine beetle activity since 2009 and bordering provincial permanent sampling plots within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta (54°39'N, 118°59'W; 950-1150 m above sea level). Canopies were dominated (≥ 80%) by even-aged (120 ± 0.4 SE years old) lodgepole pine and across plots, a gradient of beetle-induced tree mortality was captured (0 to 82% lodgepole pine basal area killed) (Cigan et al. 2015). Within stands, *Abies balsamea* (L.) Mill, *Betula papyrifera* Marshall, *Picea glauca* (Moench) Voss, *Picea mariana* Mill. Britton, Sterns, & Pogenb., and *Populus tremuloides* Michx. were interspersed in the subcanopy (0 to 14% of total basal area) along with a mixture of mostly herbaceous (e.g. *Chamerion angustifolium* (L.)) and to a lesser extent woody (e.g. *Vaccinium* spp.) vegetation in the understory (Pec et al. 2015). Soils were classified as Orthic Gray Luvisols derived from imperfectly drained glacial tills (Soil Classification Working Group, 1998). Detailed information on stand selection and description, including stand locations and structure, is presented in Treu *et al.* (2014) and Cigan *et al.* (2015).

4.2.2 Fungal community sampling

Within each plot (40 m x 40 m) in June 2012, ten 9 m x 9 m subplots were evenly distributed. Within each subplot, eight (5 cm diameter, 20 cm deep) soil cores were positioned at distances (i.e., 0.5 m, 1 m, 1.5 m, 2 m, 3 m, 4 m, 5 m) randomly radiating (51° angles between each point) from the center of each subplot (Appendix 3.1). Soil cores were sampled for fungi found on fine roots and in soil following methods in (Pec et al. 2016).

Geographical coordinates (Garmin GPSmap 60Cx; Garmin International, Olathe, KS, USA) were also recorded at each sampled soil core.

4.2.3 Biotic drivers

To determine the effect of tree mortality on the spatial structure of soil fungi, we recorded diameter at breast height (≥ 1.3 m), species identity, and health status (i.e., alive or dead, crown color and years dead) as described by (Klutsch et al. 2009) of all mature pine trees and breast height and species identity of all subordinate tree species within each subplot in June 2012. Attack by mountain pine beetle on mature lodgepole pine trees was confirmed by the presence of pitch tubes, boring dust, exit holes, and subcortical galleries (Safranyik 2006). Tree mortality was calculated as lodgepole pine basal area killed over total basal area of all trees expressed as a percentage for each subplot. Subplot values were averaged to generate estimates of tree mortality for each plot. To determine the effect of tree diversity on the spatial structure of soil fungi, we identified individual trees by species within all subplots during June 2012.

To determine understory plant diversity, we established a 1 m x 1 m micro-plot in a random cardinal direction next to the center of each subplot in May 2012 (Appendix A). In June 2012, we identified individual herbaceous and woody perennials by species within all micro-plots (see (Pec et al. 2015) for a detailed list). Micro-plot values were averaged to generate estimates of understory diversity for each plot. To determine biomass, we harvested all aboveground parts of the understory vegetation by species from each micro-plot in August 2012. Harvested plants were dried at 70°C for 48 hours, weighed, and micro-plot values were averaged for each plot. To determine the effect of belowground root biomass on the spatial

structure of soil fungi, we extracted soil cores (5 cm diameter, 20 cm deep) next to each micro-plot. Roots were washed over a 2 mm sieve and living roots were distinguished from dead roots based on the integrity and color of vascular tissue. Roots were dried at 60°C for 48 hours, weighed, and micro-plot values were averaged for each plot. We standardized estimates of root biomass by dividing the mass of each root sample by the volume of its soil core and by the basal area of trees greater than 1.5 m in height within each plot.

4.2.4 Abiotic drivers

All micro-plots were assessed for soil abiotic factors likely to influence the spatial structure of soil fungi, i.e, soil N, P, pH, moisture and phenolics (Toljander et al. 2006, Rousk et al. 2010, Erlandson et al. 2016, Pec et al. 2016). In brief, we sampled supply rates of soil N and P using Plant Root Simulator (PRS^{TM}) probes (Western Ag Innovations, Inc., SK, Canada). Probes were inserted 10 cm (length of probe) into mineral soils of the A-horizon in June 2012, removed from soil in August 2012, cleaned, and shipped to Western Ag Laboratories for analysis. Soil N concentrations were determined using an autoanalyzer while soil P concentrations were measured by inductive-coupled plasma spectrometry. Volumetric soil moisture content was measured from the upper 10 cm of the soil column using a Theta Probe soil moisture sensor (Delta-T Devices, Cambridge, UK) during June 2012. To determine soil pH and phenolics, soil samples from the top of the A-horizon were taken within two weeks of soil core sampling in early July 2012. Samples for soil pH were pooled per plot prior to being sent out for analysis to the University of Alberta Natural Resources Analytics Laboratory. Samples for soil phenolics were air-dried for 48 hours and a quantitative assay for phenolic acids and compounds was used (Tel and Covert 1992) followed by spectroscopy (Enesys 10S UV-vis Spectrophotometer, Thermo, Fisher

Scientific, Madison, WI, USA) to determine absorbance (750 nm). All micro-plot soil abiotic factors were averaged to generate estimates of soil N, P, pH, moisture, and phenolics for each plot. A more detailed description on the sampling methods is available in Cigan et al. (2015) and Pec et al. (2015).

4.2.5 Molecular characterization of fungi

Fungi occurring in soils and on roots were sampled from the soil cores described above. In total, 880 samples (8 soil cores x 10 subplots x 11 plots) were transported on ice and frozen at -20 ^{o}C until processed. Soil samples were thawed and fine roots were washed and separated using a #14 (1.4 mm opening) sieve stacked over a #80 (180 micron opening) sieve. Subsamples of 125 mg of roots as well as 125 mg of previously subsampled soil (from same soil core) were placed in a pre-chilled freeze-dryer (VirTis Freezermobile FM25XL; SP Scientific, Warminster, Pennsylvania, USA) at -45 ^{o}C and lyophilized for 24 hours. Freeze-dried roots were combined with subsampled soil, twice ground and homogenized to a fine powder using a mixer mill (Retsch Type MM 301; Retsch GmbH, Haan, Germany) for 1 minute at 25.0 Hertz. Genomic DNA was isolated from 250 mg of ground roots and soil using a CTAB protocol according to (Roe et al. 2010) with one modification: pellets were resuspended in 50 µl of nuclease-free water (Life Technologies).

A two-step PCR amplification was performed to amplify the internal transcribed spacer (ITS) 1 region of nuclear rDNA using primers ITS1F and ITS2 and sequenced on an Ion Torrent^{TM} PGM 400 Sequencing Kit and Ion 316^{TM} Chips (Life Technologies, Carlsbad, California, USA) at the Molecular Biological Sciences Facility, University of Alberta. Quality filtering, clustering of sequences, and taxonomic identities of representative

sequences were processed through bioinformatic analysis of Ion Torrent™ data using the QIIME pipeline v.1.8 (Caporaso et al. 2010) (see (Pec et al. 2016) for a detailed description of methods). Representative sequences of fungal OTUs are deposited in GenBank under accession numbers (KR584666-KR584685; KX497205-KX498025).

4.2.6 Statistical analysis

All statistical analyses were carried out using R v.3.1.2 (R Development Core Team 2015). Tree species diversity was calculated for each subplot based on species relative abundance for each tree species, represented by Shannon's diversity index, and values were averaged for each plot. Understory diversity was calculated for each micro-plot based on individual understory species biomass, represented by Shannon's diversity index, and averaged for each plot. Sequence data were first rarefied to account for uneven sequence depths, a common approach used with meta-genomic data (Gihring et al. 2012). We rarified the number of sequences in each soil core to the minimum number of sequences observed across all soil cores (305 sequences) using 1000 iterations with the *rarefy* function in the package *vegan* (Oksanen et al. 2013).

Semivariograms were used to measure how rarified OTU data (a measure of the abundance of each OTU in each soil core) by ectomycorrhizal or saprotrophic fungi were related to distance between soil samples per plot (n=11) using the *variog* function in the package *geoR* (Ribeiro Jr and Diggle 2001). Each experimental variogram provides information on the overall spatial pattern and on the estimation of spatial autocorrelation parameters: (a) variance attributed to spatial autocorrelation (C_1); (2) variance not attributed to spatial autocorrelation (C_0); (3) the proportion of variance resulting from spatial structure

($C_1/(C_0+C_1)$ with 0 indicating no measurable spatial structure and 1 indicating that all variance is caused by spatial structure; and (4) the 'range', or the distance at which data is no longer spatially autocorrelated.

To determine how variation attributed to tree mortality, vegetation, and soil abiotic factors effects the spatial structuring of soil fungi, weighted linear models were performed using the *lm* function in the *stats* package in which the models were weighted by the proportion of the total variation accounted for by the most suitable model semivariogram fit (r^2-value). The r^2-value for each plot was calculated by fitting the experimental semivariograms to theoretical semivariograms (covariance functions: e.g., exponential model, spherical model) with the most suitable model fit having the greatest r^2-value, an indicator of how well the experimental semivariogram fitted the experimental semivariogram (Legendre and Legendre 2012).

4.3 Results

Variation in community structure due to spatial positioning varied from 13 to 31 % for both ectomycorrhizal and saprotrophic fungi across plots (Appendix 3.2). The distance over which spatial autocorrelation was detected for ectomycorrhizal fungi ranged from 2.4 to 11.7 m across sites, while a similar pattern was detected for saprotrophic fungi (Appendix 3.2). The distance over which the community composition of ectomycorrhizal fungi became dissimilar (i.e., range) increased with increases in tree mortality, understory shoot productivity and soil moisture (Table 4.1; Fig. 4.1a-c). All other factors were relatively non-significant to variation in the distance over which spatial autocorrelation was detected for ectomycorrhizal fungi (Table 4.1). The range at which saprotrophic fungi became dissimilar increased with tree

mortality and higher levels of soil moisture along the same gradient (Table 4.1; Fig 4.2a-b). Although only marginally significant, the range at which saprotrophic fungi became dissimilar increased with an increase in understory diversity, while the distance over which spatial autocorrelation was detected for saprotrophic fungi remained invariant to all other biotic and abiotic factors (Table 4.1).

The proportion of variance due to spatial structure for both ectomycorrhizal and saprotrophic fungi increased with beetle-induced tree mortality (Table 4.1; Fig. 4.3a; Fig. 4a). Similar to the range at which community composition became dissimilar for ectomycorrhizal fungi, the proportion of variance due to spatial structure increased with greater aboveground productivity in the understory and increased soil moisture levels along the same mortality gradient (Table 4.1; Fig. 4.3b-c). However, the proportion of variance explaining the spatial structuring of ectomycorrhizal fungi decreased with greater levels of soil phenolics in unattacked versus beetle-killed plots (Table 4.1; Fig. 4.3d). The proportion of variance due to spatial structure for saprotrophic fungi increased with a greater diversity of understory species and increased levels of soil moisture (Table 4.1; Fig. 4.4b-c). Analogous to the change in the proportion of explained spatial structure in ectomycorrhizal fungi, the proportion of variance explaining the spatial structuring of saprotrophic fungi decreased with increased levels of soil phenolics (Table 4.1; Fig. 4.4d). Both ectomycorrhizal and saprotrophic fungi were invariant to all other biotic and abiotic factors (Table 4.1).

4.4 Discussion

Our findings show that following large-scale biotic disturbance, the distance at which both ectomycorrhizal and saprotrophic fungal communities became dissimilar increased with

tree mortality and changes to the forest structure and soil environment. Following disturbance such as fire, harvesting and insect caused-mortality, there can be a strong shift in the composition of ectomycorrhizal fungi in which rare or less dominant fungal OTUs often disappear (Barker et al. 2013, Stursova et al. 2014, Saravesi et al. 2015, Pec et al. 2016). In contrast, the frequency and abundance of saprotrophic fungi usually are higher following biotic disturbance and associated with initial massive inputs of litter and deadwood to the forest floor (Stursova et al. 2014). However, in a previous study, Pec et al. (2016) showed that saprotrophic fungi in soils declined along a similar gradient of tree mortality, with many rare fungal OTUs disappearing and the overall abundance of saprotrophic fungi becoming more evenly distributed in severely beetle-killed stands. In this study, the extent to which soil fungal communities were more similar across greater spatial distance in severely beetle-killed sites can be, in part, attributed to more niche-space availability with increased aboveground vegetative production and diversity, and soil moisture levels.

In our study, there was also an increased amount of explained variance in the spatial structure of both functional groups as tree mortality increased with subsequent increases in understory productivity and diversity as well as changes in the soil environment. Although the amount of unexplained variation was high across all sites (>60%), compared to severely beetle-killed sites, undisturbed sites explained less of the variation in the spatial structuring of soil fungi. This may indicate that both ectomycorrhizal and saprotrophic fungal community structure in undisturbed, even aged pine stands are more heterogeneous in both horizontal and vertical space, and are influenced by a complex array of interacting soil abiotic conditions (Bahram et al. 2015) in which no detectable spatial patterns in this study could be found. Alternatively, the lack of spatial structure in undisturbed versus severely

beetle-killed sites could indicate fine-scale patterning below 0.5 m (Morris 1999, Genney et al. 2006), or a greater role of dispersal limitation in undisturbed forested systems than previously anticipated (Lilleskov et al. 2004, Peay et al. 2012).

Both biotic and abiotic factors can influence the composition of soil fungal communities (e.g. (Talbot et al. 2014, Taylor et al. 2014, Pec et al. 2016)). In our study, a combination of vegetative effects and variation in environmental conditions were the main determinants in changes to the spatial patterning for both groups of soil fungal communities, with soil communities becoming more similar in severely beetle-killed than undisturbed sites. First, a direct consequence of tree mortality is a severe loss in carbon flow to ectomycorrhizal fungi (Smith and Read 2008) and an increase in deadwood and substrate availability for saprotrophic fungi (Stursova et al. 2014), which can cause compositional shifts in both groups of fungal communities (Stursova et al. 2014, Saravesi et al. 2015). Second, tree mortality often coincides with changes in forest structure, specifically with an increase in understory diversity and productivity, due to more favorable environmental conditions and release from overstory competition due to tree death (Pec et al. 2015). This increase in understory diversity and productivity, particularly of woody perennials, may provide fine root substrate for generalist soil fungi and can potentially increase the amount of litter and deadwood into soils, increasing the amount of substrate available for widely distributed saprotrophic fungi (De Bellis et al. 2007, Broeckling et al. 2008, Royer-Tardif et al. 2010). In addition, increases in understory diversity and productivity may also elevate the input of root exudates into soils, which have been shown to cause compositional shifts in soil fungal communities (Broeckling et al. 2008). Finally, tree mortality often coincides with changes in soil environmental conditions (e.g., (Cigan et al. 2015)). Environmental factors such as soil

moisture and phenolics can also influence soil fungi, with increases or declines in their relative abundances, depending on individual responses to environmental tolerance of soil abiotic conditions (Kuiters 1990, Dumbrell et al. 2009, Rousk et al. 2010).

4.5 Conclusion

Our results provide novel insight into how environmental factors determine the spatial structure of soil fungal communities and how these spatial patterns may vary following a large-scale biotic disturbance. Together, our results demonstrate that although both fungal groups vary based on their trophic strategy; large-scale biotic disturbance homogenizes the spatial patterning for both ectomycorrhizal and saprotrophic fungal communities by similar underlying biotic and abiotic factors.

Table 4.1. Models used for inference on the range and spatial structuring of ectomycorrhizal and saprotrophic fungi to beetle-induced tree mortality, variation in vegetative diversity and productivity, and soil abiotic factors.

	Ectomycorrhizal fungi				Saprotrophic fungi			
	Range		Spatial structure		Range		Spatial structure	
	$F_{1,9}$	P	$F_{1,9}$	P	$F_{1,9}$	P	$F_{1,9}$	P
Disturbance								
Tree mortality	6.213	**0.034**	9.086	**0.014**	6.330	**0.032**	5.917	**0.038**
Vegetation								
Tree diversity	0.153	0.704	0.005	0.942	0.292	0.601	0.018	0.894
Understory diversity	1.549	0.244	2.859	0.125	4.959	**0.052**	13.17	**0.005**
Understory aboveground biomass	20.95	**0.001**	7.931	**0.020**	3.711	0.086	0.651	0.440
Root biomass	1.300	0.283	1.711	0.223	3.475	0.095	1.132	0.314
Soils								
N	2.316	0.162	2.696	0.135	2.948	0.120	0.145	0.711
P	0.179	0.681	0.008	0.928	0.249	0.629	0.293	0.601
Moisture	17.43	**0.002**	12.53	**0.006**	8.673	**0.016**	5.731	**0.040**
pH	0.750	0.408	0.298	0.598	0.003	0.954	4.235	0.069
Phenolics	3.215	0.106	6.920	**0.027**	1.025	0.337	10.81	**0.009**

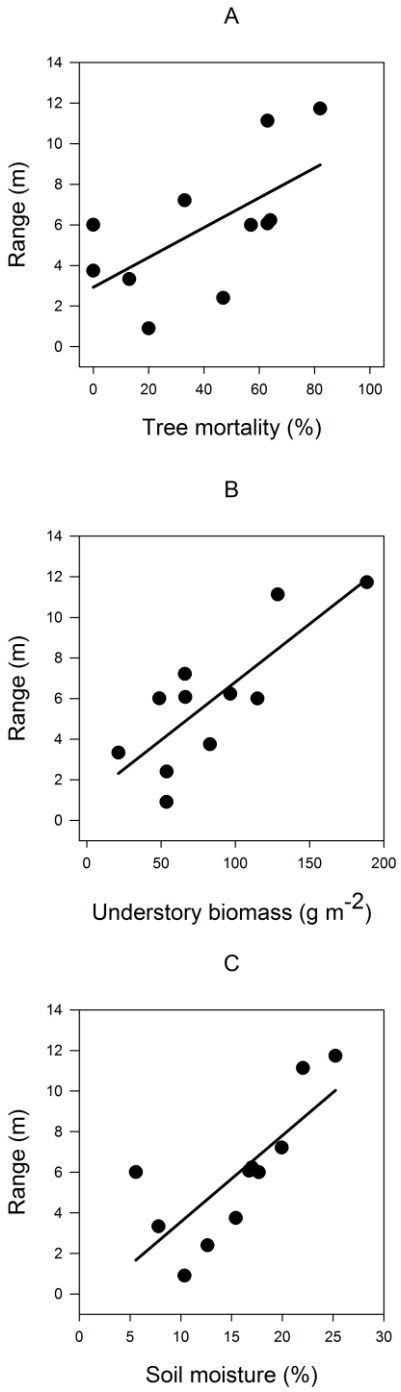

Figure 4.1. Variation in the distance at which ectomycorrhizal fungal communities are no longer spatially autocorrelated as a function of (a) mountain pine beetle-induced tree mortality, variation in (b) aboveground understory biomass, and (c) soil moisture content.

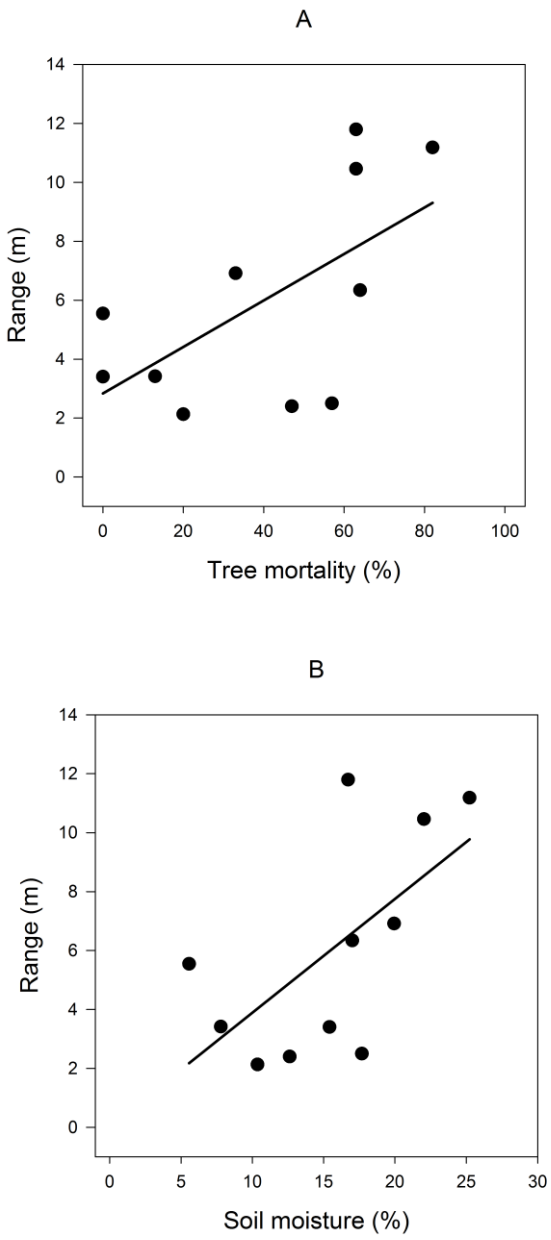

Figure 4.2. Variation in the distance at which saprotrophic fungal communities are no longer spatially autocorrelated as a function of (a) mountain pine beetle-induced tree mortality and (b) variation in soil moisture content.

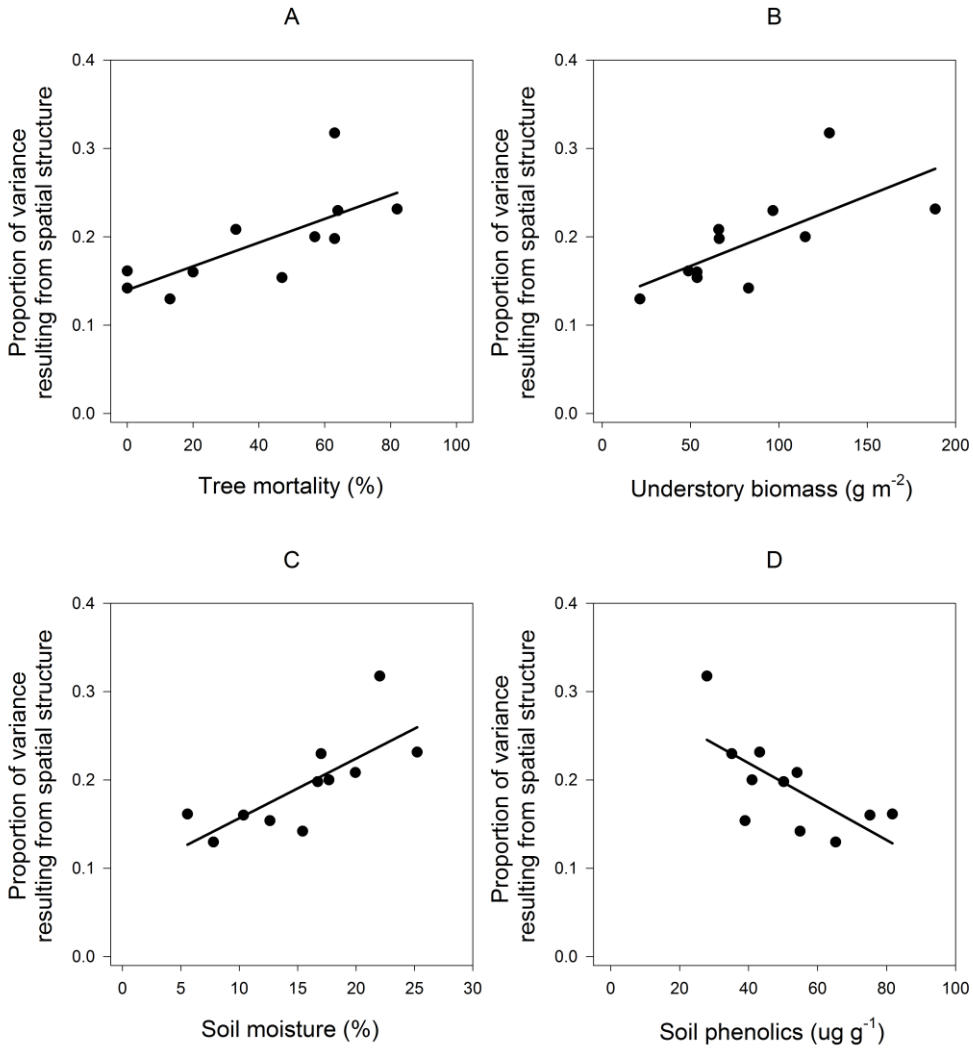

Figure 4.3. The proportion of variance due to the spatial structure of ectomycorrhizal fungal communities as a function of (a) mountain pine beetle-induced tree mortality, (b) variation in (b) aboveground understory biomass, (c) soil moisture content, and (d) soil phenolics.

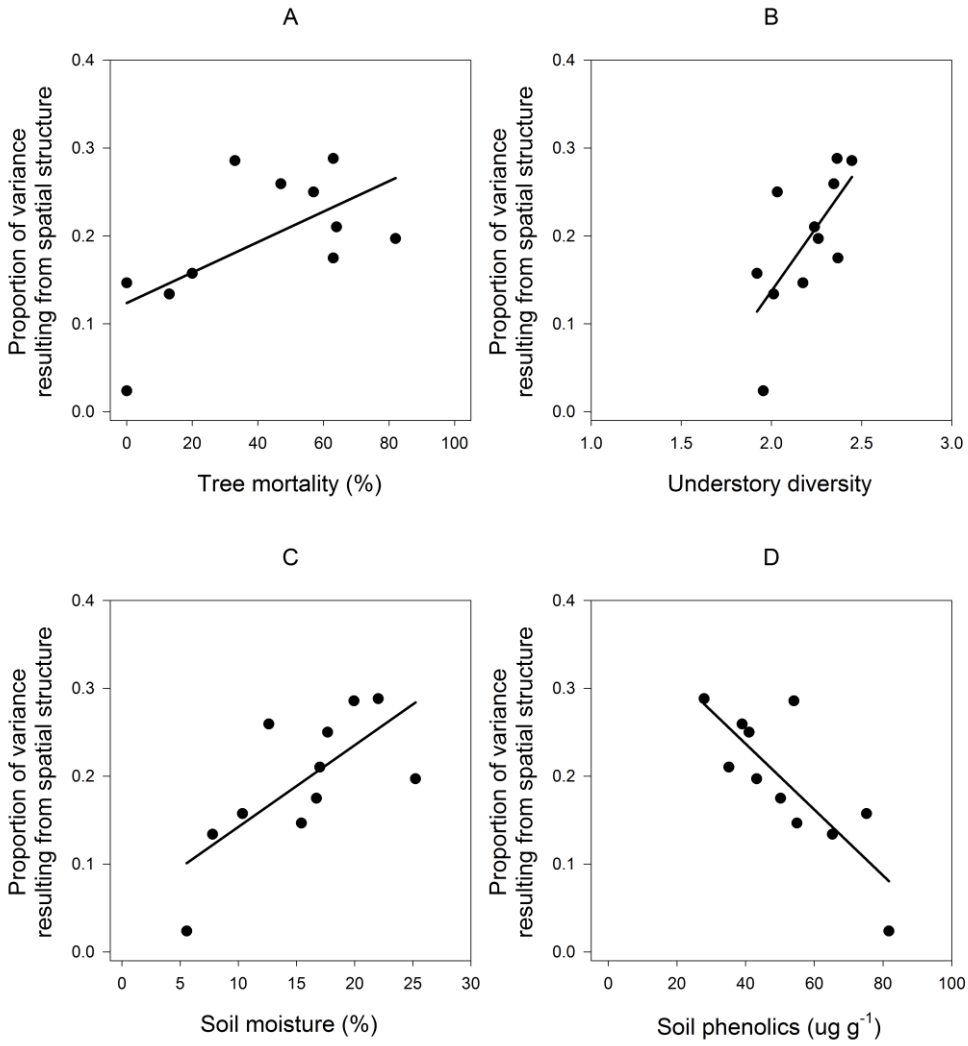

Figure 4.4. The proportion of variance due to the spatial structure of saprotrophic fungal communities as a function of (a) mountain pine beetle-induced tree mortality, (b) variation in (b) understory diversity, (c) soil moisture content, and (d) soil phenolics.

Chapter 5: Access to ectomycorrhizal fungal networks enhance seedling establishment following a mountain pine beetle outbreak

5.1 Introduction

Seedling establishment is one of the key processes that can influence the structure and functioning of ecological systems (Oliver & Larson, 1990). Many factors influence seedling establishment success, such as light and soil resource availability as well as root and shoot competition from neighboring plants (Pickett & White, 1985; Coomes & Grubb, 2000). There is increased evidence on the importance of mycorrhizal networks and their roles in improving seedling growth and survival (Horton *et al.*, 1999; Simard & Durall, 2004; Nara, 2006; McGuire, 2007). Mycorrhizal networks, fungal hyphae that connect roots of the same or different host species (Selosse *et al.*, 2006), serve as sources of fungal inoculum for seedlings and can function as channels for carbon, nutrient, and water transmission among host species (Simard & Durall, 2004; Simard *et al.*, 2012). For example, mycorrhizal networks have been shown to facilitate establishment of seedlings when resources or fungal propagules are deficient in soils (McGuire, 2007; Teste & Simard, 2008), mitigate effects of overstory competition on seedling growth (Booth & Hoeksema, 2009), and appear to facilitate natural regeneration of seedlings in association with the transfer of carbon and nitrogen from mature trees (Teste *et al.*, 2009). However, it remains unclear whether existing links between host species via their fungal partners are vulnerable to environmental stresses associated with disturbance (Simard, 2009b).

In the past several decades, insect outbreaks are occurring with increased severity and in expanded ranges, particularly in the forest ecosystems of western North America (Dordel *et al.*, 2008; Safranyik *et al.*, 2010; Diskin *et al.*, 2011). Mountain pine beetle (*Dendroctonus*

ponderosae Hopkins), an insect native to temperate conifer forests, has significantly expanded east of the Rocky Mountains into novel pine habitats (Cullingham et al. 2011; Cigan et al. 2015). Our previous work in this region has shown that the widespread mortality of pines has altered nutrient cycling (Cigan *et al.*, 2015), forest overstory and understory structure (Pec *et al.*, 2015) and the abundance of aboveground fruiting bodies and belowground ectomycorrhizal fungi (Treu et al. 2014; Pec et al. 2016). Since seedlings depend largely on ectomycorrhizal fungi to compete effectively for soil nutrients (Nara, 2006), the loss of fungal propagules as well as lack of access to an existing mycorrhizal network following insect outbreak may lead to decreased or delayed seedling establishment or overall seedling regeneration failure (Simard, 2009a).

In this study, we investigated the effects of ectomycorrhizal networks on tree seedling establishment (growth, nutrition, and survival) across varying levels of mountain pine beetle induced tree mortality. Specifically, the following questions were addressed: (1) Does the ectomycorrhizal status of tree seedlings (i.e. colonization, composition) vary with tree mortality? (2) Does greater access to an ectomycorrhizal network: (a) enhance tree seedling growth and survival? (b) increase nutrient uptake by tree seedlings, or (c) reduce water stress in tree seedlings? We predicted that ectomycorrhizal networks would degrade following stand level tree mortality and, in turn, seedling establishment would be reduced.

5.2 Methods

5.2.1 Site description

We located eleven forest stands within a 625-km^2 region experiencing mountain pine beetle activity since 2009, which bordered provincial permanent sampling plots within the

Lower Foothills natural subregion southwest of Grande Prairie, Alberta (54°39'N, 118°59'W; 950-1150 m above sea level) (see Treu *et al.* (2014) for specific stand locations). Canopies were dominated (≥ 80%) by even-aged (120 ± 0.4 SE years old) lodgepole pine (*Pinus contorta* Dougl. ex. Loud.) and across stands, a gradient of beetle-induced tree mortality was captured (0 to 82% lodgepole pine basal area killed)(Cigan *et al.*, 2015). Within and among stands, *Abies balsamea* (L.) Mill, *Betula papyrifera* Marshall, *Picea glauca* (Moench) Voss, *Picea mariana* Mill. Britton, Sterns, & Pogenb., and *Populus tremuloides* Michx. were interspersed in the subcanopy along with a mixture of understory vegetation (Pec *et al.*, 2015). White spruce (*Picea glauca*) was by far the most abundant tree species in the subcanopy (49%)(G. Pec and A. Sywenky, *unpublished data*). Since it has been shown that successful infestation of lodgepole pine by insect outbreak can promote growth of shade-tolerant conifer species, which may lead to a non-pine dominated system (Nigh *et al.*, 2008), we focused on both lodgepole pine and white spruce seedling establishment.

5.2.2 Experimental design

We used a split-plot design, which consisted of three ectomycorrhizal network (MN) treatments (MN and no roots, MN and roots, no MN or roots) and two tree species (lodgepole pine, white spruce) in a 3 x 2 factorial set of treatments to test the importance of access to ectomycorrhizal networks for the establishment of (survival, growth and nutrition) tree seedlings along a gradient of beetle-induced tree mortality. In August 2011, we installed a 900-m^2 (30 m x 30 m) plot within each of the eleven stands. Within each plot, ten evenly distributed mature lodgepole pine focal trees (>20 cm diameter at breast height) were identified and six circular subplots (15 cm diameter) were located within three meters from a focal tree in a random cardinal or intercardinal direction to allow for the likelihood of an

ectomycorrhizal network to form. The **MN** treatment and the **no MN and roots** treatment was created by placing a 44 μm and a 0.5 μm mesh bag (15 cm diameter, 35 cm deep)(Plastok® Meshes and Filtration, Ltd., Birkenhead, UK) into holes dug in the soil to a depth of 35 cm. These treatments were randomly assigned to one of the six circular holes per subplot. Each mesh bag was refilled with the previously dug field soil, keeping each of the soil layers intact. The **MN and roots** treatment was randomly assigned to another one of the six circular holes, and entailed refilling the hole with previously dug field soil but not installing a mesh bag. Both mesh sizes prevent roots from passing while allowing for diffusion of solutes, whereas the 0.5 μm mesh also prevents hyphal passage of ectomycorrhizal fungi and contact with seedlings (Teste *et al.*, 2006). Seeds sown directly into soil (no mesh) allowed for germinated seedlings to intermingle with roots and form ectomycorrhizal networks.

Because of concern that mesh would impede water and nutrient flow, we measured soil moisture levels within and directly next to each of the mesh treatments using a Theta Probe soil moisture sensor (Delta-T Devices, Cambridge, UK). We found no differences in soil moisture content levels within and directly next to a mesh treatment ($t = 0.34$, $df = 26$, $P = 0.735$) as well as among mesh treatments ($F_{2,24} = 0.101$, $P = 0.904$), which is similar to previous studies on soil water movement across mesh (Teste *et al.*, 2006; Teste *et al.*, 2009). In October 2011, twenty lodgepole pine seeds and twenty white spruce seeds were applied to each hole per treatment. Seeds were provided by Smoky Lake Forest Nursery, Alberta (seedlot number: lodgepole pine - NWB1 64-8-6-1981; white spruce – NES3 60-20-5 1983) and sourced from the same origin as where the study area was located. Bags were overwintered to allow for ectomycorrhizal networks to colonize the MN and the MN and

roots treatments. In May 2012, we found that germination was non-existent and reseeded each treatment with an additional 20 seeds. During the first growing season (2012), an open-topped cylindrical mesh (6 mm) cage was used to protect seedlings from herbivory. All holes were surrounded by a 0.5-m buffer zone and all vegetation within the buffer zone was periodically clipped throughout the growing season to eliminate interspecific plant interactions.

5.2.3 Survival, growth and nutrition measurements

Survival of germinants was assessed in May 2013 and was calculated as a proportion of live seedlings to the total number of seeds that germinated per treatment. Seedlings were thinned to two seedlings per hole in May 2013. All seedlings were destructively sampled in August 2013. Prior to harvest, height on both seedlings per mesh treatment was recorded. One of the seedlings per mesh treatment was harvested for biomass determination (shoots and roots). To determine shoot biomass, stems were cut at the soil surface, oven dried at 70°C for 48 hours and weighed. To determine root biomass, root systems of individuals were carefully removed from mesh bags with soil intact, placed in plastic bags, transported and stored at -20°C until further processing. For seedlings sown directly in soil (no mesh), root systems were carefully removed with the same volume of soil intact as from mesh bags (15 cm diameter, 35 cm deep). Roots were extracted from thawed soil by carefully washing under running tap water, oven dried at 70°C for 48 hours and weighed.

The second seedling per mesh treatment was harvested for foliar concentration of N, P, and ^{13}C as well as ectomycorrhizal colonization and community composition on seedling root tips (*See sampling and determination of EM fungi*). Shoots and roots were harvested as described for biomass determination. Conifer needles were first ground and homogenized to

a fine powder using a Brinkmann ball grinder (Retsch Type MM 220; Retsch GmbH, Haan, Germany). Foliar N was analyzed by the Dumas Combustion Method (Nelson & Sommers, 1996) using a Costech 4010 Elemental Analyzer System (Costech Analytical Technologies Inc., Valencia, CA, USA). Foliar P was analyzed by nitric acid digestion (Halloran & Cade-Menun, 2007) and determined spectrophotometrically on a SmartChem® wet chemistry discrete analyzer (Westco Scientific Instruments, Inc., Brookfield, CT, USA). The natural abundance of ^{13}C was determined using a Costech ECS 4010 Elemental Analyzer System (Costech International Strumatzione, Bremen, Germany) connected to a Conflo III & Continuous Flow Delta Plus Advantage isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany). Lab standards, calibrated against the International Reference scale (i.e. ^{13}C vs. VPDB), were used to determine sample isotopic results (Weaver, 1994). Foliar concentration of N, P, and ^{13}C was performed at the University of Alberta Natural Resources Analytical Laboratory.

5.2.4 Sampling and determination of ectomycorrhizal fungi

Roots of seedlings were carefully washed under tap water and cut into 1 cm fragments. We assessed all root tips per seedling for ectomycorrhizal colonization. Samples were morphotyped using both stereo and compound microscopes based on color, tip shape, branching pattern, and texture (Goodman *et al.*, 1998). Sanger sequencing was subsequently used to identify fungi colonizing roots of seedlings. DNA was extracted from each root tip, PCR amplification of the internal transcribed spacer (ITS) region of fungal nuclear rDNA was performed in 16 µl reactions using primers NSI1 and NLB4 (Martin & Rygiewicz, 2005), and cycle sequencing was performed in 10 µl reactions following methods outlined in Karst

et al. (2015). Sequencing reactions were cleaned using EtOH precipitation and run on an ABI 3730 DNA analyzer (Applied Biosystems).

Sequences were first edited manually by modifying the sample ID for all fasta files, ensuring uniqueness in fasta labels, and concatenating all files together for downstream bioinformatic analysis. Quality filtering, sequence clustering, and taxonomic identities of sequences were processed using the QIIME pipeline v.1.8 (Caporaso *et al.*, 2010) following methods outlined in Pec et al. (2016). Sequences of all ectomycorrhizal fungal OTUs were submitted in the GenBank database under accession numbers (KX498030-KX498065).

5.2.5 Statistical analysis

All statistical analyses were performed using R v.3.1.2 (R Development Core Team, 2015). Percentage of ectomycorrhizal colonization was calculated as the number of EM root tips divided by the total number of root tips (ectomycorrhizal and non-ectomycorrhizal) multiplied by 100. To test for differences between ectomycorrhizal fungal communities colonizing seedlings in the three mesh treatments across a gradient of mountain pine beetle induced tree mortality, a permutational multivariate analysis of variance was run using the *adonis* function in the *vegan* package (Oksanen *et al.*, 2013) with permutations set to 1000 and all other parameters as default. We used linear models to test the main effects and interaction among the mesh treatments and mountain pine beetle induced tree mortality on seedling survival, growth and nutrition. Model assumptions were checked with diagnostic plots of the residuals. Post hoc tests on differences based on mesh treatments were done with least significant difference tests ($\alpha = 0.05$).

5.3 Results

A total of 31 ectomycorrhizal taxa were found on lodgepole pine seedling root systems and 30 ectomycorrhizal taxa on white spruce seedling root systems, with 26 ectomycorrhizal taxa shared between both seedling root systems (Fig. 5.1). Ectomycorrhizal fungal community composition differed among lodgepole pine and white spruce seedlings (permANOVA: $F = 32.75$, $P = 0.01$; Appendix 4.1). Many ectomycorrhizal taxa were abundant on both lodgepole pine and white spruce seedlings (Fig. 5.1); however, *Laccaria bicolor, Thelephoraceae 1, Thelephora terrestris, Tomentellopsis submollis,* and *Tuber pacificum* were only found on lodgepole pine seedling root systems (Fig. 5.1). Colonization rates of ectomycorrhizal fungi on lodgepole pine and white spruce seedlings were similar across all three MN treatments (lodgepole pine, $F = 2.862$, $P = 0.080$; white spruce, $F = 1.580$, $P = 0.229$). Although, colonization rates on lodgepole pine seedlings increased across the gradient of tree mortality, while colonization rates remained similar across the same gradient for white spruce (lodgepole pine, $F = 4.334$, $P = 0.048$; white spruce, $F = 0.575$, $P = 0.456$). Ectomycorrhizal fungal community composition on lodgepole pine and white spruce seedlings (independent of each other) were similar among all three MN treatments (permANOVA: lodgepole pine, $F = 0.011$, $P = 0.68$; white spruce, $F = 1.008$, $P = 0.38$) and across the gradient of tree mortality (permANOVA: lodgepole pine, $F = 0.010$, $P = 0.23$; white spruce, $F = 0.012$, $P = 0.90$).

Lodgepole pine seedling survival decreased with beetle-induced tree mortality, while white spruce seedlings were invariant across the same gradient (Table 5.1; Fig. 5.2). Both lodgepole pine and white spruce seedling survival did not differ among the MN treatments (Table 5.1; Fig. 5.2). Height of both lodgepole pine and white spruce seedlings did not differ

between MN treatments (Table 5.1; Fig. 5.3); however, lodgepole pine seedling became taller across the tree mortality gradient (Table 5.1; Fig. 5.3). Both lodgepole pine and white spruce seedlings increased in biomass with tree mortality. Lodgepole pine seedlings increased one-fold with access to an EM fungal network; while, white spruce seedlings did not differ in biomass among the MN treatments (Table 5.1; Fig. 5.3).

Both lodgepole pine and white spruce seedling foliar N concentrations increased with tree mortality (Table 5.2; Fig. 5.4 a, b). Lodgepole pine seedlings had one-fold greater foliar N concentrations in severely beetle-killed stands with access to an EM fungal network, while white spruce foliar N concentrations remained similar among MN treatments (Table 5.2; Fig. 5.4 a, b). Lodgepole pine seedlings also differed among MN treatments in foliar P concentrations. Seedlings with access only to an EM fungal network had increased foliar P and these concentrations increased across the tree mortality gradient (Table 5.2; Fig. 5.4 c, d). In contrast, white spruce seedling foliar P did not differ among MN treatments or across the tree mortality gradient (Table 5.2; Fig. 5.4 c, d). Foliar ^{13}C concentrations were similar across MN treatments in both lodgepole pine and white spruce seedlings (Table 5.2; Fig. 5.4 e, f). However, foliar ^{13}C concentrations in lodgepole pine seedlings became less negative across the gradient of tree mortality (Table 5.2; Fig. 5.4 e, f).

5.4 Discussion

Following widespread tree mortality, seedling biomass and nutrition were improved where there was access to ectomycorrhizal networks, but these benefits disappeared in the presence of root competition. Our results are similar, in part, to field manipulative experiments in dry conifer forests (Booth & Hoeksema, 2009; Teste *et al.*, 2009) and tropical forests (Corrales *et al.*, 2016) on the beneficial effects of ectomycorrhizal networks for

seedling establishment. Our results also highlight that seedlings in beetle-killed stands do not appear limited by access to ectomycorrhizal networks, however the effects of these networks on lodgepole pine and white spruce seedling establishment following insect-induced tree mortality were more complex.

In our study, survival of white spruce seedlings was greater than that of lodgepole pine. Both lodgepole pine and white spruce are widely distributed tree species within boreal forests of North America (Perry, 2008). Following stand replacing disturbance, lodgepole pine has much faster juvenile growth rate and establishment compared to white spruce. As canopy closure ensues through time, white spruce is able to increase in growth in the understory due to more favorable environmental conditions (Despain, 2001; Gärtner *et al.*, 2011). However, insect induced tree mortality has little physical effect on understory vegetation and soils, as these are intact following disturbance (Burton, 2008). In our study, greater survival of white spruce across the tree mortality gradient may be due more to favorable seedbeds, less deteriorated mineral soils, and thicker organic matter layers (Simard *et al.*, 1998; Purdy *et al.*, 2002; Paudel *et al.*, 2015) and less due to ectomycorrhizal network connectivity (Kranabetter, 2005). In contrast, the decline in lodgepole pine seedling survival across the tree mortality gradient may be due, in part, to increased residual vegetative productivity with increasing resource availability in the understory post-disturbance (Despain, 2001).

It has been suggested that mycorrhizal network response can vary with site productivity (Simard, 2009a; Simard, 2009b) as predicted by the stress-gradient hypothesis, where increased facilitation among plants has been shown with environmental stress (Maestre *et al.*, 2009). In our study, seedling growth increased but survival was unaffected

with access to an ectomycorrhizal network. This might suggest that ectomycorrhizal networks benefit seedling growth more than survival in areas with increased resource availability (Simard, 2009a). In our attacked versus undisturbed stands, light, soil moisture, and nutrients were more plentiful and readily available (Cigan *et al.*, 2015) allowing lodgepole pine seedlings to quickly establish and stratify in height. In our study, access to an ectomycorrhizal network seems to be important, in part, to seedling growth (height and productivity) in more shaded and productive areas, possibly by supplementing carbon or nutrient supply (Simard & Vyse, 2006). Furthermore, although light varied across the gradient of tree mortality (Cigan *et al.*, 2015), height, total biomass and survival of lodgepole pine and white spruce seedlings did not change with increased light availability (Appendix 4.2). Similar to increases in light levels, soil moisture was greater in beetle-killed versus undisturbed stands (Cigan *et al.*, 2015; Pec *et al.*, 2015). However, white spruce seedlings did not respond in height, total biomass or survival to the MN treatments or increased soil moisture. In contrast, lodgepole pine seedling height increased while survival decreased as soil moisture levels rose across the gradient of tree mortality (Appendix 4.2). This suggests the increase in ^{13}C for lodgepole pine seedlings across the tree mortality gradient was due to an increase in photosynthetic capacity, not due to increase in water use efficiency. With increased light and foliar N across the tree mortality gradient, seedlings would have greater photosynthetic capacity, leading to more positive ^{13}C values.

The importance of residual trees as refugia for ectomycorrhizal fungal colonization has been shown to be vital to the increased survival and growth of seedlings (Kranabetter, 2000; Smith & Read, 2008). Residual trees, either conspecific or heterospecific tree species, growing in the subcanopy of forest stands can potentially serve as surrogate hubs for

networking fungi to establishing seedlings (Simard, 2009a; Beiler *et al.*, 2010; Beiler *et al.*, 2015). For example, ectomycorrhizal networks of residual trees have been shown to facilitate regeneration of *Pseudotsuga menziesii* seedlings under drought and root competition in interior dry forests of British Columbia (Bingham & Simard, 2012). In our study, residual trees in the subcanopy of beetle-killed stands may be acting as legacy trees for ectomycorrhizal fungi following disturbance, providing a robust network for the establishment, survival and growth of seedlings. Of lodgepole pine seedlings that survived in beetle-killed stands, connecting to the ectomycorrhizal network of living trees improved biomass and foliar nutrition.

In our study, ectomycorrhizal colonization did not differ among networking treatments, however did differ between tree species, with greater colonization of lodgepole pine than spruce seedlings in beetle-killed versus undisturbed stands. Both lodgepole pine and white spruce seedlings are colonized quickly (Despain, 2001; Gärtner *et al.*, 2011) and are able to associate with a broad range of ectomycorrhizal fungi (Molina *et al.*, 1992; Taylor & Sinsabaugh, 2015). A lack of MN treatment effect may be likely due to high ectomycorrhizal inoculum (i.e., spores, sclerotia, network hyphae) potential across the gradient of tree mortality. This may have led to rapid colonization of seedling rooting systems within MN treatments. Ectomycorrhizal inoculum occur at high levels following other disturbances such as clear-cutting (Jones *et al.*, 2003) and fire (Purdy *et al.*, 2002) provided there are trees or plant present to host the fungi.

Along with greater colonization, lodgepole pine seedling root systems hosted a greater diversity and different community of ectomycorrhizal fungi compared to white spruce seedlings (LM: F=111.37, P<0.0001; lodgepole pine: mean Shannon diversity: 2.37±0.05;

white spruce: mean Shannon diversity: 1.45±0.19). Greater diversity of ectomycorrhizal fungi has been proposed as a mechanism of facilitating seedling establishment (Newman, 1988; Simard & Durall, 2004). Previously, Nara (2006) demonstrated that different ectomycorrhizal fungi could affect seedling growth and N acquisition in volcanic soils. Similarly, Jones et al. (2009) found in clearcuts that N uptake by *Picea engelmannii* seedlings was associated with colonization and composition of ectomycorrhizal fungal communities (Jones *et al.*, 2009). In our study, lodgepole pine seedling growth and nutrition were enhanced with greater access to an ectomycorrhizal network, but only in the most severely beetle-disturbed stands and when root competition was excluded in the MN treatment. In contrast, white spruce seedlings had a lower diversity of ectomycorrhizal fungi and differed in ectomycorrhizal fungal composition on the rooting systems. This difference in host preference between lodgepole pine and white spruce seedlings may have accounted for the changes seen in growth and nutrition in lodgepole pine versus white spruce seedlings in the MN treatments.

5.5 Conclusion

Following widespread tree mortality, seedling establishment (growth, nutrition, and survival) was positively influenced by access to ectomycorrhizal networks. Contrary to our prediction, ectomycorrhizal networks seemed to not be degraded with stand level tree mortality potentially due to an ample supply of ectomycorrhizal inoculum (Teste *et al.*, 2009; Simard *et al.*, 2012) and maintained mycorrhizal network connectivity by remaining residual trees acting as refugia for ectomycorrhizal fungi (Simard, 2009a). Our results are also in congruence with those from field (Bingham & Simard, 2012) and greenhouse conditions (Song *et al.*, 2015) demonstrating that the beneficial effects of ectomycorrhizal networks are

reduced when in the presence of root competition. Song *et al.* (2015) also showed that competing roots could have been better scavengers for resources than the mycorrhizal networks alone. This recurring result where root competition appears to reduce network benefits begs further study. However, as disturbances, such as insect outbreaks, continue to intensify in forested systems of western North America (Weed *et al.*, 2013), our research points to mycorrhizal networks positioned to play an important role for enhancing seedling establishment in disturbed forests.

Table 5.1. Summary of linear models testing response of growth and survival of lodgepole pine and white spruce seedlings to mountain pine beetle induced tree mortality across three MN treatments.

	MN		**Tree mortality**		**MN x tree mortality**	
	$F_{1,9}$	P	$F_{1,9}$	P	$F_{1,9}$	P
Lodgepole pine						
Survival	1.92	0.168	20.95	**<0.001**	0.09	0.906
Height	0.75	0.482	34.15	**<0.001**	0.55	0.538
Total biomass	4.11	**0.029**	4.26	**0.049**	4.43	**0.022**
White spruce						
Survival	0.04	0.952	0.56	0.459	0.70	0.505
Height	4.04	**0.032**	1.30	0.265	0.08	0.921
Total biomass	0.91	0.417	10.02	**0.004**	0.40	0.675

Notes: Significant differences are in **bold.**

Table 5.2. Summary of linear models testing foliar N, foliar P, and foliar δ ^{13}C concentrations in lodgepole pine and white spruce seedlings to mountain pine beetle induced tree mortality across three MN treatments.

	MN		**Tree mortality**		**MN x tree mortality**	
	$F_{1,9}$	*P*	$F_{1,9}$	*P*	$F_{1,9}$	*P*
Lodgepole pine						
Foliar δ ^{13}C	0.11	0.895	16.16	**<0.001**	0.04	0.955
Foliar N	15.66	**<0.001**	22.65	**<0.001**	5.54	**0.010**
Foliar P	4.17	**0.027**	0.11	0.734	3.58	**0.043**
White spruce						
Foliar δ ^{13}C	0.16	0.850	0.30	0.586	0.76	0.475
Foliar N	0.68	0.516	9.76	**0.005**	0.68	0.516
Foliar P	0.50	0.608	0.09	0.921	0.38	0.684

Notes: Significant differences are in **bold.**

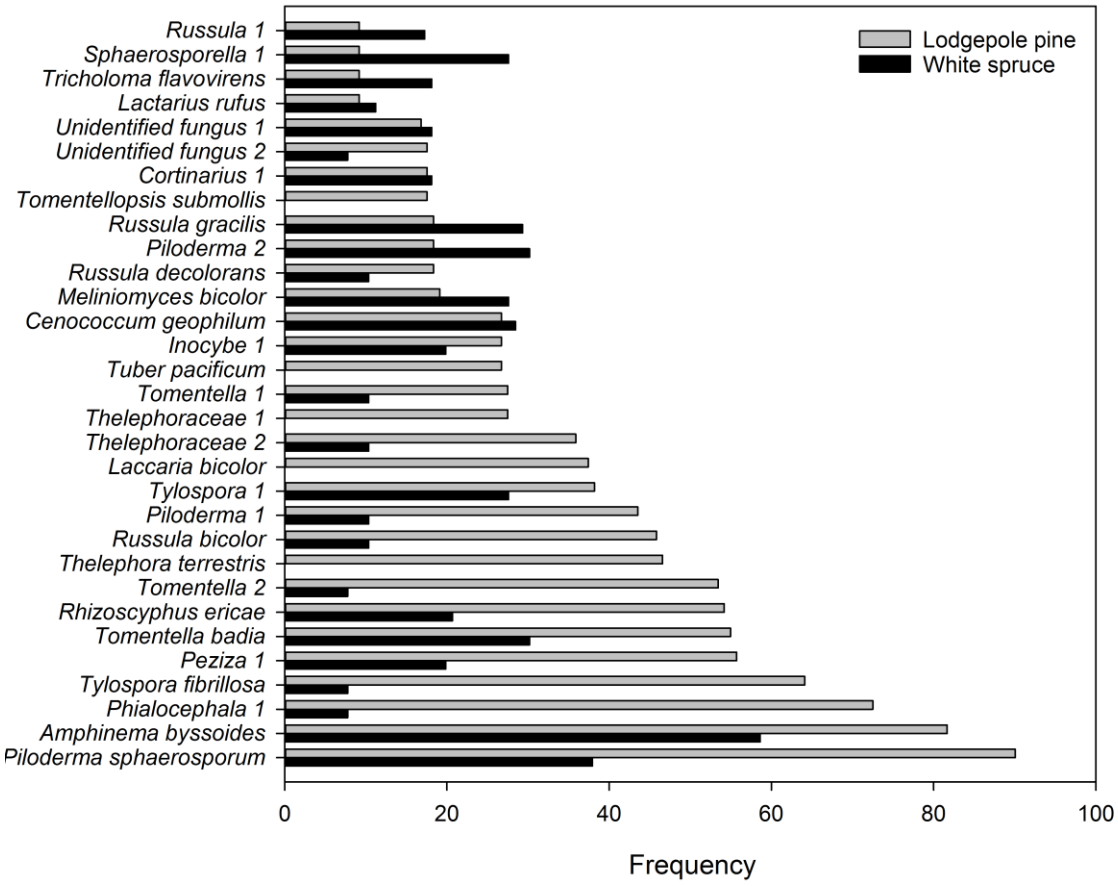

Figure 5.1. Frequency of occurrence of ectomycorrhizal taxa found on lodgepole pine and white spruce seedlings.

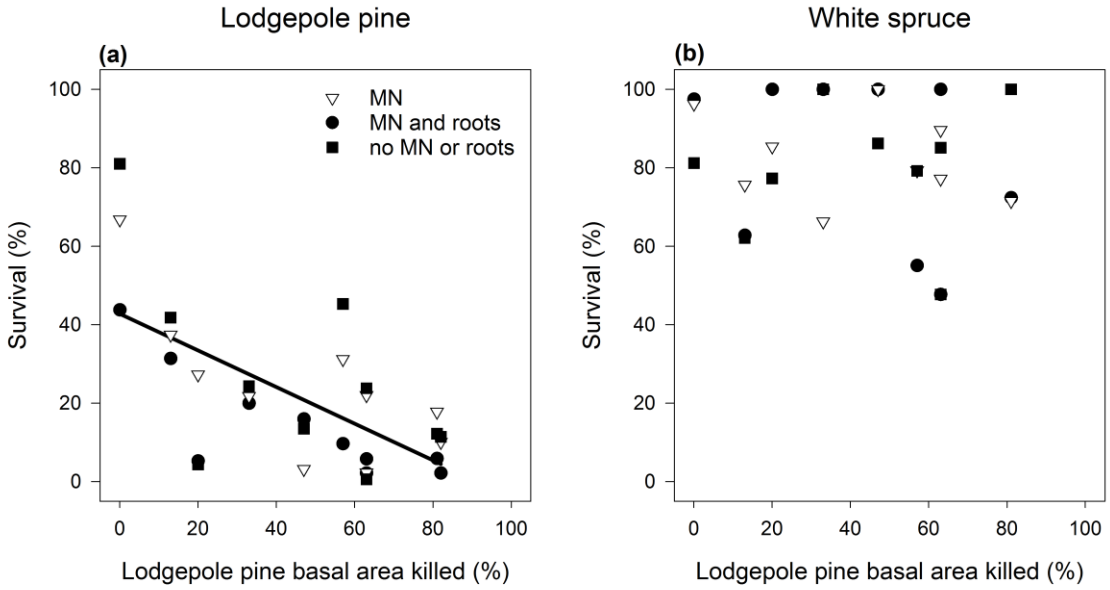

Figure 5.2. Survival of (a) lodgepole pine and (b) white spruce seedlings on each of three ectomycorrhizal network treatments across a gradient of lodgepole pine basal area killed (%) by mountain pine beetle.

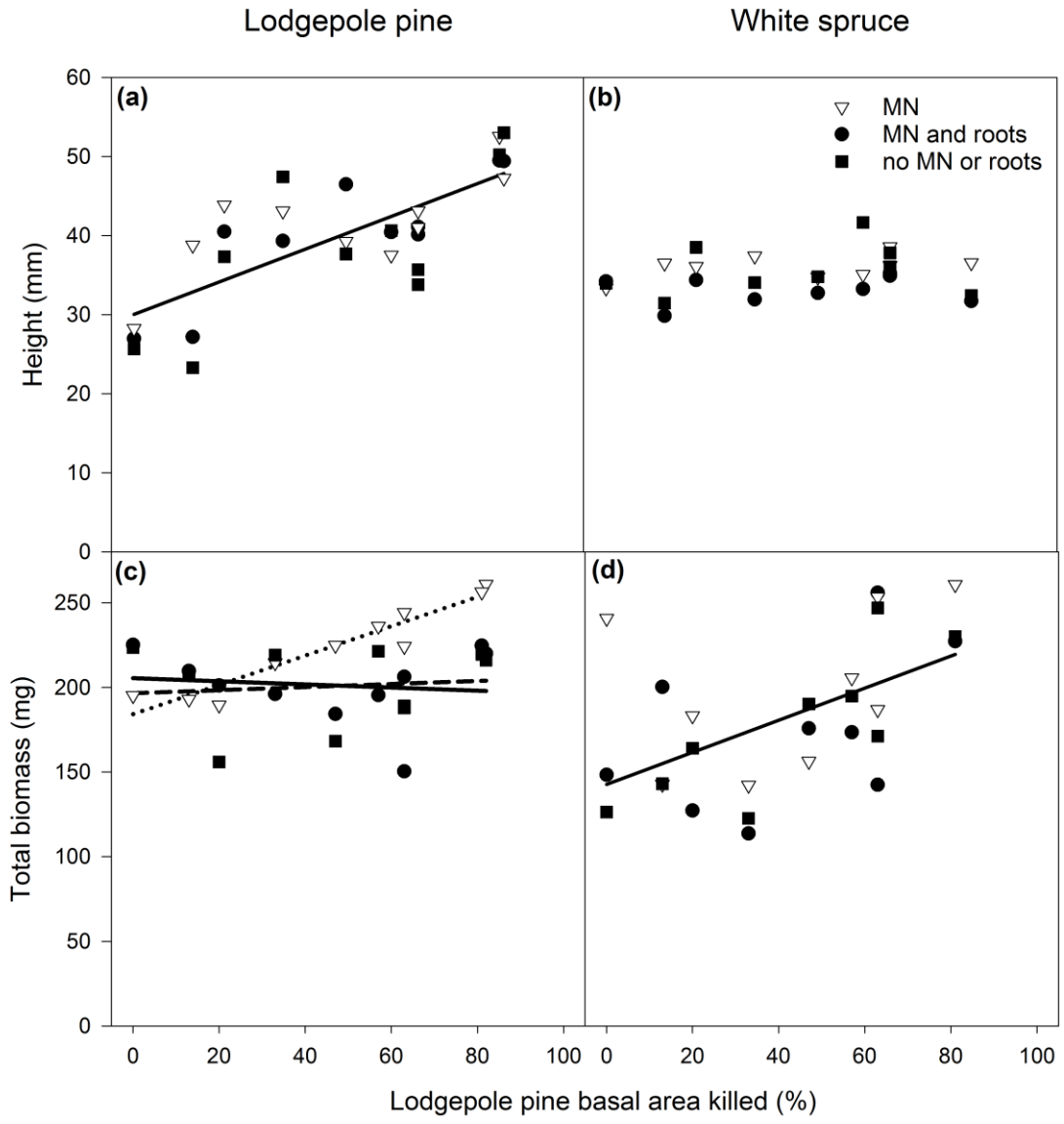

Figure 5.3. Height and total biomass of lodgepole pine (a, c) and white spruce (b, d) seedlings on each of three ectomycorrhizal network treatments across a gradient of lodgepole pine basal area killed (%) by mountain pine beetle.

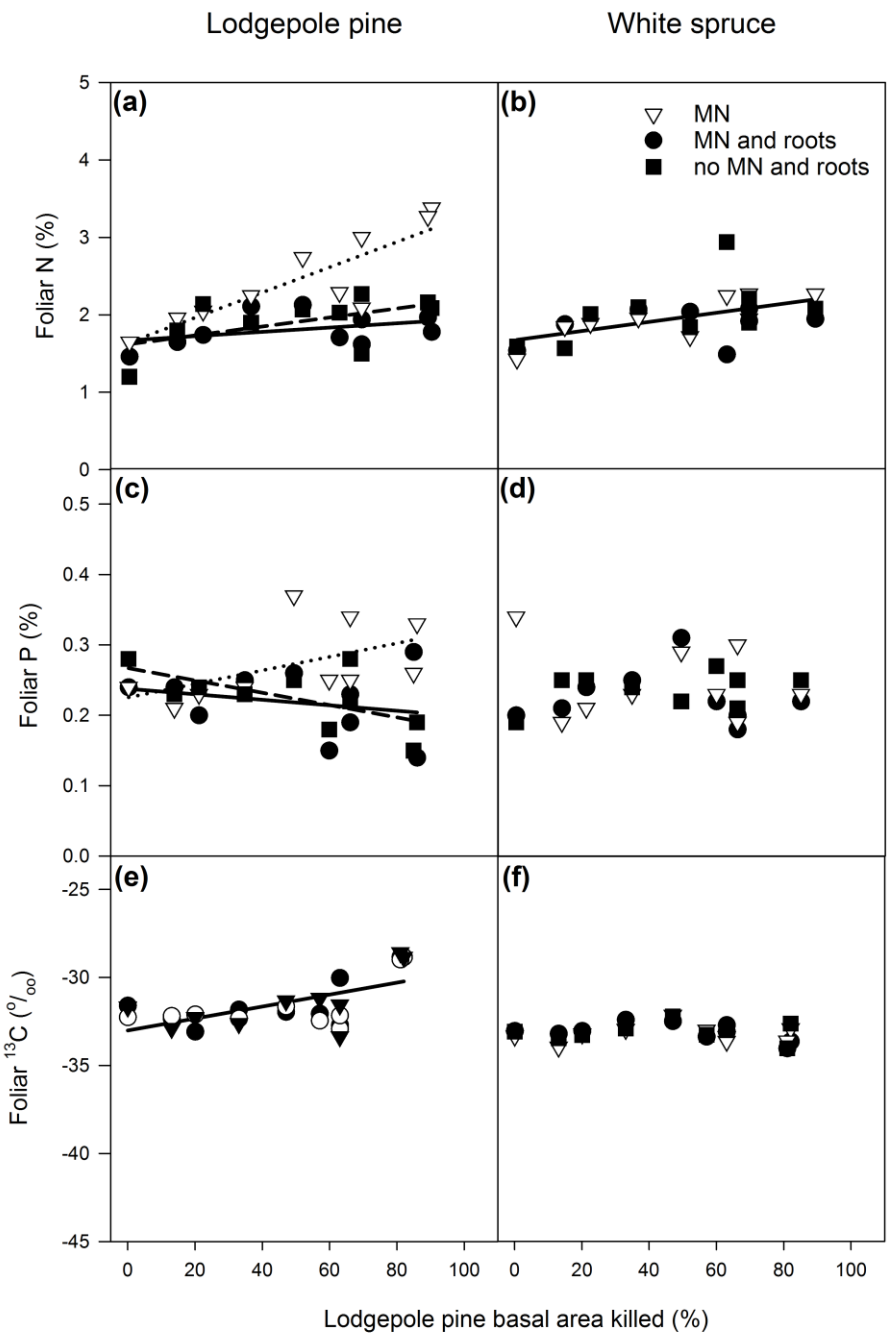

Figure 5.4. Foliar chemistry (N, P, ^{13}C) of lodgepole pine (a, c, e) and white spruce (b, d, f) seedlings on each of three ectomycorrhizal network treatments across a gradient of lodgepole pine basal area killed (%) by mountain pine beetle.

Chapter 6: General discussion and conclusion

The objectives of this thesis were to disentangle the relative importance of tree mortality following biotic disturbance (i.e., insect outbreak) from changes in soil chemistry following tree death, and in turn, its effect on: (1) the diversity and productivity of understory plant communities (Chapter 2); (2) the richness and composition of belowground fungal communities (Chapter 3); (3) the spatial structuring of ectomycorrhizal and saprotrophic fungal communities (Chapter 4) and (2) the functional importance of ectomycorrhizal fungal networks on tree seedling establishment (Chapter 5).

In Chapter 2, overall understory community diversity and productivity increased across the gradient of increased tree mortality. Richness of herbaceous perennials increased with tree mortality as well as with soil moisture and nutrient levels. In contrast, the diversity of woody perennials did not change across the same gradient. Understory vegetation, namely herbaceous perennials, showed an immediate response to improved growing conditions caused by increases in tree mortality. Similar observations of an increased response in advanced understory regeneration following *D. ponderosae*-induced tree mortality have been reported (Romme et al. 1986, Amoroso et al. 2013) and are worth further investigation for understory establishment and dispersal strategies. My findings suggest that in sites with increased vegetation following *D. ponderosae*-induced tree mortality, tree seedling recruitment and forest recovery may be delayed due to the potential release from belowground competition of beetle-killed trees. Further, how this increased pulse in understory richness and productivity affects future forest trajectories, particularly with respect to nutrient retention in the residual understory vegetation should be an important next step worth investigating.

In Chapter 3, the richness of ectomycorrhizal and saprotrophic fungi declined and the overall composition was altered by beetle-induced tree mortality. Soil nutrients, phenolics, and geographical location influenced the community structure of soil fungi; however, the relative importance of these factors differed between ectomycorrhizal and saprotrophic fungi. The independent effects of tree mortality, soil phenolics, and geographical location influenced the community composition of ectomycorrhizal fungi, while the community composition of saprotrophic fungi was weakly but significantly correlated with geographical location of plots. Taken together, my results indicate that both deterministic and stochastic processes structure soil fungal communities following landscape-level insect outbreaks and reflect the independent roles tree mortality, soil chemistry, and geographical location play in regulating the community composition of soil fungi.

In Chapter 4, I found that the spatial structuring of soil fungal communities was influenced by tree mortality, understory diversity and productivity, and soil moisture. Communities of ectomycorrhizal and saprotrophic fungi became more similar with increased tree death, while understory shoot productivity and diversity and soil moisture availability, which increased along the same gradient of tree mortality, also had a strong impact on both groups of soil fungal communities. Together, my results demonstrate that although ectomycorrhizal and saprotrophic fungi vary based on their trophic lifestyle, large-scale biotic disturbance essentially homogenizes the spatial patterning for both groups of soil communities by similar underlying environmental factors.

In Chapter 5, I found that lodgepole pine seedling survival decreased with beetle-induced tree mortality, while white spruce seedling survival was invariant across the same

gradient. Access to ectomycorrhizal networks improved growth and nutrition of lodgepole pine seedlings, but had not effect on survival of either pine or white spruce seedlings along the tree mortality gradient. Lodgepole pine seedlings with access to ectomycorrhizal networks had higher biomass and foliar N and P in stands with high tree mortality than in undisturbed stands. My results demonstrate that lodgepole pine and white spruce have differential regeneration strategies following insect-induced tree mortality. Contrary to my prediction, ectomycorrhizal networks seemed to not be degraded with stand level tree mortality potentially due to a ready supply of ectomycorrhizal inoculum and maintained ectomycorrhizal network connectivity by remaining residual trees, which acted as refugia for these fungi.

Conclusion

Taken together, this thesis demonstrates the far-reaching effects of biotic disturbance and emphasizes the interconnectedness between aboveground understory vegetation, trees, soils, and belowground soil fungi (Fig. 6.1). It remains uncertain what the successional trajectory of these forested systems will follow. However, based on this research, the occurrence of mountain pine beetle attacks in pine dominated stands may indirectly affect tree seedling recruitment through disrupted belowground mutualisms and shifts in overall soil fungal community composition, resulting in potentially more structurally and compositionally diverse stands aboveground. Whereas, belowground, I suggest that the consequences of biotic disturbance on overall shifts in the abundance and community composition of soil fungi extend beyond a single cohort of trees. Soil fungi play vital roles in ecosystem functions such as carbon flow and nutrient cycling as well as in forest

regeneration and succession (Read and Perez-Moreno 2003, Smith and Read 2008, Clemmensen et al. 2013). Variation in soil fungal community structure and function following landscape-level biotic disturbance may lead to multiple successional pathways for the boreal forest via their influence not only on host plant performance, but also ecosystem processes such as carbon and nutrient cycling and decomposition of organic matter.

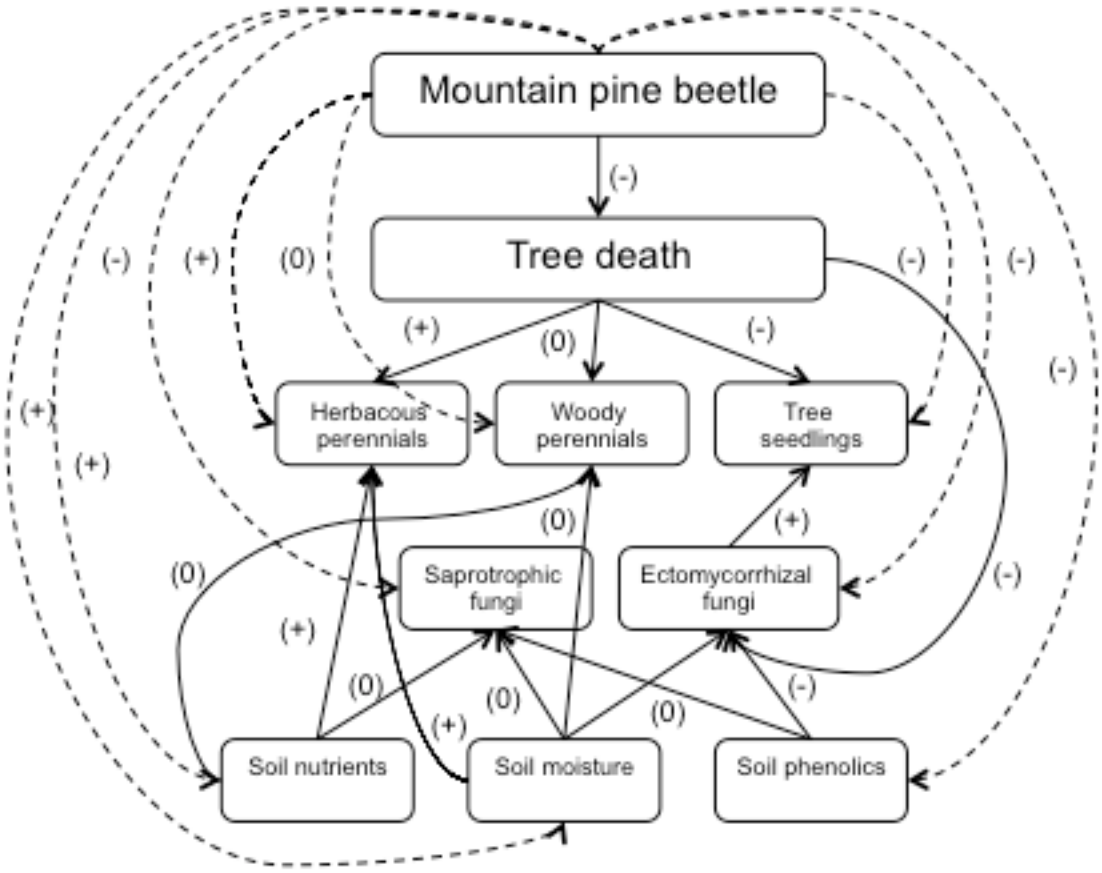

Figure 6.1. Direct and indirect effects of tree mortality on understory vegetation, soil fungi and soils following mountain pine beetle outbreak. Direct effects are represented by a solid line while indirect effects are represented by a dashed line with positive (+), negative (-) or neutral (0) symbols representing the direction of a given relationship.

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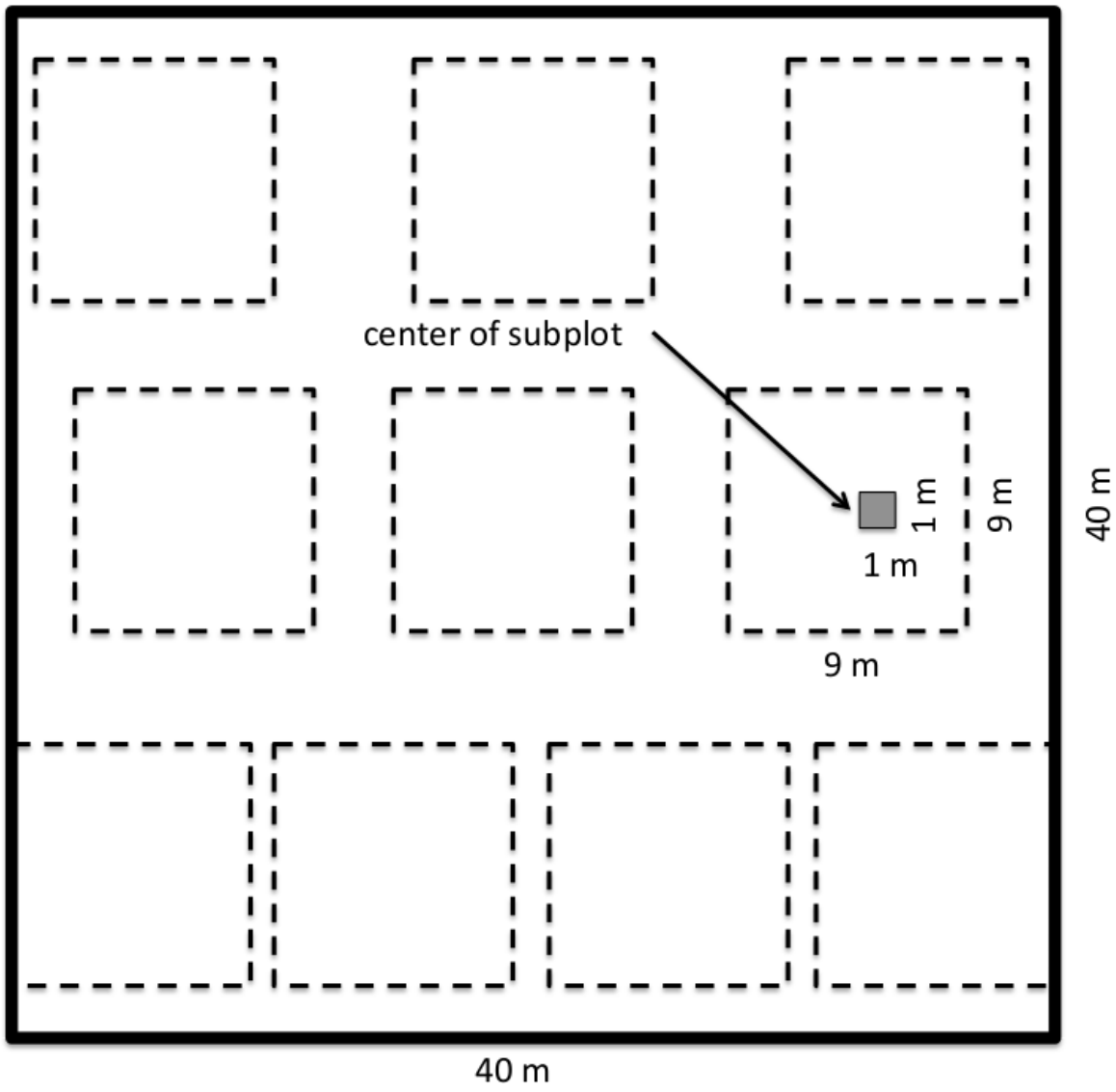
Appendices

Appendix 1.1. Representative site design for sampling understory diversity and productivity following recent mountain pine beetle activity (since 2009) across eleven sites located within the Lower Foothills natural subregion of west central Alberta.

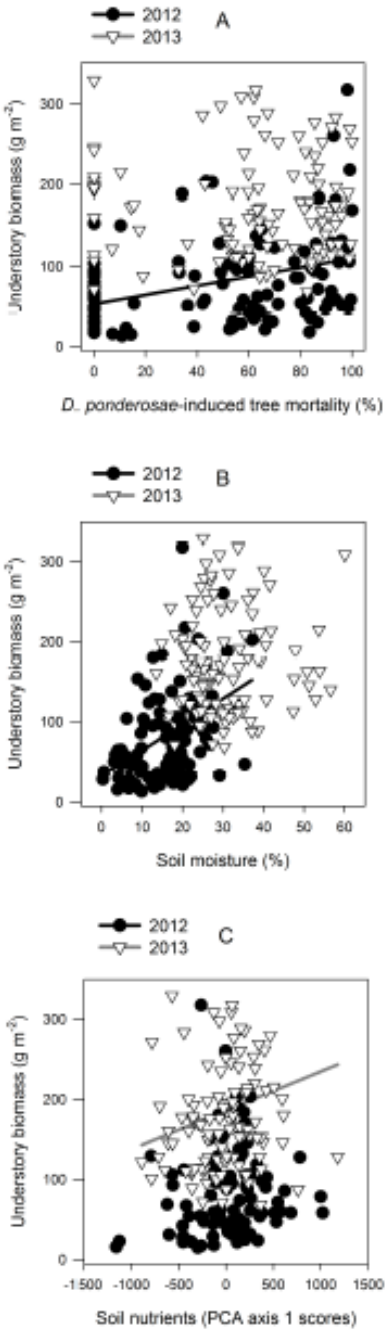


Appendix 1.2. A table of vascular plants present in sample plots along a gradient of lodgepole pine killed by mountain pine beetle.

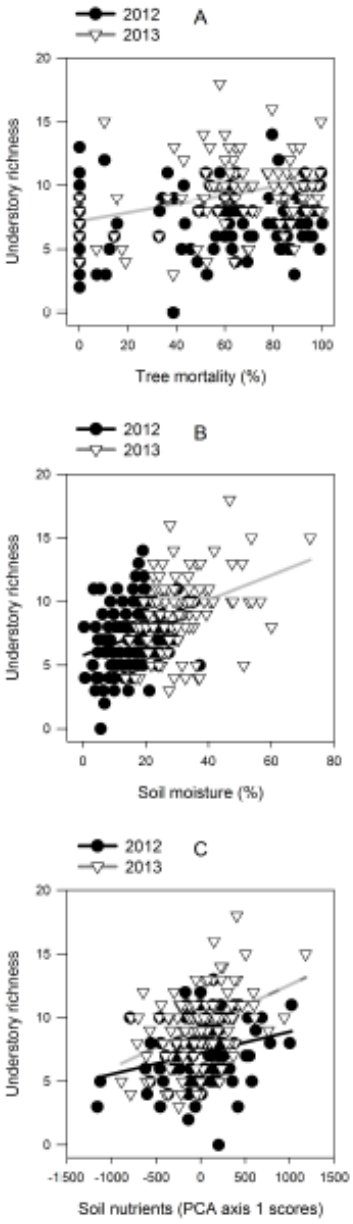
Species	Family	Functional group
Actaea rubra	Ranunculaceae	Herbaceous
Amelanchier alnifolia	Rosaceae	Woody
Aralia nudicaulis	Araliaceae	Herbaceous
Arnica cordifolia	Asteraceae	Herbaceous
Aster ciliolatus	Asteraceae	Herbaceous
Athyrium filix-femina	Dryopteridaceae	Herbaceous
Cornus canadensis	Cornaceae	Herbaceous
Disporum trachycarpum	Liliaceae	Herbaceous
Epilobium angustifolium	Onagraceae	Herbaceous
Equisetum pratense	Equisetaceae	Herbaceous
Eurybia conspicua	Asteraceae	Herbaceous
Galium triflorum	Rubiaceae	Herbaceous
Goodyera repens	Orchidaceae	Herbaceous
Gymnocarpium dryopteris	Dryopteridaceae	Herbaceous
Lathyrus ochroleucus	Fabaceae	Herbaceous
Ledum groenlandicum	Ericaceae	Woody
Linnaea borealis	Caprifoliaceae	Woody
Listera cordata	Orchidaceae	Herbaceous
Lonicera involucrata	Caprifoliaceae	Woody
Lycopodium annotinum	Lycopodiaceae	Herbaceous
Lycopodium complanatum	Lycopodiaceae	Herbaceous
Maianthemum candense	Liliaceae	Herbaceous
Mertensia paniculata	Boraginaceae	Herbaceous
Mitella nuda	Saxifragaceae	Herbaceous
Oplopanax horridus	Araliaceae	Woody
Orthilia secunda	Pyrolaceae	Woody
Petasites palmatus	Asteraceae	Herbaceous
Pyrola asarifolia	Pyrolaceae	Woody
Pyrola chlorantha	Pyrolaceae	Woody
Pyrola uniflora	Pyrolaceae	Herbaceous
Ribes lacustre	Grossulariaceae	Woody
Rosa acicularis	Rosaceae	Woody
Rubus parviflorus	Rosaceae	Woody

Rubus pedatus	Rosaceae	Herbaceous
Rubus pubescens	Rosaceae	Herbaceous
Smilacina racemosa	Liliaceae	Herbaceous
Spiraea betulifolia	Rosaceae	Woody
Streptopus amplexifolius	Liliaceae	Herbaceous
Tiarella trifoliata	Saxifragaceae	Herbaceous
Trifolium pratense	Fabaceae	Herbaceous
Vaccinium caespitosum	Ericaceae	Woody
Vaccinium myrtilloides	Ericaceae	Woody
Vaccinium vitis-idaea	Ericaceae	Woody
Viburnum edule	Caprifoliaceae	Woody
Viola renifolia	Violaceae	Herbaceous

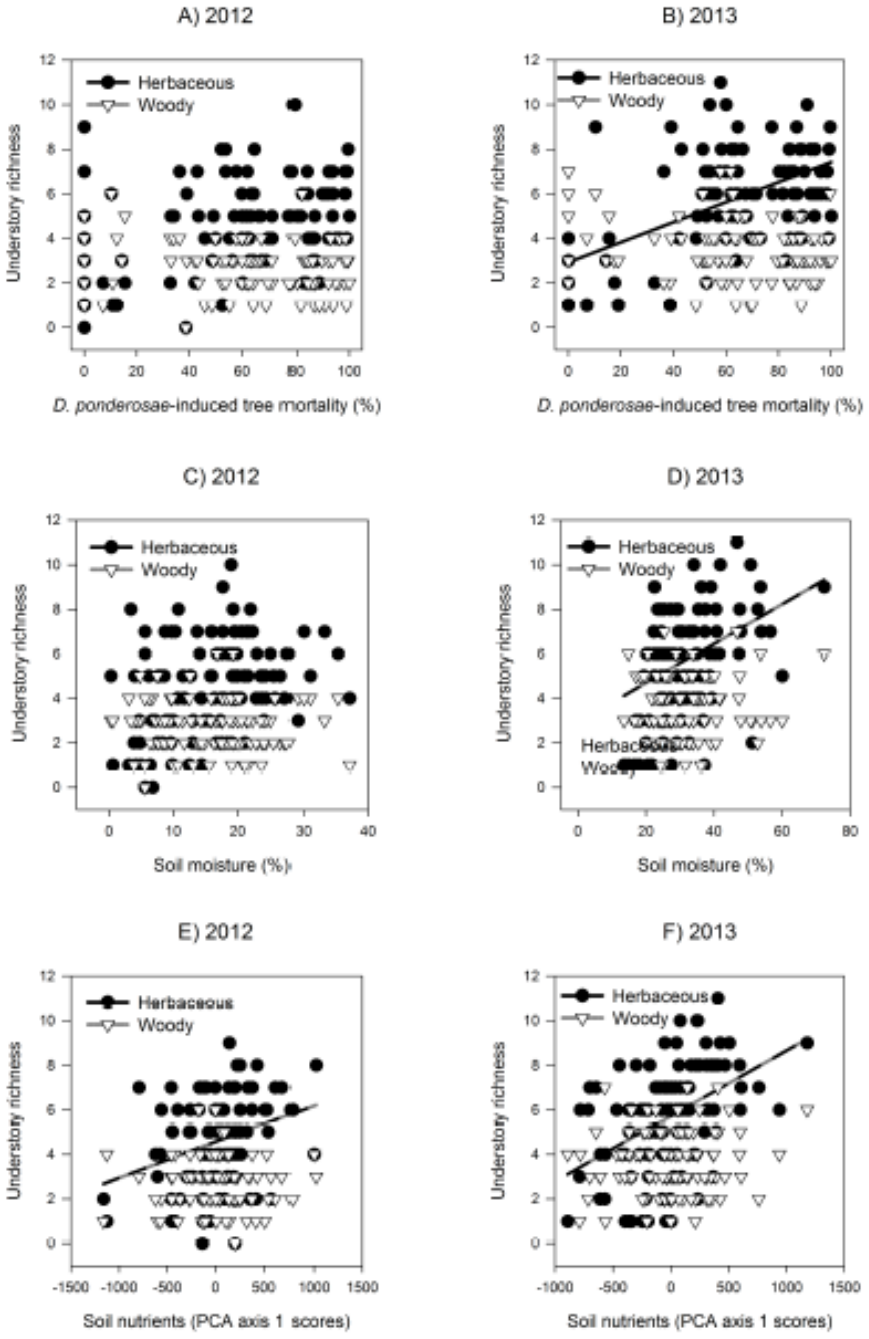
Appendix 1.3. Understory plant community biomass (g m^{-2}) in 2012 and 2013 as a function of (A) *Dendroctonus ponderosae*-induced tree mortality in *Pinus contorta* forests, (B) percent soil moisture, and (C) soil nutrients.



Appendix 1.4. Understory plant community richness in 2012 and 2013 as a function of (A) *Dendroctonus ponderosae*-induced tree mortality in *Pinus contorta* forests, (B) percent soil moisture, and (C) soil nutrients.



Appendix 1.5. Richness of herbaceous and woody perennials as a function of *Dendroctonus ponderosae*-induced tree mortality in (A) 2012 and (B) 2013, (B) percent soil moisture in (C) 2012 and (D) 2013 and soil nutrients in (E) 2012 and (F) 2013.



Appendix 2.1. Next-generation sequencing of fungi occurring in soils across a gradient of beetle-induced tree mortality in lodgepole pine (*Pinus contorta*) forests in west-central Alberta, Canada.

Molecular analysis

Genomic DNA was isolated from 250 mg of ground roots and soil using the CTAB protocol of (Roe *et al.*, 2010), a modification of the (Chang *et al.*, 1993) protocol. In brief, CTAB buffer (700 µL) was added to each sample, followed by 10 µL of proteinase K (600 mAU/mL; Qiagen Inc., Mississauga, ON, Canada). Samples were incubated at 65°C for 1 hour, cooled to 21°C, and 600 µL of 24:1 chloroform–isoamyl alcohol were added to sample tubes. Sample tubes were centrifuged for 5 minutes (17000 × ***g*** and 21°C). Aqueous supernatant was mixed with 600 µL isopropanol and chilled at −20 °C for 2 h. Samples were centrifuged for 15 min and supernatant discarded. 500 µL 95% ethanol (v/v) was added to the pellet, vortexed and centrifuged for 3 minutes and repeated with 500 µL 70% ethanol (v/v). Pellets were resuspended in 50 µl of nuclease-free water (Life Technologies) with gentle agitation.

Extracted DNA was quantified and checked for purity by spectrophotometric absorbance (A 260/280 nm)(Nanodrop, Thermo Fisher Scientific, MA, USA). A two-step PCR amplification was performed to amplify the internal transcribed spacer (ITS) 1 region of nuclear rDNA. The forward primer for the first PCR amplification was comprised of the A-adapter and the ITS1-F primer (Gardes & Bruns, 1993), whereas the reverse primer was comprised of the trP1 adapter and the ITS2 primer (White *et al.*, 1990). The forward primer for the second PCR amplification was comprised of a specific multiplex identifier (MID)

barcode and the A-adapter, whereas the reverse primer was comprised of the trP1 adapter. Ion Torrent PCR mixtures for the first PCR amplification contained 19.0 µl of Platinum PCR SuperMix High Fidelity (Invitrogen; ready-to-use mixture, Life Technologies, Carlsbad, CA, USA), 0.5 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, and 5 µl of DNA template. Ion Torrent™ PCR mixtures for the second PCR amplification contained 19.0 µl of Platinum PCR SuperMix High Fidelity (Invitrogen; ready-to-use mixture, Life Technologies, Carlsbad, CA, USA), 3.0 µl of 1.7 µM forward primer, 0.5 µl of 10.0 µM reverse primer, and 2.5 µl of template (1:25 dilution of PCR1). Negative control reactions contained the same mixtures with 5 µl of sterile water replacing the DNA template in the first PCR amplification. Thermocycler conditions used for the first PCR amplification were as follows: one cycle of 94ºC for 2 minutes; then 30 cycles of 94ºC for 30 seconds, 45ºC for 30 seconds, and 68ºC for 60 seconds; and ending with one cycle of 68ºC for 7 minutes. Thermocycler conditions used for the second PCR amplification were as follows: one cycle of 94ºC for 2 minutes; then 35 cycles of 94ºC for 30 seconds, 50ºC for 30 seconds, and 68ºC for 60 seconds; and ending with one cycle of 68ºC for 7 minutes. Successful amplification was confirmed using gel electrophoresis (1.5% agarose gel, 80V, 1.5 hours). Bands between 150-400 bp were excised from the gel and PCR products from successful amplifications were purified using Qiaquick gel extraction kit (Qiagen Inc., Mississauga, ON, Canada). Following gel extraction cleanup, products were quantified fluorescently using a dsDNA HS Assay kit on a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and pooled into equimolar concentrations. A second gel extraction cleanup was conducted on the pooled products using a Qiaquick gel extraction kit (Qiagen Inc., Mississauga, ON, Canada). Pooled products were quantified and diluted prior to emulsion PCR. An emulsion PCR quality check was

conducted prior to sequencing using an Ion OneTouch™ system (Life Technologies, Carlsbad, CA, USA) following manufacturer protocols. Amplicon library sequencing was performed on an Ion Torrent™ PGM 400 Sequencing Kit using 10 Ion 316™ Chips (Life Technologies, Carlsbad, CA, USA) at the Molecular Biological Sciences Facility, University of Alberta.

Bioinfomatic analysis

Ion Torrent™ data were processed using the QIIME pipeline v.1.8 (Caporaso *et al.*, 2010). Initial sequence processing and sample assignments were performed using the *split_libraries.py* script with a minimum sequence cutoff of 200 bp, maximum number of homopolymers of 10, maximum number of errors in barcodes of 0, maximum primer mismatches of 0, and minimum quality score of 25. Of the total sequence pool generated, we detected 0% of samples from PCR controls following initial quality filtering. Additional quality filtering was performed as part of the *pick_otus.py* script. Chimeric sequences were removed (12.3%) using a combination of *de novo* and reference-based chimera checks using the UCHIME algorithm (Edgar *et al.*, 2011) and referenced against the UNITE database (v.6) (Koljalg *et al.*, 2013). Sequences were clustered using the USEARCH algorithm (v.5.2.236) (Edgar, 2010) into operational taxonomic units (OTUs) using a 97% similarity threshold and a minimum cluster size of 2. Additionally, we excluded all global singletons and clusters with fewer than five reads were also removed to reduce artificially inflating richness due to sequencing error. Taxonomic affiliations were assigned by searching representative sequences from each OTU against GenBank and UNITE+INSD databases using the BLAST option in the *assign_taxonomy.py* script. OTUs were assigned to two groups

(ectomycorrhizal or saprotrophic) based on their genus and/or family affiliation, trophic mode and functional guild as described in (Branco *et al.*, 2013), referring to reviews by (Tedersoo *et al.*, 2010), (Tedersoo & Smith, 2013), and (Tedersoo *et al.*, 2014), and using the FUNGuild database (Nguyen *et al.*, 2016). OTUs were placed into either ectomycorrhizal or saprotrophic groupings only if assignments were deemed as highly probable (= absolute certain) or probable (= fairly certain) based on default parameters in the Guilds database (http://www.stbates.org/guilds/app.php). Non-fungal OTUs were excluded from further analyses while fungal OTUs that were assigned a genus affiliation though their trophic mode and/or functional guild was uncertain were evaluated as "unresolved fungi".

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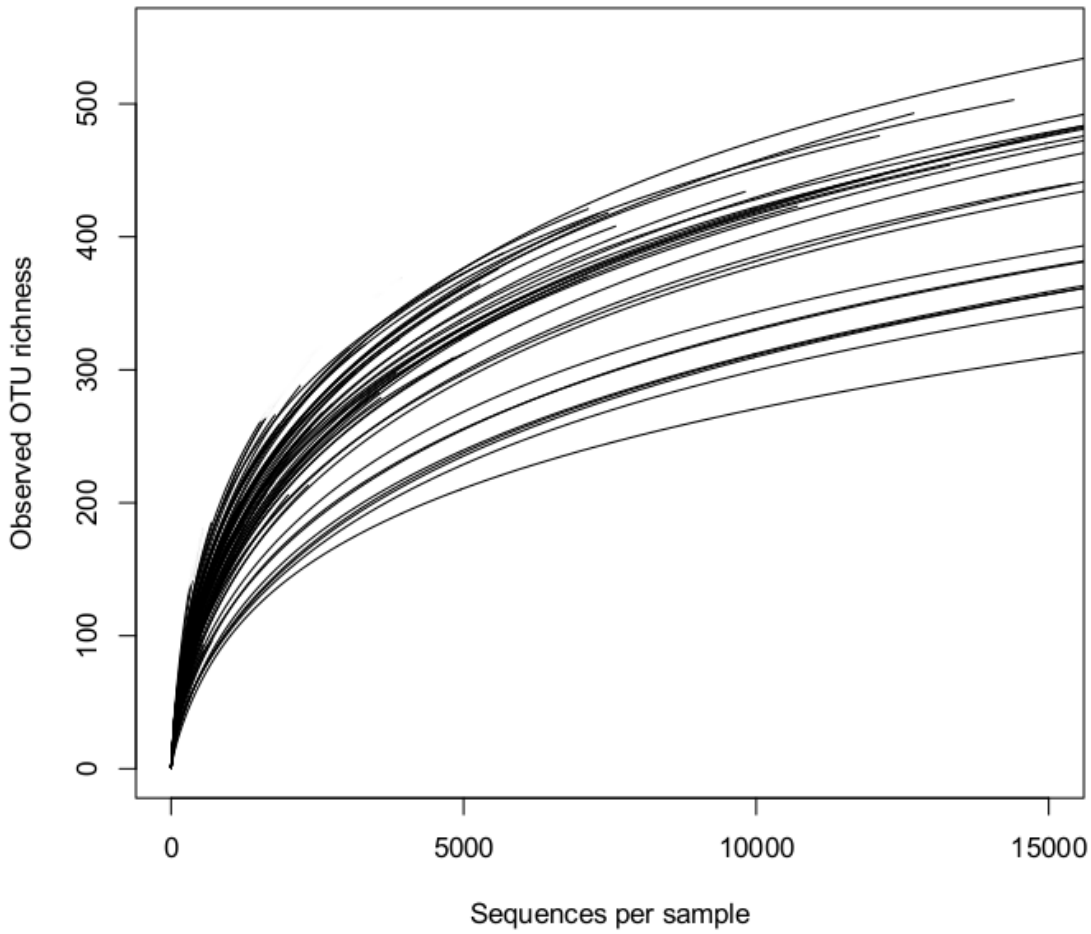
Appendix 2.2. Rarefaction curve of observed OTU richness as a function of the number of sequences per sample.



Appendix 2.3. Grouping assignments for soil fungi present in soil cores from stands within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta, Canada. Grouping assignments include: am = arbuscular mycorrhizal fungi, ecto = ectomycorrhizal fungi, pat = pathogenic fungi, sap = saprotrophic fungi, un = unidentified fungi, un-re = unresolved fungi.

OTU#	Taxonomic affiliation	Grouping
6	*Glomus* sp.5	am
19	Glomeromycota2	am
20	*Glomus* sp.2	am
80	*Glomus* sp.3	am
154	*Glomus* sp.4	am
159	Glomeraceae sp.1	am
203	*Scutellospora savannicola*	am
300	*Claroideoglomus* sp.1	am
310	Archaeosporales1	am
329	Glomeromycota4	am
423	*Glomus* sp.1	am
471	Glomeromycota5	am
591	*Glomus* sp.6	am
725	Glomeromycota1	am
750	Glomeromycota3	am
796	*Glomus indicum*	am
4	*Suillus flavidus*	ecto
7	*Inocybe* sp.2	ecto
9	*Russula* sp.5	ecto

10	*Cortinarius* sp.10	ecto
12	*Piloderma* sp.5	ecto
13	*Cortinarius* sp.9	ecto
24	*Elaphomyces* sp.1	ecto
36	*Pseudotomentella* sp.1	ecto
37	*Piloderma* sp.13	ecto
51	*Russula odorata*	ecto
52	*Wilcoxina* sp.1	ecto
54	*Tomentella* sp.6	ecto
56	*Piloderma* sp.8	ecto
57	*Russula brevipes*	ecto
61	*Inocybe* sp.3	ecto
65	*Tomentella bryophila*	ecto
77	*Inocybe sororia*	ecto
88	*Amphinema* sp.2	ecto
91	*Tomentella sublilacina*	ecto
101	*Tylospora* sp.3	ecto
103	*Cortinarius casimiri*	ecto
109	*Russula sanguinea*	ecto
114	*Inocybe lacera*	ecto
116	*Cortinarius* sp.13	ecto
126	*Lactarius rufus*	ecto
127	*Tylospora asterophora*	ecto
135	*Cenococcum* sp.2	ecto
136	*Cortinarius flexipes*	ecto
139	*Inocybe* sp.1	ecto

151	*Cenococcum* sp.1	ecto
153	*Russula americana*	ecto
162	*Cortinarius decipiens*	ecto
168	*Tomentella cinerascens*	ecto
172	*Cortinarius* sp.17	ecto
174	Hygrophorus sp.2	ecto
175	*Hygrophorus flavodiscus*	ecto
179	*Tomentella ramosissima*	ecto
185	*Russula* sp.5	ecto
188	*Amphinema byssoides*	ecto
197	*Sphaerosporella* sp.1	ecto
199	*Cortinarius* sp.8	ecto
207	*Cortinarius colymbadinus*	ecto
209	*Piloderma sphaerosporum*	ecto
210	*Laccaria bicolor*	ecto
229	*Tomentella* sp.2	ecto
239	*Tomentella* sp.5	ecto
240	*Russula* sp.1	ecto
250	*Cortinarius* sp.12	ecto
257	*Amphinema* sp.3	ecto
265	*Piloderma* sp.2	ecto
266	*Wilcoxina* sp.1	ecto
278	*Piloderma* sp.1	ecto
279	*Cortinarius* sp.1	ecto
294	*Russula* sp.4	ecto
295	*Piloderma* sp.10	ecto

305	*Russula* sp.10	ecto
318	*Tomentella* sp.1	ecto
319	*Piloderma lanatum*	ecto
325	*Tomentella* sp.7	ecto
326	*Tricholoma* sp.1	ecto
327	*Piloderma* sp.12	ecto
340	*Wilcoxina rehmii*	ecto
342	*Cortinarius* sp.10	ecto
348	*Sebacina vermifera*	ecto
352	*Wilcoxina* sp.3	ecto
358	*Piloderma* sp.2	ecto
362	*Inocybe sindonia*	ecto
371	*Russula bicolor*	ecto
374	*Russula foetens*	ecto
380	*Piloderma* sp.3	ecto
385	*Rhizopogon luteorubescens*	ecto
389	*Cenococcum geophilum*	ecto
390	*Pseudotomentella humicola*	ecto
396	*Lactarius* sp.1	ecto
402	*Tomentella ellisii*	ecto
403	*Cortinarius ochropallens*	ecto
409	*Tomentella subclavigera*	ecto
416	*Sebacina* sp.1	ecto
417	*Hymenogaster* sp.1	ecto
422	*Cortinarius* sp.5	ecto
426	*Tylospora* sp.2	ecto

429	*Cortinarius* sp.2	ecto
434	*Russula lutea*	ecto
436	*Tomentella* sp.3	ecto
438	*Amphinema* sp.4	ecto
470	*Cortinarius* sp.7	ecto
472	*Inocybe jacobi*	ecto
473	*Cortinarius* sp.14	ecto
474	*Tomentella terrestris*	ecto
481	*Cortinarius* sp.11	ecto
485	*Russula* sp.6	ecto
503	*Piloderma* sp.14	ecto
517	*Piloderma* sp.1	ecto
524	*Russula integra*	ecto
527	*Cortinarius* sp.3	ecto
529	*Tylospora* sp.4	ecto
531	*Cortinarius cf. saniosus*	ecto
533	*Piloderma* sp.9	ecto
536	*Amphinema* sp.1	ecto
548	*Tylospora* sp.1	ecto
550	*Thelephoraceae* sp.1	ecto
551	*Piloderma* sp.11	ecto
552	*Russula* sp.4	ecto
554	*Tricholoma atroviolaceum*	ecto
558	Thelephoraceae1	ecto
579	*Lactarius* sp.2	ecto
581	*Russula* sp.8	ecto

585	*Cortinarius* sp.16	ecto
588	*Russula* sp.3	ecto
589	*Cenococcum* sp.3	ecto
595	*Cortinarius* sp.4	ecto
596	*Tomentella* sp.9	ecto
599	*Cenococcum* sp.4	ecto
602	*Cortinarius* sp.15	ecto
603	*Piloderma byssinum*	ecto
609	*Wilcoxina* sp.2	ecto
613	*Tomentella badia*	ecto
622	*Russula versicolor*	ecto
623	*Hygrophorus* sp.1	ecto
631	*Russula* sp.7	ecto
635	*Cortinarius* sp.6	ecto
636	*Sebacina* sp.2	ecto
638	*Cortinarius uraceus*	ecto
640	*Russula xerampelina*	ecto
644	*Russula aeruginea*	ecto
645	*Suillus variegatus*	ecto
652	*Tylospora fibrillosa*	ecto
653	*Tomentella lapida*	ecto
658	*Inocybe giacomi*	ecto
662	*Tylospora* sp.1	ecto
667	*Russula* sp.9	ecto
674	*Tomentella* sp.3	ecto
676	*Tomentella* sp.2	ecto

677	*Piloderma* sp.6	ecto
682	*Piloderma* sp.16	ecto
691	*Hygrophorus* sp.1	ecto
693	*Russula* sp.2	ecto
694	*Cortinarius uraceomajalis*	ecto
698	*Russula* sp.3	ecto
706	*Tomentella* sp.1	ecto
710	*Russula* sp.8	ecto
713	*Russula* sp.2	ecto
715	*Pseudotomentella tristis*	ecto
721	*Sebacina* sp.4	ecto
733	*Piloderma olivaceum*	ecto
740	*Russula decolorans*	ecto
741	*Russula* sp.7	ecto
744	*Suillus* sp.1	ecto
747	*Russula* sp.1	ecto
748	*Piloderma* sp.17	ecto
751	Thelephoraceae2	ecto
752	*Amphinema* sp. 5	ecto
763	*Russula emetica*	ecto
766	*Tomentella* sp.8	ecto
783	*Piloderma* sp.7	ecto
785	*Tomentella* sp.4	ecto
794	*Piloderma* sp.15	ecto
799	*Cortinarius* sp.18	ecto
800	*Piloderma* sp.4	ecto

801	*Tomentella coerulea*	ecto
803	*Cortinarius aurantiobasis*	ecto
811	*Cortinarius laniger*	ecto
812	*Russula* sp.6	ecto
821	*Suillus tomentosus*	ecto
829	*Cortinarius brunneus*	ecto
836	*Tomentella subtestacea*	ecto
857	*Sebacina* sp.3	ecto
858	*Tuber pacificum*	ecto
2	*Tremella encephala*	pat
14	Hyaloscyphaceae sp.1	sap
16	*Cryptosporiopsis brunnea*	sap
33	*Trichosporon porosum*	sap
34	Zygomycete sp.2	sap
39	*Mortierella* sp.1	sap
44	*Umbelopsis* sp.2	sap
48	*Mortierella parvispora*	sap
64	*Umbelopsis isabellina*	sap
89	*Hymenoscyphus* sp.1	sap
98	Saccharomycetales5	sap
107	*Metschnikowia* sp.1	sap
108	*Phialea strobilina*	sap
117	*Sporobolomyces productus*	sap
128	*Mortierella* sp.4	sap
144	*Mortierella* sp.5	sap
149	Saccharomycetales6	sap

150	*Chalara holubovae*	sap
155	Dermateaceae2	sap
186	*Cladophialophora* sp.2	sap
194	*Mortierella* sp.2	sap
200	*Cladophialophora chaetospira*	sap
201	*Fibulobasidium murrhardtense*	sap
214	*Cryptococcus terricola*	sap
228	*Yarrowia* sp.1	sap
242	*Geoglossum barlae*	sap
246	Magnaporthaceae1	sap
247	*Monacrosporium bembicodes*	sap
248	*Mortierella pulchella*	sap
254	*Phoma herbarum*	pat
259	*Polyporus mikawai*	sap
263	Saccharomycetales4	sap
291	*Sugiyamaella paludigena*	sap
302	*Hypocrea pachybasioides*	sap
306	*Mortierella amoeboidea*	sap
308	Rhodotorula1	sap
312	*Dasyscyphella longistipitata*	sap
315	Xenasmataceae1	sap
332	*Leohumicola verrucosa*	sap
343	Dermateaceae3	sap
346	*Xenopolyscytalum pinea*	sap
357	*Mortierella* sp.1	sap
364	*Cryptococcus podzolicus*	sap

366	*Hymenoscyphus* sp.2	sap
376	Magnaporthaceae sp.1	sap
393	*Pichia fermentans*	sap
395	*Cladophialophora* sp.1	sap
408	Sordariomycetidae1	sap
414	*Lophium mytilinum*	sap
415	*Umbelopsis* sp.3	sap
418	*Inflatostereum aff. Glabrum*	sap
421	*Craterocolla cerasi*	sap
424	*Mortierella* sp.3	sap
432	*Mortierella* sp.3	sap
443	Leotiomycetes sp.2	sap
457	*Clavaria acuta*	sap
476	Dermateaceae1	sap
477	*Mortierella* sp.6	sap
482	Leotiomycetes1	sap
487	*Ilyonectria crassa*	pat
491	*Mortierella* sp.2	sap
501	*Lachnum* sp.2	sap
502	*Lachnum* sp.1	sap
519	*Umbelopsis* sp.1	sap
534	*Cladophialophora* sp.2	sap
540	*Verticillium* sp.1	pat
543	*Venturia* sp.1	pat
557	*Ramariopsis* sp.1	sap
565	Hyaloscyphaceae1	sap

577	*Hyaloscypha aureliella*	sap
590	Leotiomycetes sp.3	sap
593	*Chaetomella* sp.1	sap
605	*Leohumicola minima*	sap
612	Saccharomycetales3	sap
615	*Galactomyces candidum*	sap
619	Saccharomycetales2	sap
637	*Mortierella macrocystis*	sap
657	*Cryptococcus filicatus*	sap
680	Umbelopsidaceae sp.1	sap
686	*Cladophialophora* sp.3	sap
688	*Hyaloscypha* sp.1	sap
692	*Cryptococcus* sp.1	sap
701	*Mortierella minutissima*	sap
707	*Hypocrea parapilulifera*	sap
712	Saccharomycetales1	sap
726	Magnaporthaceae2	sap
754	*Botrytis caroliniana*	sap
758	*Lachnum* sp.3	sap
765	*Yarrowia lipolytica*	sap
779	*Mortierella globulifera*	sap
780	*Mycena flavoalba*	sap
781	*Penicillium bialowiezense*	sap
789	*Rhodotorula cresolica*	sap
792	*Verticillium fungicola*	pat
818	Saccharomycetales sp.1	sap

823	*Mortierella* sp.1	sap
827	*Hymenoscyphus* sp.1	sap
828	*Mortierella* sp.1	sap
852	*Lachnellula calyciformis*	sap
855	*Cryptococcus victoriae*	sap
859	*Dactylella mammillata*	sap
860	*Mortierella longigemmata*	sap
1	unidentified fungus sp.431	un
3	unidentified fungus sp.289	un
5	unidentified fungus sp.65	un
8	unidentified fungus sp.298	un
11	unidentified fungus sp.369	un
15	unidentified fungus sp.168	un
17	unidentified fungus sp.317	un
18	unidentified fungus sp.138	un
21	Pezizales3	un
22	unidentified fungus sp.393	un
23	Ascomycota11	un
25	unidentified fungus sp.224	un
26	Agaricales2	un
27	unidentified fungus sp.335	un
28	unidentified fungus sp.172	un
29	unidentified fungus sp.11	un
30	unidentified fungus sp.119	un
31	unidentified fungus sp.137	un
32	Herpotrichiellaceae1	un

35	unidentified fungus sp.124	un
38	unidentified fungus sp.145	un
40	Ascomycota6	un
41	unidentified fungus sp.263	un
42	unidentified fungus sp.422	un
43	unidentified fungus sp.437	un
45	unidentified fungus sp.343	un
46	unidentified fungus sp.123	un
47	unidentified fungus sp.254	un
49	unidentified fungus sp.154	un
50	Ascomycota12	un
53	unidentified fungus sp.351	un
55	unidentified fungus sp.284	un
58	unidentified fungus sp.151	un
59	unidentified fungus sp.55	un
60	unidentified fungus sp.353	un
62	unidentified fungus sp.446	un
63	Basidiomycota sp.1	un
66	unidentified fungus sp.69	un
67	unidentified fungus sp.90	un
69	unidentified fungus sp.426	un
71	unidentified fungus sp.12	un
72	Helotiales1	un
73	unidentified fungus sp.24	un
74	unidentified fungus sp.326	un
75	unidentified fungus sp.406	un

76	unidentified fungus sp.51	un
78	unidentified fungus sp.83	un
79	unidentified fungus sp.33	un
81	Ascomycota5	un
82	Agaricomycetes11	un
83	unidentified fungus sp.192	un
84	unidentified fungus sp.313	un
85	unidentified fungus sp.344	un
86	unidentified fungus sp.67	un
87	Helotiales18	un
90	unidentified fungus sp.257	un
92	Agaricomycetes1	un
93	unidentified fungus sp.204	un
94	unidentified fungus sp.334	un
95	unidentified fungus sp.312	un
96	unidentified fungus sp.445	un
97	unidentified fungus sp.357	un
99	Basidiomycota8	un
100	unidentified fungus sp.75	un
102	unidentified fungus sp.3	un
104	unidentified fungus sp.285	un
105	unidentified fungus sp.20	un
106	unidentified fungus sp.306	un
110	unidentified fungus sp.129	un
111	unidentified fungus sp.249	un
112	unidentified fungus sp.10	un

113	unidentified fungus sp.19	un
118	unidentified fungus sp.319	un
119	unidentified fungus sp.157	un
120	unidentified fungus sp.160	un
121	unidentified fungus sp.188	un
122	unidentified fungus sp.233	un
123	unidentified fungus sp.366	un
124	unidentified fungus sp.64	un
125	unidentified fungus sp.60	un
129	unidentified fungus sp.440	un
130	unidentified fungus sp.162	un
131	unidentified fungus sp.27	un
132	unidentified fungus sp.308	un
133	unidentified fungus sp.148	un
134	unidentified fungus sp.397	un
137	unidentified fungus sp.386	un
138	unidentified fungus sp.248	un
140	unidentified fungus sp.81	un
141	unidentified fungus sp.418	un
142	unidentified fungus sp.45	un
143	unidentified fungus sp.89	un
145	unidentified fungus sp.361	un
146	unidentified fungus sp.101	un
147	unidentified fungus sp.99	un
148	unidentified fungus sp.424	un
152	unidentified fungus sp.427	un

156	unidentified fungus sp.383	un
157	unidentified fungus sp.97	un
158	unidentified fungus sp.37	un
160	unidentified fungus sp.370	un
161	unidentified fungus sp.276	un
164	Helotiales10	un
165	unidentified fungus sp.333	un
166	unidentified fungus sp.96	un
167	unidentified fungus sp.4	un
169	unidentified fungus sp.63	un
170	unidentified fungus sp.410	un
171	unidentified fungus sp.223	un
173	Ascomycota8	un
176	unidentified fungus sp.118	un
177	unidentified fungus sp.180	un
178	unidentified fungus sp.282	un
180	unidentified fungus sp.395	un
181	unidentified fungus sp.22	un
182	unidentified fungus sp.195	un
184	unidentified fungus sp.35	un
187	unidentified fungus sp.174	un
189	unidentified fungus sp.372	un
190	Lecanoromycetidae1	un
192	Basidiomycota2	un
195	unidentified fungus sp.381	un
196	unidentified fungus sp.201	un

198	unidentified fungus sp.212	un
202	unidentified fungus sp.110	un
204	unidentified fungus sp.179	un
206	unidentified fungus sp.87	un
208	unidentified fungus sp.68	un
211	Boletales1	un
212	unidentified fungus sp.7	un
213	unidentified fungus sp.362	un
215	unidentified fungus sp.421	un
216	Pezizales6	un
217	unidentified fungus sp.115	un
218	unidentified fungus sp.44	un
219	unidentified fungus sp.278	un
220	unidentified fungus sp.73	un
222	unidentified fungus sp.331	un
223	unidentified fungus sp.238	un
225	unidentified fungus sp.186	un
226	unidentified fungus sp.17	un
227	unidentified fungus sp.261	un
230	unidentified fungus sp.365	un
231	unidentified fungus sp.208	un
232	unidentified fungus sp.392	un
233	unidentified fungus sp.330	un
234	unidentified fungus sp.328	un
235	unidentified fungus sp.6	un
236	unidentified fungus sp.246	un

237	unidentified fungus sp.78	un
238	unidentified fungus sp.403	un
241	unidentified fungus sp.401	un
243	unidentified fungus sp.364	un
244	unidentified fungus sp.336	un
245	unidentified fungus sp.363	un
249	unidentified fungus sp.420	un
251	unidentified fungus sp.402	un
252	unidentified fungus sp.367	un
253	unidentified fungus sp.42	un
255	Ascomycota17	un
256	unidentified fungus sp.135	un
258	unidentified fungus sp.318	un
260	unidentified fungus sp.128	un
261	unidentified fungus sp.382	un
262	unidentified fungus sp.80	un
264	unidentified fungus sp.149	un
267	unidentified fungus sp.216	un
268	unidentified fungus sp.191	un
269	Ascomycota7	un
270	unidentified fungus sp.345	un
271	unidentified fungus sp.217	un
272	Agaricomycetes4	un
273	unidentified fungus sp.79	un
274	Agaricomycetes6	un
276	unidentified fungus sp.280	un

277	unidentified fungus sp.84	un
280	unidentified fungus sp.423	un
281	unidentified fungus sp.450	un
282	Helotiales16	un
283	Auriculariales1	un
284	unidentified fungus sp.267	un
285	unidentified fungus sp.355	un
287	unidentified fungus sp.21	un
288	unidentified fungus sp.163	un
289	unidentified fungus sp.239	un
290	unidentified fungus sp.52	un
292	unidentified fungus sp.32	un
293	unidentified fungus sp.347	un
296	Atheliaceae 2	un
297	Agaricomycetes2	un
298	unidentified fungus sp.169	un
299	Helotiales3	un
301	unidentified fungus sp.415	un
303	unidentified fungus sp.209	un
304	unidentified fungus sp.120	un
307	unidentified fungus sp.213	un
309	unidentified fungus sp.325	un
311	unidentified fungus sp.18	un
313	unidentified fungus sp.405	un
314	unidentified fungus sp.143	un
316	unidentified fungus sp.274	un

317	unidentified fungus sp.377	un
320	Basidiomycota6	un
321	Agaricomycetes9	un
322	unidentified fungus sp.388	un
323	unidentified fungus sp.309	un
324	Basidiomycota5	un
328	unidentified fungus sp.176	un
330	unidentified fungus sp.130	un
331	unidentified fungus sp.412	un
333	unidentified fungus sp.181	un
334	Helotiales9	un
335	unidentified fungus sp.231	un
336	Ascomycota4	un
337	unidentified fungus sp.288	un
338	Ceratobasidiaceae sp.1	un
339	unidentified fungus sp.77	un
341	unidentified fungus sp.256	un
344	unidentified fungus sp.332	un
345	Ascomycota3	un
347	unidentified fungus sp.74	un
349	unidentified fungus sp.226	un
350	unidentified fungus sp.374	un
351	Agaricomycetes3	un
354	unidentified fungus sp.305	un
355	unidentified fungus sp.107	un
356	unidentified fungus sp.371	un

359	unidentified fungus sp.219	un
360	Agaricales1	un
361	Sebacinales2	un
363	unidentified fungus sp.414	un
365	unidentified fungus sp.98	un
367	Pezizales2	un
368	Pezizales10	un
369	unidentified fungus sp.9	un
370	unidentified fungus sp.131	un
372	unidentified fungus sp.251	un
373	unidentified fungus sp.56	un
375	unidentified fungus sp.206	un
377	Agaricales3	un
378	unidentified fungus sp.72	un
379	unidentified fungus sp.438	un
381	Ascomycota sp.1	un
382	unidentified fungus sp.300	un
384	unidentified fungus sp.352	un
386	unidentified fungus sp.173	un
387	unidentified fungus sp.23	un
388	unidentified fungus sp.210	un
391	unidentified fungus sp.449	un
392	unidentified fungus sp.265	un
394	Pezizales9	un
397	unidentified fungus sp.242	un
398	unidentified fungus sp.316	un

399	unidentified fungus sp.41	un
400	unidentified fungus sp.245	un
401	Ascomycota19	un
404	unidentified fungus sp.271	un
405	unidentified fungus sp.296	un
406	unidentified fungus sp.112	un
407	Helotiales6	un
410	unidentified fungus sp.350	un
411	unidentified fungus sp.240	un
412	unidentified fungus sp.13	un
413	unidentified fungus sp.193	un
419	Pezizales sp.1	un
420	unidentified fungus sp.400	un
425	unidentified fungus sp.277	un
427	unidentified fungus sp.109	un
428	unidentified fungus sp.222	un
430	unidentified fungus sp.159	un
431	unidentified fungus sp.152	un
433	unidentified fungus sp.66	un
435	unidentified fungus sp.340	un
437	Pezizales7	un
439	Helotiales13	un
440	unidentified fungus sp.229	un
441	unidentified fungus sp.104	un
442	unidentified fungus sp.196	un
444	Tremellales1	un

445	unidentified fungus sp.62	un
446	unidentified fungus sp.273	un
447	unidentified fungus sp.448	un
448	unidentified fungus sp.354	un
450	unidentified fungus sp.264	un
451	unidentified fungus sp.425	un
452	unidentified fungus sp.390	un
453	unidentified fungus sp.189	un
454	unidentified fungus sp.384	un
455	unidentified fungus sp.85	un
456	unidentified fungus sp.134	un
458	unidentified fungus sp.349	un
459	unidentified fungus sp.447	un
460	unidentified fungus sp.214	un
461	unidentified fungus sp.404	un
462	unidentified fungus sp.286	un
464	Sebacinales1	un
465	unidentified fungus sp.218	un
466	unidentified fungus sp.451	un
467	unidentified fungus sp.61	un
468	unidentified fungus sp.146	un
469	unidentified fungus sp.250	un
475	unidentified fungus sp.385	un
479	unidentified fungus sp.389	un
480	unidentified fungus sp.136	un
483	unidentified fungus sp.391	un

484	unidentified fungus sp. 23	un
486	unidentified fungus sp.227	un
488	unidentified fungus sp.170	un
489	unidentified fungus sp.47	un
490	unidentified fungus sp.122	un
492	Ascomycota15	un
493	unidentified fungus sp.153	un
494	unidentified fungus sp.320	un
495	unidentified fungus sp.294	un
496	unidentified fungus sp.164	un
497	unidentified fungus sp.435	un
498	unidentified fungus sp.71	un
499	unidentified fungus sp.144	un
500	Ascomycota10	un
504	unidentified fungus sp.434	un
505	Basidiomycota9	un
506	unidentified fungus sp.295	un
507	unidentified fungus sp.54	un
508	unidentified fungus sp.31	un
509	Pezizales4	un
510	unidentified fungus sp.268	un
511	unidentified fungus sp.46	un
512	unidentified fungus sp.262	un
513	unidentified fungus sp.399	un
514	unidentified fungus sp.442	un
515	unidentified fungus sp.237	un

516	unidentified fungus sp.133	un
518	unidentified fungus sp.411	un
520	Agaricomycetes7	un
521	unidentified fungus sp.125	un
522	unidentified fungus sp.409	un
523	unidentified fungus sp.235	un
525	unidentified fungus sp.202	un
526	unidentified fungus sp.111	un
528	unidentified fungus sp.141	un
530	unidentified fungus sp.25	un
532	unidentified fungus sp.116	un
535	unidentified fungus sp.53	un
537	unidentified fungus sp.255	un
538	unidentified fungus sp.58	un
539	unidentified fungus sp.301	un
541	unidentified fungus sp.453	un
542	Helotiales17	un
544	unidentified fungus sp.258	un
545	unidentified fungus sp.167	un
546	unidentified fungus sp.38	un
549	Hysterangiales 1	un
553	unidentified fungus sp.356	un
555	unidentified fungus sp.339	un
556	unidentified fungus sp.396	un
559	unidentified fungus sp.419	un
560	unidentified fungus sp.378	un

561	unidentified fungus sp.430	un
562	unidentified fungus sp.327	un
563	unidentified fungus sp.324	un
564	unidentified fungus sp.156	un
566	Pezizales8	un
567	unidentified fungus sp.142	un
568	unidentified fungus sp.293	un
569	unidentified fungus sp.30	un
570	unidentified fungus sp.16	un
571	unidentified fungus sp.444	un
572	unidentified fungus sp.373	un
573	unidentified fungus sp.247	un
574	unidentified fungus sp.346	un
575	unidentified fungus sp.114	un
576	unidentified fungus sp.398	un
578	unidentified fungus sp.158	un
580	unidentified fungus sp.106	un
583	unidentified fungus sp.387	un
584	Atheliaceae 1	un
586	unidentified fungus sp.368	un
587	Galactomyces1	un
592	unidentified fungus sp.70	un
594	unidentified fungus sp.178	un
597	unidentified fungus sp.150	un
598	unidentified fungus sp.375	un
600	unidentified fungus sp.48	un

601	unidentified fungus sp.290	un
604	unidentified fungus sp.279	un
606	Ascomycota16	un
607	unidentified fungus sp.441	un
608	unidentified fungus sp.408	un
610	Helotiales12	un
611	unidentified fungus sp.34	un
614	unidentified fungus sp.359	un
616	unidentified fungus sp.161	un
617	unidentified fungus sp.225	un
618	Ascomycota13	un
620	unidentified fungus sp.40	un
621	Helotiales14	un
624	unidentified fungus sp.297	un
625	unidentified fungus sp.270	un
626	Helotiales11	un
627	unidentified fungus sp.59	un
628	Lecanorales1	un
629	unidentified fungus sp.2	un
630	unidentified fungus sp.341	un
632	unidentified fungus sp.175	un
633	Helotiales sp.1	un
634	Agaricomycetes5	un
639	unidentified fungus sp.358	un
641	unidentified fungus sp.43	un
642	Pezizales1	un

643	Ascomycota sp.3	un
646	Helotiales4	un
647	Helotiales2	un
648	leptodontium sp.2	un
650	Ascomycota14	un
651	Basidiomycota7	un
654	unidentified fungus sp.436	un
655	unidentified fungus sp.413	un
656	unidentified fungus sp.417	un
660	Basidiomycota10	un
661	unidentified fungus sp.394	un
663	unidentified fungus sp.117	un
664	unidentified fungus sp.165	un
665	unidentified fungus sp.323	un
666	unidentified fungus sp.314	un
668	unidentified fungus sp.140	un
669	unidentified fungus sp.86	un
670	Basidiomycota4	un
671	unidentified fungus sp.57	un
672	unidentified fungus sp.198	un
673	unidentified fungus sp.322	un
675	unidentified fungus sp.315	un
678	Agaricomycetes10	un
679	unidentified fungus sp.190	un
683	unidentified fungus sp.287	un
685	unidentified fungus sp.439	un

687	unidentified fungus sp.376	un
689	unidentified fungus sp.292	un
690	unidentified fungus sp.49	un
695	unidentified fungus sp.310	un
696	unidentified fungus sp.236	un
697	Agaricomycetidae1	un
699	unidentified fungus sp.50	un
700	unidentified fungus sp.94	un
703	unidentified fungus sp.342	un
704	unidentified fungus sp.91	un
705	unidentified fungus sp.215	un
708	unidentified fungus sp.28	un
709	unidentified fungus sp.127	un
711	unidentified fungus sp.182	un
714	unidentified fungus sp.200	un
716	unidentified fungus sp.5	un
717	unidentified fungus sp.303	un
718	unidentified fungus sp.321	un
719	unidentified fungus sp.8	un
720	unidentified fungus sp.360	un
722	unidentified fungus sp.102	un
723	unidentified fungus sp.283	un
724	unidentified fungus sp.92	un
727	unidentified fungus sp.199	un
728	unidentified fungus sp.428	un
729	Pyronemataceae1	un

730	unidentified fungus sp.243	un
731	unidentified fungus sp.281	un
732	unidentified fungus sp.307	un
735	unidentified fungus sp.452	un
736	unidentified fungus sp.432	un
737	Ascomycota20	un
738	unidentified fungus sp.103	un
739	unidentified fungus sp.220	un
742	Helotiales sp.2	un
743	unidentified fungus sp.259	un
745	unidentified fungus sp.93	un
746	unidentified fungus sp.234	un
749	unidentified fungus sp.82	un
753	unidentified fungus sp.221	un
756	unidentified fungus sp.260	un
757	unidentified fungus sp.407	un
759	unidentified fungus sp.113	un
760	Clavicipitaceae sp.1	un
761	unidentified fungus sp.291	un
762	unidentified fungus sp.433	un
764	unidentified fungus sp.155	un
767	unidentified fungus sp.132	un
768	unidentified fungus sp.187	un
769	unidentified fungus sp.207	un
770	unidentified fungus sp.211	un
771	unidentified fungus sp.241	un

772	unidentified fungus sp.100	un
773	unidentified fungus sp.14	un
774	unidentified fungus sp.269	un
775	unidentified fungus sp.232	un
776	unidentified fungus sp.185	un
777	Agaricomycetes8	un
778	unidentified fungus sp.108	un
782	unidentified fungus sp.139	un
784	unidentified fungus sp.302	un
786	unidentified fungus sp.76	un
787	unidentified fungus sp.26	un
788	unidentified fungus sp.95	un
790	Agaricomycetidae2	un
791	unidentified fungus sp.197	un
795	unidentified fungus sp.230	un
797	Ascomycota sp.2	un
802	unidentified fungus sp.105	un
804	unidentified fungus sp.183	un
805	unidentified fungus sp.275	un
806	unidentified fungus sp.228	un
807	unidentified fungus sp.253	un
808	unidentified fungus sp.380	un
810	unidentified fungus sp.171	un
813	Helotiales5	un
814	unidentified fungus sp.205	un
815	unidentified fungus sp.1	un

816	unidentified fungus sp.166	un
817	unidentified fungus sp.272	un
819	unidentified fungus sp.36	un
820	unidentified fungus sp.337	un
822	Rhytismatales1	un
824	unidentified fungus sp.252	un
825	unidentified fungus sp.299	un
826	unidentified fungus sp.203	un
830	unidentified fungus sp.184	un
831	unidentified fungus sp.39	un
832	Helotiales8	un
833	Basidiomycota3	un
834	unidentified fungus sp.379	un
835	unidentified fungus sp.121	un
837	unidentified fungus sp.244	un
838	unidentified fungus sp.15	un
839	unidentified fungus sp.443	un
840	unidentified fungus sp.29	un
841	Helotiales7	un
842	Pezizales5	un
843	Ascomycota18	un
844	unidentified fungus sp.177	un
845	unidentified fungus sp.266	un
846	unidentified fungus sp.147	un
847	unidentified fungus sp.194	un
848	Pezizales11	un

849	unidentified fungus sp.429	un
850	unidentified fungus sp.88	un
851	unidentified fungus sp.311	un
853	Helotiales15	un
854	unidentified fungus sp.338	un
856	unidentified fungus sp.348	un
861	unidentified fungus sp.329	un
862	unidentified fungus sp.126	un
863	unidentified fungus sp.416	un
864	Ascomycota9	un
865	unidentified fungus sp.304	un
68	*Archaeorhizomyces borealis*	un-re
70	*Sistotrema* sp.1	un-re
115	*Archaeorhizomyces finlayi*	un-re
163	*Meliniomyce*s sp.1	un-re
183	*Meliniomyces* sp.4	un-re
191	*Mollisia minutella*	un-re
193	*Chroogomphus vinicolor*	un-re
205	*Oidiodendron pilicola*	un-re
221	*Mollisia dextrinospora*	un-re
224	*Meliniomyces* sp.2	un-re
275	*Sistotrema* sp.2	un-re
286	*Cuphophyllus lacmus*	un-re
353	*Cadophora* sp.1	un-re
383	*Meliniomyces* sp.2	un-re
449	*Degelia* sp.1	un-re

463	*Meliniomyces* sp.5	un-re
478	*Hygrocybe* sp.2	un-re
547	*Oidiodendron* sp.1	un-re
582	*Pseudogymnoascus roseus*	un-re
649	*Phialocephala fortinii*	un-re
659	*Rhizoscyphus ericae*	un-re
681	*Mollisia* sp.1	un-re
684	*Meliniomyces variabilis*	un-re
702	*Meliniomyces bicolor*	un-re
734	*Hygrocybe* sp.1	un-re
755	*Hygrocybe mucronella*	un-re
793	*Goidanichiella sphaerospora*	un-re
798	*Meliniomyces* sp.3	un-re
809	*Tetracladium* sp.1	un-re

Appendix 2.4. Results from Pearson product-moment correlations determining the strength and direction of relationships between (a) tree mortality, soil nutrients, soil moisture, and soil phenolics and (b) macronutrients: calcium (Ca), potassium (K), nitrogen (N), phosphorus (P), and sulfur (S).

(A)

Correlations	Tree mortality	Soil nutrients	Soil moisture	Soil phenolics
Tree mortality	1.00	0.20	0.27	-0.45
Soil nutrients	0.20	1.00	0.39	-0.30
Soil moisture	0.27	0.39	1.00	-0.31
Soil phenolics	-0.45	-0.30	-0.31	1.00

(B)

Correlations	Ca	K	N	P	S
Ca	1.00	-0.82*	-0.34*	-0.45*	0.60*
K	-0.82*	1.00	0.42*	0.48*	-0.58*
N	-0.34*	0.42*	1.00	0.34*	-0.26*
P	-0.45*	0.48*	0.34*	1.00	-0.49*
S	0.60*	-0.58*	-0.26*	-0.49*	1.00

Notes: Significance (P<0.05) of correlation is indicated by *.

Appendix 2.5. Summary of soil parameters used in testing the response in the richness and composition of soil fungi to variation in soil moisture, nutrients and phenolics following beetle-induced tree mortality across stands in west-central Alberta, Canada.

Stand ID	Soil core ID	Soil moisture	Soil nutrients					Soil phenolics
			Ca	K	N	P	S	
60	1	40.3	1410.7	112.6	3.7	11.2	31.6	75.5
60	2	36.5	617	367.6	9.2	13.1	11.4	65.2
60	3	40.8	592.5	426.2	13.1	25	16.1	80.9
60	4	33.8	1573.8	93.6	2.7	31.4	29.1	66.1
60	5	42.4	1455.9	134.1	6.2	16.9	25.4	75.8
60	6	38.2	1347.3	145.3	5.5	18.5	33.3	65.2
60	7	43.5	1899.5	15.3	3.1	1.8	105.8	55.2
60	8	62.0	1278.3	224.6	8.6	16.8	33.6	44.8
60	9	42.0	1259.4	164.4	6.0	17.1	75	65.2
60	10	38.0	1792.6	69	7.2	3.1	43.9	58.5
57	11	36.5	1741.3	142.4	6.8	39.5	36	68.2
57	12	39.4	1753.5	88.2	7.7	15	91.8	54.1
57	13	41.7	2710.9	24.2	4.7	2	158.2	59.6
57	14	47.7	2085.9	55.1	3.9	2.6	127.2	58.1
57	15	42.5	1962	45.6	6.0	8.1	140.2	42.2
57	16	43.8	2281	24.2	2.1	5.4	115.5	54.1
57	17	39.8	1727	131.5	5.1	38.2	55	59.9
57	18	41.0	2062.4	37.4	2.4	16.4	73.5	45.1
57	19	35.7	2145	40.9	8.4	9.7	31.1	54.1
57	20	43.2	1574.4	80.8	3.5	14.6	29.5	45.1
59	21	18.9	1647.1	60.6	2.7	15	26.5	126.0
59	22	29.8	1754	109.5	2.6	14.2	28.6	91.7
59	23	30.2	1296.8	166.7	3.6	8.2	28.6	91.7
59	24	26.3	1559.6	83.1	10.8	8.3	160.2	139.7
59	25	22.6	1437.1	119.3	7.2	19.3	64.1	122.4
59	26	30.2	1581.1	74.2	4.9	17.2	31.7	66.1
59	27	25.8	1258.2	151.8	8.9	18.9	26.8	91.7
59	28	28.2	1495.1	217	9.2	37.5	39.2	59.2
59	29	30.2	1799.6	42.7	6.2	16.1	36.4	50.2
59	30	28.1	1468	185.3	9.0	13.4	48.2	78.4
61	31	49.8	1816.2	44.9	10.4	47.1	59.1	116.6
61	32	32.2	1835.6	24.6	4.1	17.9	127.3	75.2
61	33	42.2	1816.1	31.8	8.5	26.1	121.3	72.9
61	34	37.6	1912.9	28.7	5.4	8.6	242	75.2
61	35	31.8	2460.8	11.7	4.7	0.6	399.2	64.6

61	36	31.0	1952.4	26.2	4.1	17.7	160.8	75.2
61	37	33.0	2338.5	81	1.9	15.6	82.3	72.6
61	38	35.0	2251.1	30.2	10.5	23	137.3	73.7
61	39	40.6	1571	284.1	15.9	38.7	30.6	57.1
61	40	39.0	1714.3	104.7	5.7	47.6	40.6	69.0
62	41	39.3	1968.7	68.1	7.1	40.1	41.1	28.2
62	42	36.4	2669.5	17.4	3.5	7.3	767	33.6
62	43	39.8	1776.1	94.3	5.4	24.8	54.6	25.3
62	44	47.3	2221.5	41.3	6.7	41	84.6	27.9
62	45	59.1	1756	135.6	7.7	29.4	85.6	30.0
62	46	36.3	1683.7	242.6	9.6	25.2	31.1	27.9
62	47	34.3	1926.7	146.4	9.9	44.5	64.1	31.8
62	48	41.3	1363.3	364.3	10.7	14.6	15.1	24.2
62	49	40.4	1724.7	178	10.9	31.2	42.1	27.9
62	50	38.5	1625.8	187.6	16.4	17.7	92	22.4
63	51	30.0	2245.1	76.8	15.9	11.9	107.3	33.9
63	52	79.3	2025.2	43.5	6.2	30.9	148.9	37.2
63	53	43.5	1881.2	63.2	12.2	18.1	80.4	47.3
63	54	55.3	971.5	463.2	9.4	57.9	31.2	40.4
63	55	39.7	2122.5	51.7	10.6	11.9	144.2	39.7
69	61	30.8	1472.7	271.6	8.4	18.4	73.9	47.7
69	62	37.0	1553.2	139.3	13.0	34.6	32.8	39.7
69	63	38.0	1631.4	214.7	3.2	30.9	31.7	46.6
69	64	48.5	1941.6	142	5.7	26.9	44	52.2
69	65	44.0	1734	100.1	6.2	29.7	23.7	35.4
69	66	36.2	1909.1	95.5	5.7	15.3	26.4	52.4
69	67	43.2	1725.6	124.3	5.5	27.9	18.5	22.4
69	68	35.2	1622.6	94	4.1	13.7	26.3	43.2
69	69	38.3	1649.7	142.1	8.8	15.6	29.6	43.2
69	70	48.9	1929	148	5.5	25.3	18.3	45.9
70	71	22.1	2095.7	54.2	4.9	10	18	45.1
70	72	30.4	1971.3	74.3	8.2	22.3	20.3	42.2
70	73	34.2	1827.8	66.5	13.7	36.1	19.2	45.5
70	74	29.8	2365.3	13.7	7.4	4.2	56.7	37.9
70	75	37.5	1923.5	59.8	5.8	14.5	389.1	40.1
62a	81	35.9	1783.4	232.7	5.9	38.9	52.3	69.7
62a	82	53.0	1651.3	66.5	7.9	30.7	38.5	35.4
62a	83	35.2	1901.4	25.8	7.4	12.3	37.8	51.7
62a	84	32.5	1810.9	50	6.8	12.6	39.5	55.2
62a	85	33.0	1951.3	17.8	6.9	40	60.5	51.7
62a	86	42.9	2036.6	9.8	5.5	11.2	128.3	46.6
70a	91	37.2	1270.3	219.1	16.5	5.9	119.2	45.1
70a	92	35.5	1863.2	129.6	8.0	35.8	27.4	35.1
70a	93	36.1	1191.9	384.5	11.5	28.4	21.9	35.1
70a	94	41.6	1601.7	121.4	10.3	35.5	29.7	33.6

70a	95	29.4	1137.3	112.6	32.7	16.6	22.5	26.4
70a	96	34.0	1274.3	284.1	5.1	39.4	20.9	39.4
70a	97	39.2	1732.1	180.4	3.5	19.6	25	35.1
70a	98	36.7	1410.7	141.7	14.6	22.6	28.2	49.5
70a	99	34.2	1851.8	88	6.3	37	35.3	24.6
70a	100	33.8	1854.9	90.6	7.9	22.9	26.6	27.4
Kb1	101	40.3	1116.5	297.6	16.5	15.7	19	65.4
Kb1	102	43.8	1171.5	311	5.5	49.6	21.8	78.4
Kb1	103	51.6	1784.7	132.7	6.5	33.2	29.6	18.8
Kb1	104	47.0	1568.9	193.2	5.4	36	33.6	54.9
Kb1	105	42.1	1847.7	47.8	3.4	20.5	67.2	54.9
Kb1	106	36.5	1854.2	40.1	5.1	29.1	33.4	49.5
Kb1	107	37.5	1772.2	75.2	7.9	24	51.1	54.9
Kb1	108	34.8	1608.7	199	5.0	66.5	35.3	48.8
Kb1	109	35.2	1939.3	59.7	6.7	35.2	34.1	65.4
Kb1	110	32.3	1697.5	214.9	3.7	35.2	46.4	58.5

Notes: Volumetric soil moisture content is expressed as a percentage. Individual soil nutrient supply rates are expressed as the weight of nutrient absorbed per surface area of ion-exchange membrane over time (μg nutrient/10 cm^2 ion-exchange membrane surface area/time of burial (67 days)). Soil phenolics are expressed as μg phenolics per g of soil.

Appendix 2.6. Operational taxonomic units (OTUs) of fungal phyla and orders gained and lost with tree mortality.

Phylum	Order	Gain/Lost ratio
Basidiomycota		0.89 *
	Agaricales	0.83 *
	Atheliales	0.84 ***
	Russulales	1.08
	Thelephorales	1.02
	Sebacinales	0.88 ***
	Boletales	0.70 ***
	Other Basidiomycota[1]	0.88 *
	Unidentified	0.95
Ascomycota		1.02
	Helotiales	0.98
	Pezizales	1.23 *
	Saccharomycetales	1.24 **
	Hypocreales	0.80 ***
	Other Ascomycota[2]	0.92
	Unidentified	0.98
Basal lineages		0.89
	Mortierellales	0.98
	Mucorales	0.80 *
Glomeromycota		0.87 *
	Glomerales	0.76 **
	Diversisporales	0.60 *
	Archaeosporales	0.96
Unidentified		1.00

Notes: Gain/loss ratio reflects the number of OTUs gained or lost for a particular phylum or order by dividing the total number of gains by the total number of losses. Values greater than one indicate that gains exceeded losses and values less than one indicate that losses exceeded gains. Nonparametric *t*-tests with 999 Monte Carlo permutations were used to statistically evaluate the gain and loss of fungal phyla or orders present in soil cores from undisturbed (n = 23) compared to severely beetled-killed (n = 30) (>60% *Pinus contorta* killed basal area) stands. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

[1] Includes orders Polyporales, Tremellales, Auriculariales, Cantharellales, and Filobasidiales of the phylum Basidiomycota.

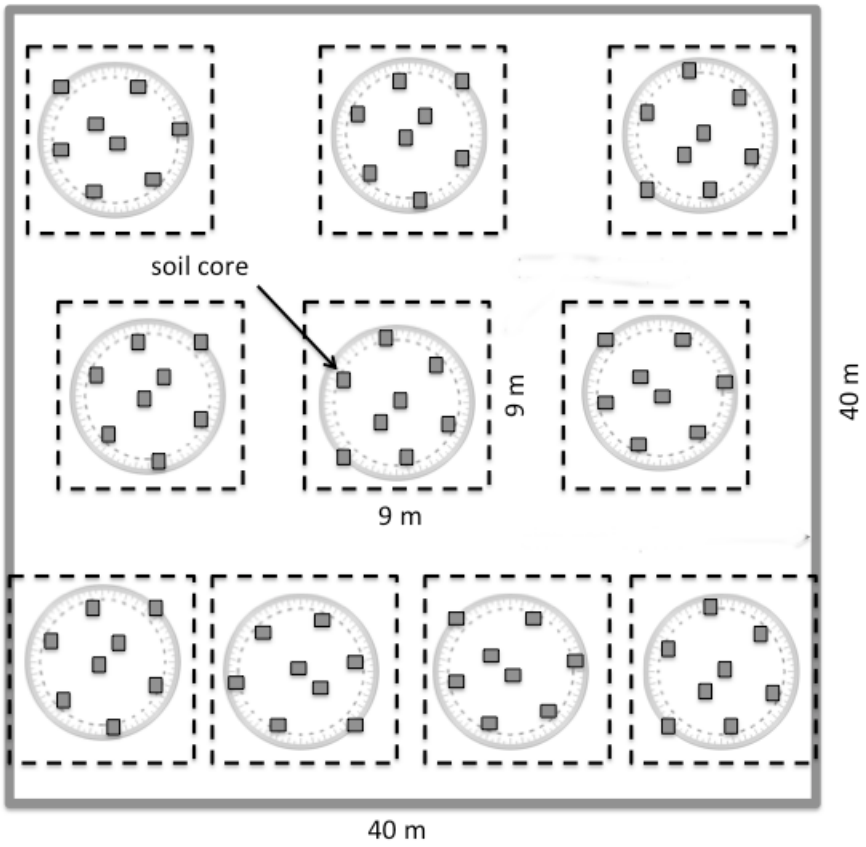
[2] Includes orders Magnaporthales, Chaetothyriales, Eurotiales, Rhytismatales, Capnodiales, Pleosporales, Venturiales, Geoglossales, Peltigerales, Orbiliales, and Archaeorhizomycetales of the phylum Ascomycota.

Appendix 2.7. List of indicator fungal taxa present in soil cores from undisturbed and severely beetled-killed (>60% *Pinus contorta* killed basal area) stands within the Lower Foothills natural subregion southwest of Grande Prairie, Alberta.

Tree mortality	Taxon	Indicator value[+]
Undisturbed (*n*=23)	*Sebacina* sp. 1	0.97**
	Cenococcum sp. 1, 2	0.91**
	Glomus sp. 2	0.89**
	Phialocephala sp. 1	0.84**
	Suillus sp. 1	0.84*
	Cortinarius sp. 4, 5	0.60-0.75*
	Piloderma sp. 3	0.63*
Severely attacked (*n*=30)	Saccharomycetales 1, 2, 4	0.83-0.93**
	Verticillium sp. 1	0.87**
	Tremella sp. 1	0.79**
	Inocybe sp. 1	0.71*
	Cryptococcus sp. 1, 5	0.61-0.69**
	Tomentella sp. 2	0.56*

Note: * $P<0.05$. ** $P<0.01$. [+] When an OTU could not be identified to species, a range of indicator values is given. An indicator value of 1 indicates a species found in all plots of one group and no plots in any other group. *P* values were calculated based on a Monte Carlo significance test of observed maximum indicator values for each species

Appendix 3.1. Representative site design for sampling of soil cores to determine changes in spatial structure of soil fungal communities following recent mountain pine beetle activity (since 2009) across eleven sites in pine forests in west-central Alberta, Canada.



Appendix 3.2. Semivariance analysis of the distribution of ectomycorrhizal and saprotrophic fungi along a gradient of lodgepole pine killed by mountain pine beetle (*n*=11 sites) in pine forests of west central Alberta, Canada.

Functional group	Site ID	Structured variance (C_1)[1]	Nugget variance (C_0)[2]	Spatial structure $C_1/(C_0+C_1)$[3]	Model fit (r^2)[4]	Range (m)[5]
Ectomycorrhizal fungi						
	1	0.071	0.478	0.130	0.331	3.327
	2	0.050	0.190	0.208	0.390	7.207
	3	0.090	0.468	0.161	0.197	6.007
	4	0.085	0.446	0.160	0.140	0.906
	5	0.181	0.389	0.318	0.235	11.132
	6	0.040	0.220	0.154	0.377	2.400
	7	0.175	0.581	0.231	0.388	11.731
	8	0.010	0.040	0.200	0.149	5.998
	9	0.174	0.705	0.198	0.231	6.068
	10	0.161	0.540	0.230	0.422	6.228
	11	0.084	0.508	0.142	0.238	3.743
Saprotrophic fungi						
	1	0.068	0.442	0.134	0.331	3.418
	2	0.060	0.150	0.286	0.395	6.913
	3	0.010	0.411	0.024	0.193	5.548
	4	0.082	0.439	0.157	0.136	2.129
	5	0.149	0.368	0.288	0.225	10.460
	6	0.070	0.200	0.259	0.372	2.400
	7	0.128	0.522	0.197	0.366	11.184
	8	0.010	0.030	0.250	0.144	2.500
	9	0.145	0.684	0.175	0.235	11.796
	10	0.132	0.496	0.210	0.402	6.338
	11	0.078	0.454	0.147	0.232	3.402

Notes: [1] Variance attributed to spatial autocorrelation. [2] Variance not attributable to spatial autocorrelation. [3] Proportion of variance due to spatial structure. [4] Proportion of the total variation accounted for by fitting the experimental semivariograms to theoretical semivariograms. [5] Distance at which data is no longer spatially autocorrelated.

Appendix 4.1. GenBank accession numbers of operational taxonomic units of soil fungi identified on roots of lodgepole pine and white spruce seedlings growing in undisturbed and beetle-killed stands in west-central Alberta, Canada.

OTU	Best GenBank match	Best GenBank match accession number	Percent identity	Query coverage
Amphinema byssoides	*Amphinema byssoides* isolate FFP590	JQ711816	98%	99%
Cenococcum geophilum	*Cenococcum geophilum* isolate FFP820	JQ711879	99%	99%
Cortinarius 1	*Cortinarius* sp. 5 RT-2012 FFP544	JQ711811	94%	98%
Inocybe 1	*Inocybe* sp. 6 RT-2012 isolate FFP490	JQ711849	98%	99%
Laccaria bicolor	*Laccaria bicolor* voucher SM1211	FJ845417	97%	98%
Lactarius rufus	*Lactarius rufus* isolate OUC97250	DQ097868	99%	98%
Meliniomyces bicolor	*Meliniomyces bicolor* isolate pkc34	AY394885	99%	96%
Peziza 1	*Peziza* sp. B276	FN669234	98%	98%
Phialocephala 1	*Phialocephala* sp. RT-2012 isolate FFP1134	JQ711934	98%	93%
Piloderma 1	*Piloderma* sp. 10 RT-2012 isolate FFP754	JQ711870	97%	99%
Piloderma 2	*Piloderma* sp. 9 RT-2012 isolate FFP651	JQ711824	98%	97%
Piloderma sphaerosporum	*Piloderma sphaerosporum* isolate FFP884	JQ11884	94%	100%
Rhizoscyphus ericae	*Rhizoscyphus ericae* isolate FFP254	JQ711771	98%	95%
Russula 1	*Russula* sp. d25	KM517246	94%	98%

Russula bicolor	*Russula bicolor* isolate ST-7 voucher UBC F19771	HQ604845	98%	99%
Russula decolorans	*Russula decolorans* voucher SMI265	FJ845432	94%	99%
Russula gracilis	*Russula gracilis* voucher SMI258	FJ845431	93%	97%
Sphaerosporella 1	*Sphaerosporella* sp. RT-2012 isolate FFP321	JQ711781	96%	100%
Thelephora terrestris	*Thelephora terrestris* isolate FFP1250	JQ712012	96%	97%
Thelephoraceae 1	*Thelephoraceae* sp. P104	FN669257	97%	96%
Thelephoraceae 2	*Thelephoraceae* sp. B146	FN66928	93%	95%
Tomentella 1	*Tomentella* sp. 3 RT-2012 isolate FFP593	JQ711817	99%	97%
Tomentella 2	*Tomentella* sp. 4 RT-2012 isolate FFP690	JQ711829	98%	99%
Tomentella badia	*Tomentella badia* isolate FFP856	JQ11882	93%	97%
Tomentellopsis submollis	*Tomentellopsis submollis* isolate FFP892	JQ711898	96%	99%
Tricholoma flavovirens	*Tricholoma flavovirens* voucher DAVFP:26245	JF899574	98%	96%
Tuber pacificum	*Tuber pacificum* isolate FFP578	JQ711989	96%	97%
Tylospora 1	*Tylospora* sp. 4 RT-2012 isolate FFP649	JQ711823	99%	100%
Tylospora fibrillosa	*Tylospora fibrillosa* isolate FFP1064	JQ712008	98%	97%
Unidentified fungus 1	Uncultured ectomycorrhizal fungus clone STILRO10	EU645617	94%	98%
Unidentified fungus 2	Uncultured fungus clone	KC965648	96%	97%

87_NA3_P32_07

Note: OTU – Operational Taxonomic Unit; Percent identity = Percent of identical bases in the alignment for which all sequence are identical; Query coverage = Percent of the query sequence covered by hit.

Appendix 4.2. Summary of linear models testing the response of lodgepole pine and white spruce seedlings to light availability and soil moisture levels across three MN treatments.

	MN		**Light**		**MN x light**	
	$F_{1,9}$	P	$F_{1,9}$	P	$F_{1,9}$	P
Lodgepole pine						
Survival	0.281	0.756	1.517	0.229	0.124	0.884
Height	0.235	0.792	0.153	0.669	0.228	0.797
Total biomass	0.402	0.673	2.652	0.116	0.225	0.799
White spruce						
Survival	3.230	0.060	0.032	0.859	3.410	0.052
Height	0.360	0.701	0.970	0.335	0.215	0.808
Total biomass	0.138	0.871	2.492	0.094	0.067	0.935

	MN		**Soil moisture**		**MN x soil moisture**	
Lodgepole pine						
Survival	0.621	0.545	12.59	**0.001**	0.442	0.647
Height	0.001	0.999	6.839	**0.015**	0.008	0.991
Total biomass	0.177	0.838	0.101	0.753	0.677	0.517
White spruce						
Survival	0.249	0.781	0.583	0.453	0.302	0.742
Height	0.108	0.898	2.269	0.146	0.312	0.735
Total biomass	0.491	0.618	0.123	0.729	0.328	0.723

Notes: Significant differences are in **bold.**