Characterization, restoration, and assembly of fungal communities in lodgepole pine forests impacted by recent disturbances

by

Jean Carlos Rodríguez Ramos

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Forest Biology and Management

Department of Renewable Resources

University of Alberta

Abstract

Novel disturbance regimes have impacted boreal forest with unknown consequences for belowground communities and underlying ecological processes. Soil fungi are an integral component of belowground communities and are particularly sensitive to disturbances. In the Canadian boreal forest, common overstory trees, such as lodgepole pine (*Pinus contorta* var. *latifolia*), form mutualisms with ectomycorrhizal (EcM) fungi, while many understory plants tend to associate with arbuscular mycorrhizal (AM) fungi. These fungal symbionts influence the establishment and growth of their host by mediating nutrient availability, while also interacting with saprotrophic and pathogenic fungi. The response of these different fungal guilds to individual disturbances and the response of seedlings to changes fungal communities is poorly understood. Furthermore, it is unclear what processes underlie EcM fungal community assembly and whether different disturbances affect the relative contribution of neutral and deterministic assembly processes. The objectives of this thesis are to (1) characterize soil fungal communities in lodgepole pine forests following a range of disturbances including bark beetle outbreak, wildfire, clear-cut logging, and salvage-logging, (2) test whether the disrupting soil organic matter structures fungal communities belonging to different guilds (3) determine whether soil transfers from intact (control) lodgepole pine forests into regenerating conspecific forests amend soil fungal communities and, in turn, affect the performance of pine seedlings, and (4) examine whether root-associated EcM fungi assemble at fine spatial scales based on neutral or deterministic processes across disturbances.

I found that wildfire, clear-cut, and salvage-logging changes the community composition of EcM fungi and shifted the dominance from EcM to saprotrophic fungi compared to control forests. However, despite MPB outbreak declined the EcM fungal relative abundance, compared

to controls, the effects were not as strong as in the other disturbances. Disruption of the soil organic layer with disturbances correlated with the decline of EcM and the increase of AM fungi. In addition, wildfire changed the community composition of pathogenic fungi but did not affect their proportion or diversity. Fungal biomass declined with the same disturbances that also disrupt the soil organic layer, specifically wildfire, clear-cut, and salvage-logging. Soil transfers from control stands did not change the composition of the resident fungal community in soils and lodgepole pine seedling roots across the disturbances. Instead, I found that the variation in the EcM fungal community was explained largely by disturbance type. Furthermore, soil transfers did not affect seedling survival or performance. For EcM fungi colonizing roots of seedlings grown in the different disturbances, a neutral model that uses species abundance in the soil metacommunity to predict their occurrence on roots predicted the abundance of 58% to 64% of root-associated EcM fungal taxa. This finding suggests that both neutral and deterministic processes are important in fine-scale assembly of EcM fungi regardless of disturbance type. I also found that the fungal communities of taxa assigned as neutral or deterministic were similar across the disturbance types. Traits, including host-specificity of EcM fungi, large production of resistant spores, and uncertain ecological roles of taxa commonly identified as EcM fungi could explain their deviations from neutrality. Collectively, these results suggest that while disturbances alter the community composition and abundance of soil fungal guilds and root-associated EcM fungi, lodgepole pine seedlings can be relatively insensitive to the *in-situ* variation in fungal communities across disturbances that assemble in roots largely by probabilistic dispersal from soil. Therefore, under novel disturbance regimes, soil and root-associated fungal communities can show resilience through a response diversity that may provide functions regarding seedling establishment and performance.

Preface

A version of Chapter 2 of this thesis has been published as Rodriguez-Ramos, J. C., Cale, J. A., Cahill, J. F., Simard, S. W., Karst, J., & Erbilgin, N. (2021). Changes in soil fungal community composition depend on functional group and forest disturbance type. *New Phytologist*: doi: 10.1111/nph.16749. J.C.R.R., J.A.C., N.E., J.K., J.F.C., and S.W.S. planned and designed the research. J.C.R.R. and J.A.C. conducted field and laboratory work. J.C.R.R. analyzed the data and wrote the manuscript together with J.A.C., J.F.C., J.K., and N.E.

Chapter 3 was planned and designed by Rodriguez-Ramos, J. C., Cale, J. A., Cahill, J. F., Simard, S. W., Erbilgin, N., & Karst, J. Field and laboratory analysis was conducted by J.C.R.R., as well as the data analysis. J.C.R.R. wrote the chapter with guidance from J.K. and N.E.

Chapter 4, under the guidance of my committee, I conducted the field work and conducted the analysis with collaboration from Dr. Bachar Cheaib from University of Glasgow. J.C.R.R. wrote the chapter with guidance from N.E., J.K., and J.F.C.

Acknowledgements

I would first and foremost like to express my sincere gratitude to my supervisors Drs. Nadir Erbilgin and Justine Karst for their outstanding mentoring throughout my graduate degree and dissertation. Their insight, support, and guidance helped me study in depth the topics that I am most passionate about. Thank you for believing in me and for your encouragement to stay positive in times of hardship and rejection. I am not the same person who moved to Edmonton in 2016 and for that you are partially to thank. Your leadership, ethics, and compassion are part of the legacy I will carry forward in my future endeavours. I would also like to extend my thanks to Dr. James F. Cahill Jr., for providing thoughtful feedback throughout these years as part of my supervisory committee. You all have guided and helped me grow into the scientist that I am today by challenging me to think critically about my projects; the road would have not been the same without you.

I would also like to thank the members of the Erbilgin and Karst Lab groups including Dr. Jonathan Cale and Dr. Jennifer Klutsch for the many queries they helped me navigate. I am particularly grateful to have met, become friends, and/or share office with Sydne Guevara-Rosso, Dr. Shiyan Zao, Jackson Beck, Melanie Mullin, James Franklin, Joshua Wasyliw, with whom I had many venting sessions, coffee breaks, and science discussion. Furthermore, I am thankful to all the staff in the Molecular Biology Facility (MBSU) at the University of Alberta for their guidance on integral molecular methods of my research, ensuring the success of my experiments.

In addition, I am immensely thankful for the love and support of my family. Thank you to my wonderful partner, Ian Stefanuk, who celebrated every single one of my milestones and provided the best of comforts in times of difficulty. I am forever grateful for your patience, positivity, and encouragement; words could never explain how much you mean to me and how

much you contributed to my success. Thank you to my parents for always pushing me to achieve my best at a personal and professional level, and to my siblings for being part of my support and example of ethical and hard work. I love you and I hope I have made you all proud.

Table of Contents

List of Figures

variants (ASVs) that occur more or less frequently than predicted by the model are shown as circles, and those whose abundance conforms model predictions, crosses. Green shade represents 95% confidence intervals around the neutral model prediction. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

Figure 4.2 Non-metric multidimensional scaling plot using Bray-Curtis distance among root-associated ectomycorrhizal fungal communities of amplicon sequence variants (ASVs) assigned as neutral or non-neutral across disturbances. Neutral is defined as ASVs with frequencies conforming to Sloan neutral community model predictions, and non-neutral as those fitting above or below the model prediction. Mountain pine beetle (MPB, *Dendroctonus ponderosae*) outbreak communities not shown due to low neutral model fit on data. Significant difference of communities at $\alpha = 0.05$ was tested using perMANOVA.

List of Tables

Chapter 1

Thesis Introduction

One of ecology's fundamental objectives is to understand the factors influencing the structure of communities at various spatial and temporal scales (Whittaker, 1960; MacArthur, 1965; Vellend, 2016). Natural and anthropogenic disturbances are among the factors shaping community assemblages across spatiotemporal scales (Pickett & White, 1985). The power of disturbances to alter forest ecosystems but also prompt community succession is conspicuous to anyone observing the aftermath of disturbances such as stand-replacing fires. The Canadian boreal forest is a disturbance-prone ecosystem, and therefore, events such as wildfires and insect outbreaks, shape the landscape and the species community composition. Despite extensive understanding on how disturbances impact aboveground plant communities, characterizing the effects of disturbances on the structure and function of soil microbial communities has largely lagged.

In the past several years, we have gained much information about the pivotal role of soil microbes in ecosystem processes. For instance, soil microbes such as fungi can be considered biological controllers, ecosystem regulators, and organic matter decomposers or modifiers (Frac et al., 2018). As biological controllers, some fungi can regulate plant diseases and growth of other organisms in the soil (Baum et al., 2015; El-Komy et al., 2015), as ecosystem regulators, they can affect the soil structure (Siddiky et al., 2012), and given their ability to exude a diverse set of extracellular enzymes, fungi can modify or break down complex organic substances (Lindahl & Clemmensen, 2016). Thus, characterizing soil microbial communities and identifying the mechanisms structuring them helps us understand how ecosystem processes might be affected by different disturbances.

This thesis aims to examine how frequent boreal disturbances alter the community composition and assembly of belowground fungi, both in forest soils and associated with the roots of a common, widespread tree species, lodgepole pine (*Pinus contorta* Douglas var. *latifolia* Englm.). The thesis expands on previous efforts to characterize fungal communities in pine forests by comparing multiple fungal guilds—a group of species that exploit a resource in a similar way—across intact forests and forests disturbed by wildfire, clear-cut logging, mountain pine beetle (MPB. *Dendroctonus ponderosae* Hopkins), and salvage-logging. Climate change has increased the frequency and intensity of these disturbances, which are known to affect aboveground plant communities with cascading effects on belowground soil microbial communities. Recent research indicates that seedling establishment may be limited following cumulative disturbances and MPB (Mcintosh & Macdonald, 2013; Hansen & Turner, 2019). As such, I investigate how soil fungi are affected by different disturbances and whether we can restore forest fungal communities and improve lodgepole pine seedling establishment and performance in disturbed forests. Furthermore, I examine the relative roles of neutral and deterministic processes in shaping the fungal community composition of fungal taxa associated with lodgepole pine seedling roots.

4.1 The boreal forest

Throughout its circumpolar distribution, boreal zones cover 1.9 billion ha worldwide in the northern hemisphere, with 552 million ha occurring in Canada (Natural Resources Canada, 2019). The climate in the Canadian boreal forest is characterized by large ranges in annual temperatures, including cool short summers with considerable amounts of precipitation and cold long winters (Brandt et al., 2013). Given its extensive geographical cover in Canada, this zone is

also subjected to large changes in photoperiod and climate gradients. The main tree species of this forest are from the genera *Abies*, *Larix*, *Pinus*, *Picea*, *Populus*, and *Betula*, occurring either as closed-canopy coniferous stands, pure deciduous stands, or intermixed conifer-deciduous stands (Burton at al., 2013). Around 25% of the forested area is managed for industrial forestry purposes, representing approximately 7% of the country's exports (Natural Resources Canada, 2019).

The boreal forest is not only economically important but also ecologically to global biodiversity and carbon storage. From tree crowns to belowground, this forest supports a diverse flora and fauna and is a long-term reservoir of significant amounts of C. Some studies estimate 60–80 Pg of total C stored in its circumpolar distribution (Tarnocai et al., 2009; DeLuca & Boisvenue, 2012). The majority of the stored C is found in the soil (Kurz et al., 2013), and it is fundamental for several ecosystem functions including improving soil physical properties and biological activity (Trivedi et al., 2018). In addition, the Canadian boreal forest is periodically impacted by both natural and anthropogenic disturbances, including wildfires, insect outbreaks, tree harvesting, and mining. These disturbances contribute to the spatial heterogeneity, and are main drivers of forest renewal, structure, and biodiversity.

4.1.4 *Lodgepole pine forests*

Lodgepole pine forests cover extensive areas in western North America, including the boreal forest. With a wide latitudinal and elevational range, its four distinct varieties extend from Alaska, U.S.A., to Baja California, Mexico, throughout the Pacific Coast and the Rocky Mountains (Wheeler & Guries, 1982; Klinka et al., 1999). This pine is an early successional species characterized by low shade tolerance and its ability to establish and grow in a wide

variety of soil types (Dhar et al., 2016). In western Canada, *Pinus contorta* var. *latifolia* is considered a pioneer species that quickly colonizes areas following wildfire, due to its serotinous cones that release large amounts of seeds when exposed to heat (Teste et al., 2011). This seed-release mechanism typically results in even-aged pine-dominated stands that are later replaced by shade-tolerant species in the absence of disturbances. Due to its predominance in the landscape and high timber value, lodgepole pine is one of the most planted and harvested species in western North America. In its range, lodgepole pine is subject to a variety of disturbances, including clear-cut logging, wildfire, MPB outbreaks, and salvage harvesting following the MPB outbreak.

4.1 Forest disturbances

Despite trees being sessile organisms, their populations are dynamic with changing population densities and age structures across spatial and temporal scales (Sousa, 1984). One of the causal agents of change in the stand structure and composition, as well as the overall function of forest ecosystems, is the occurrence of various types of disturbances. Disturbances are defined as a "relatively discrete event that disrupts the structure of an ecosystem, community, or population, and changes resource availability or the physical environment" (Sousa, 1984). Disturbances create intricate landscape patterns that set the stage for ecological succession (Pickett & White, 1985). Disturbance frequency, size, and magnitude influence the extent of the impacts on the ecosystem and define the regime of the events. However, the capacity of disturbances to interact with each other in the same spatial and temporal scale is fundamental to how disturbance regimes are changing in the time of the Anthropocene (Newman, 2019). Disturbance interactions, as the case of droughts followed by wildfire, can synergistically impact ecosystems

resulting in non-linear ecosystem behaviours that threaten recovery and resilience of forests (Buma & Wessman, 2011).

The Canadian boreal forest has been historically shaped by disturbances such as wildfires, droughts, and insect outbreaks, affecting millions of hectares every year (Volney & Fleming, 2000). In western Canada, approximately 647,140 ha burned in the last decade, while insect outbreaks such as MPB have impacted over 18 million ha of lodgepole pine since the beginning of the outbreak in 1999 (Natural Resources Canada, 2019). Disturbance regimes have largely shifted in their characteristics over the last decades, in part due to changes in global climate patterns. For example, the land area impacted by wildfires increased by 50% in Alaska in the 2000s compared to the previous decade and, in Canada, up to 40% increase is expected over the next two decades compared to current regimes (Kasischke et al., 2010; Wotton et al., 2017). Furthermore, mortality rates caused by drought have increased yearly by 4.9% in western Canada (Peng et al., 2011), and both MPB and spruce budworm (*Choristoneura fumiferana* [Clem.]) outbreaks have rapidly expanded (Taylor et al., 2006; Cullingham et al., 2011; Pureswaran et al., 2015). Climate models project further changes in disturbance regimes (Wotton et al., 2010; Wang et al., 2017). Taken together, the increased magnitude and frequency of disturbances, including interactions among disturbances, will potentially impact forest ecological processes and timber supplies (Boucher et al., 2018).

1.2.1 Wildfire

Forest fires release large quantities of energy in the form of heat as they oxidate and consume the organic matter present in forests. Wildfires play critical roles in structuring forest landscapes, communities, and populations as the heating force results in injury or mortality and also alters

species composition (Landres et al., 1999). This disturbance not only affects communities but also changes the chemical and physical properties of soils. By combusting the aboveground biomass, fires increase the abundance of mineral elements such as nitrogen and remove the forest floor, which then tends to increase soil surface temperatures (Agee, 1993). The effects of fires on ecosystems are determined based on the magnitude of individual events, which include their intensity (amount of heat released) and/or their severity (post-disturbance assessment) (Agee, 1993). Fires vary in their severity, where a high severity fire result in the death of the overstory combined with combustion of the organic layer and exposure of the mineral layer, and a low severity fire leaves some live residual overstory with an incomplete or uneven soil disruption (Belillas & Feller, 1998; Peterson & Arbaugh, 1986).

Multiple boreal plant species have adapted to the ubiquity and reoccurrence of wildfire in their forest habitat. For instance, recolonization by lodgepole and jack pine (*Pinus banksiana* Lamb.), black spruce (*Picea mariana* (Mill.) B.S.P.), paper birch (*Betula papyfera* Marsh.), and aspen (*Populus tremuloides* Michx.) is immediate following fires (Agee, 1993). Lodgepole pine, in particular, provides an example of a fire-adapted species. Its thick bark can prevent mortality during low-intensity fires, and its serotinous cones are dependent on the heat from fires to melt the resin and release the seeds from within the cone scales. In addition, the overstory mortality and exposure of the mineral soil that comes with the combustion of biomass and organic matter provides a suitable seedbed for lodgepole pine seeds to germinate and survive in the absence of overstory shade. Therefore, fires largely mediate the main factors of the "natural reproduction triangle," sufficient seed source, suitable seedbed, and a compatible environment with germination and seedling establishment (Roe et al., 1970).

1.2.2 Mountain pine beetle outbreak

Mountain pine beetle is a widely distributed bark beetle in western North America, where lodgepole and ponderosa pines (*Pinus ponderosa* Lawson & C. Lawson) are its most abundant hosts. Despite the co-evolutionary history of western coniferous forests with MPB, in recent decades, outbreaks have occurred at unprecedented magnitudes and in novel habitats (Safranyik et al., 2010). Today, MPB has significantly expanded its range, impacting novel lodgepole pine habitats, both north of its historical range and east of the Rocky Mountains into Alberta (Cudmore et al., 2010). As the beetle's range continues expanding eastwardly, it raises concerns of further adaptation and expansion into jack pine forests, a dominant species covering forests from eastern Alberta to eastern Canada (Cullingham et al., 2011). As a result of successful and dense beetle attacks, pine trees undergo a relatively long-term mortality process that has cascading effects on soil biological, physical, and chemical properties (Cigan et al., 2015; Pec et al., 2017).

1.2.3 Clear-cut and salvage-logging

Clear-cut logging of lodgepole pine stands is a common practice given the importance of this species in the local economy and the even age forest stands in which they occur. Clear-cut logging results in the complete removal of mature pine trees from the landscape as well as the disruption of the soil organic layer. Salvage-logging is an increasingly common practice used following natural disturbances such as wildfire, insect outbreaks, and windstorms to capture timber before it deteriorates, reduce fuel loads, and improve site conditions. In contrast to wildfire, MPB outbreak, and clear-cut logging, salvage-logging is a compounded disturbance, which may result in unpredictable ecosystem responses due to the interaction between individual

disturbance events (Buma & Wessman, 2011, 2012). Overall, the removal and/or mortality of overstory trees caused by disturbances influence heterotrophic organisms through the cessation of belowground photosynthate flow that drive soil nutrient cycles and soil microbial community composition and function.

4.1 Soil fungi

Soil fungi play prominent roles in the ecology of the boreal forest, where fungal : bacterial ratio (Tedersoo et al., 2014) and fungi : plant diversity ratio (Fierer et al., 2009) are considerably higher than most other biomes. As opposed to plants, fungi are heterotrophic organisms that require external sources of carbon *via* multiple, but not necessarily mutually exclusive, nutritional strategies. Through multiple trophic guilds, fungi mediate carbon and nitrogen dynamics by decomposing organic materials (saprotrophic fungi) and, by forming mutualistic symbioses (biotrophic fungi) with plant roots, they influence the hosts' nutrient availability and growth (Smith & Read, 2008; Baldrian, 2016). Furthermore, pathogenic fungi are a main causal agent of plant diseases and can increase hosts' susceptibility to other fungal infections and insect infestations (Sturrock et al., 2011).

For most plant species, biotrophic fungi are arguably the most important group. Approximately 90% of plants associate with some form of mycorrhizal fungi, interactions that tend to increase the nutritional status of both partners (Smith & Read, 2008). Whereas the fungi provide the plant with mineral nutrients, water, and disease resistance, the plant transfers carbohydrates to the mycorrhizal fungi colonizing the roots (Smith & Read, 2008). In the Canadian boreal forest, most overstory trees form mutualisms with ectomycorrhizal fungi, while understory plants tend to associate with arbuscular mycorrhizal fungi (Öpik et al., 2008). Soil

fungal communities, in particular ectomycorrhizal fungi, have shown to facilitate the establishment of seedlings, an integral step for forest regeneration following disturbances (Horton et al., 1999; Nara, 2006). Pine seedlings become colonized by mycorrhizal fungi quickly after root formation, providing the host access to increased mineral nutrition and, sometimes, increasing seedling growth (Horton & van der Heijden, 2018). In fact, reforestation of degraded sites that include soil transfers from late-successional forests have increased the amount of mycorrhizal colonization in seedlings and sometimes improve reforestation efforts (Colinas et al., 1994; Policelli et al., 2020).

1.4 Community assembly

How communities assemble and the mechanisms driving species abundance have been a central questions in ecology. The field of community ecology has gone through vigorous debates regarding the significance of deterministic versus stochastic processes in determining global, regional, and local community composition (Clements, 1936; Gleason, 1939). Through the lens of niche-based theory, deterministic factors such as environmental conditions and species interactions govern the composition and structure of natural communities. Therefore, the species comprising a community differ in their ecological traits, allowing the partitioning of niches (Leibold & McPeek, 2006). In contrast, neutral theories assume that all co-occurring individuals from the same trophic mode are ecologically equivalent; therefore, the observed community composition results from stochastic processes (Hubbell, 2001). It was not until recently that Vellend (2010) conceptualized these theories and introduced a new framework that studied community assembly based on the influence of four main classes of processes: selection, drift, diversification, and dispersal. Selection is a deterministic process and occurs when there are

differences in fitness between taxa; ecological drift refers to stochastic changes in the relative abundance of organisms; diversification is the evolutionary process by which new genetic variation arises; and dispersal refers to the movement of individuals across space (Nemergut et al., 2013)

Understanding how these assembly processes influence microbial communities has lagged those of other higher organisms such as plants; however, advances in molecular biology enable us to characterize diverse microbial communities at high resolution, which was previously limited to culture-dependent methods. Despite many studies showing the effects of deterministic factors such as disturbances and nutrient levels on the assembly of soil fungi at global and regional scales (Kipfer et al., 2011; Barker et al., 2013; Tedersoo et al., 2014; Saravesi et al., 2015), we still do not know the relative roles of stochastic and deterministic processes in the assembly of ectomycorrhizal fungal communities at fine scales (i.e., plant roots). Understanding the mechanisms underlying ectomycorrhizal fungal assembly would elucidate what specific taxa and corresponding traits are being selected for or against by the host plant and would inform us on how we can better predict the community composition of root colonizing fungi from that of the surrounding metacommunity. Elucidating what taxa assemble neutrally or are selected for or against by the hosts under given environmental gradients could guide restoration efforts to predict what microbial communities will likely associate with species of interests.

1.5 Thesis overview

To broadly address the impacts of boreal forest disturbances on soil fungal communities, fungal community assembly, and forest regeneration (Fig. 1.1), this thesis contains three chapters followed by general discussion. In Chapter 2, I test whether soil fungal biomass and the

community composition of distinct fungal guilds differentially respond to disturbances, and whether disruption of the soil organic layer mediates fungal responses. Specifically, I investigate how the composition of ectomycorrhizal, saprotrophic, pathogenic, and arbuscular mycorrhizal fungal communities compare among intact forests and those impacted by wildfire, clear-cut logging, MPB outbreak, and salvage-logging of beetle-attacked sites. In Chapter 3, I transfer soils from intact to disturbed forests and introduce lodgepole pine seedlings and test whether soil fungal community composition can be restored in sites recovering and regenerating following disturbances, and if community restoration impacts the performance of pine seedlings *in situ*. In Chapter 4, I use the analytical framework of neutral community models to test whether the community composition of root-associated fungi can be predicted from that of soils, thereby, assessing the relative contribution of neutral and deterministic processes in the community assembly of root fungi across different disturbed sites.

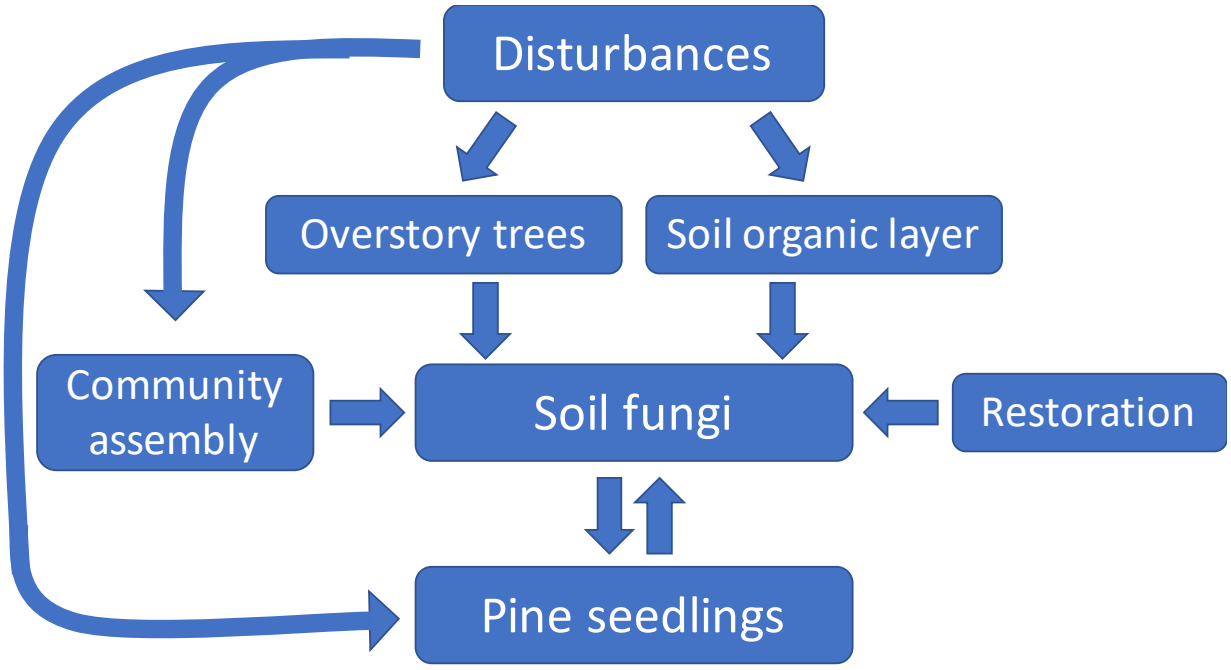


Figure 1.1. Conceptual diagrams of the linkages among overstory trees, soil organic layer, soil fungi, pine seedlings and their restoration, and fungal community assembly following forest disturbances.

Chapter 2

Changes in soil fungal community composition depend on functional group and forest disturbance type

2.1 Introduction

Forests are disturbed over a wide range of temporal and spatial scales (Pickett & White, 1985). Although ecosystems are adapted to their natural disturbance regimes (Turner et al., 1999; Johnstone et al., 2016), climate models anticipate novel regimes with an increased frequency and intensity of disturbances including insect outbreaks, drought, and wildfire (Allen et al., 2010; Wotton et al., 2010, 2017; Schneider, 2013; Bolton et al., 2015; Raffa et al., 2017; Erbilgin, 2019). Shifts in disturbance regimes, in particular the onset of compounded disturbances, raise concerns that abrupt and persistent ecological changes will cause ecosystems to diverge from historical trajectories (Paine et al., 1998; Johnstone et al., 2016; Ratajczak et al., 2018; Zscheischler et al., 2018; Rillig et al., 2019). In northern coniferous forests, the combination of warmer and drier climates has already amplified insect outbreaks, e.g., mountain pine beetle (MPB; *Dendroctonus ponderosae*), and wildfires, disturbances now reaching unprecedented levels that can erode resiliency—i.e., an ecosystem's ability to reorganize after a disturbance, and recover the same dominant forest cover—(Bale et al., 2002; Buma & Wessman, 2011; Johnstone et al., 2016; Raffa et al., 2017; Erbilgin, 2019). Together with anthropogenic activities (e.g., timber harvesting), novel disturbance regimes can impact the structure, function, and long-term successional dynamics of above- and belowground species assemblages in unprecedented ways (Paine et al., 1998; Buma & Wessman, 2011; Buma, 2015). Specifically, it is unknown

how various disturbances that alter aboveground forest structure impact the community assembly, abundance, and diversity of soil fungi, organisms with wide-ranging effects on forest health, biogeochemistry, and regeneration (Frąc et al., 2018).

Fungi are of particular interest as they represent a significant fraction of the soil microbial community and influence several ecosystem processes (Treseder & Lennon, 2015; Baldrian, 2016; Frąc et al., 2018). Through multiple trophic guilds, fungi mediate carbon and nitrogen dynamics by decomposing organic materials and, by forming mutualistic symbioses with plant roots, they influence the hosts' nutrient availability and growth (Smith & Read, 2008; Baldrian, 2016). Furthermore, pathogenic fungi can increase hosts' susceptibility to fungal infections and insect infestations (Sturrock et al., 2011). In the Canadian boreal forest, common overstory trees, such as lodgepole pine (*Pinus contorta* var. *latifolia*), form mutualisms with ectomycorrhizal (EcM) fungi. In contrast, most understory plants in boreal coniferous forests tend to associate with arbuscular mycorrhizal (AM) fungi (Öpik et al., 2008). Tree mortality induced by disturbances and the subsequent effects on soil mycobiota, will thus likely have consequences for forests' ecosystem function.

Within forest soils, organic horizons hold much of the fungal diversity, especially for EcM fungi that stabilize soil organic matter by slowing saprotrophic decomposition (Averill & Hawkes, 2016; Jacobs et al., 2018). Both theoretical and field studies have demonstrated that EcM fungi mine organic N, which indirectly decreases the soil carbon respired by free-living decomposers, consequently, increasing soil carbon storage (Orwin et al., 2011; Averill et al., 2014; Averill & Hawkes, 2016). In contrast, AM fungi cultivate an inorganic nutrient-based economy dominated by the activity of free-living decomposers and characterized by a rapid C turnover, and thus, saprotrophic and AM fungi prevail in mineral soil layers (Phillips et al., 2013;

Frey, 2019). Chronosequence studies show that 50 to 70% of the C sequestered in boreal forest soils is derived from the continuous belowground allocation of C assimilates to roots and associated fungi (Clemmensen et al., 2013). The production of EcM fungal biomass represents a substantial pool of soil C, constituting up to 238 kg C ha^{-1} $year^{-1}$ (Wallander et al., 2004; Ekblad et al., 2016). Degradation of soils, specifically the loss of organic horizons following disturbances, can reduce overall fungal biomass (Holden & Treseder, 2013), and differentially impact fungal guilds (Barker et al., 2013; Wilhelm et al., 2017). Thus, understanding how different disturbances disrupt forest floors is necessary to predict changes in the abundance of different fungal guilds, and potential losses in soil C.

Previous studies have examined how individual disturbances impact the community composition of soil fungi in coniferous forests (Byrd et al., 2000; Dahlberg et al., 2001; Saravesi et al., 2008, 2015; Kipfer et al., 2011; Barker et al., 2013; Treu et al., 2014; Hartmann et al., 2014; Karst et al., 2015). However, these studies have primarily focused on EcM fungi, disregarding other fungal guilds with which they interact and synergize to carry out multiple ecosystem processes (Talbot et al., 2013; Bödeker et al., 2016; Crowther et al., 2019). In fact, few studies have included more than two disturbances or fungal guilds (Hartmann et al., 2012; Holden et al., 2013; Fichtner et al., 2014; Holden et al., 2016; Pec et al., 2017; Marín et al., 2017; Kohout et al., 2018; Day et al., 2019), yet to test whether common or different filters operate across disturbances it is imperative to compare how multiple disturbances impact distinct guilds. Overall, these studies have reported that tree mortality and the subsequent changes in edaphic factors—moisture, nutrients, pH, temperature—have detrimental effects on EcM fungal communities, while saprotrophic fungi become more abundant. However, it is still unclear how

disturbances affect AM and pathogenic fungi, and how the disruption of the forest floor following disturbances impacts particular fungal communities (van der Heyde et al., 2017).

Here, I examined the impact of different biotic (MPB outbreak) and abiotic disturbances (wildfire, clear-cut logging, and salvage-logging) on the soil fungal communities of lodgepole pine forests in Alberta, Canada. I further compared disturbed sites to paired controls, characterized the composition, structure, and diversity of multiple fungal guilds (EcM, AM, saprotrophic, and pathogenic fungi) and quantified the total soil fungal biomass. I tested the following three hypotheses. First, fungal guilds will differentially respond to forest disturbances; fungi that depend on living pines for their C source—EcM fungi—should decrease after stand-replacing disturbances such as wildfire and logging, but less so in disturbances causing moderate tree mortality, such as MPB outbreak. Second, the disruption to the forest floor mediates changes in the frequency and diversity of particular fungal guilds; guilds that depend on C supplied by overstory trees and play roles in the accumulation of soil organic matter—EcM fungi—may decrease, impacting the proportion and/or frequency of guilds that promote opposite C dynamics—saprotrophic and AM fungi. Lastly, soil fungal biomass would vary across disturbances; disturbances that strongly disrupt forest (wildfire, clear-cut, and salvage-logging) will result in more pronounced declines in soil fungal biomass compared to disturbances that leave an intact forest floor (MPB outbreak).

2.2 Materials and Methods

2.2.1 Field site selection and sample collection

During the summer of 2016, I selected 28 sites in west-central Alberta, Canada, to capture a range of disturbances (Appendix 2.1)**.** Within six years prior to my sampling, 14 of these sites

were disturbed by either clear-cut logging (n=4), MPB outbreak (n=3), wildfire (n=3), or salvage-logging after MPB outbreak (n=4). All of the disturbed sites were previously mature forests dominated by lodgepole pine with a minimum of 70% stand basal area. For each disturbed site, I selected a paired control site located within 10 km with no signs of insect outbreak, burn, thinning, or any other type of mortality, and with similar stand age, basal dominance, and forest structure to the pre-disturbance conditions of its pair (Appendix 2.2).

At each site, I established one 900 m^2 (30 × 30 m) plot with a 25-point grid (7.5 m between points) for sampling (Appendix 2.3). Plots were located at least 100 m away from the edge of the disturbed patch and any roads. In the case of MPB, where the disturbance edge is not evident, I established the plot in a central location within the attacked/dead forest stand. Over June to July 2016, a soil core (1.9 cm diameter; 23 cm depth) was collected at each grid point in all 28 sites to account for potential spatial heterogeneity within sites (Anderson et al., 2014), for a total of 700 soil samples. I placed soil cores in separate bags and kept them at 4 °C for less than 48 hrs until stored at -20 °C for further use in fungal biomass and DNA analysis. I cleaned and flamed all field tools between sites to avoid cross-contamination. In each plot, I also established two parallel 7.5 m wide transects (Appendix 2.3) within which I measured the diameter at breast height (1.3 m) of trees comprising the forest overstory, counted understory plant individuals, and estimated the percentage of ground covered by moss, graminoids, shrubs, forbs, leaf litter, woody debris, and bare ground at three different 1 m^2 squares per transect. Additionally, I measured the depth of the organic layer at each core location using a scale ruler. Soil nutrients and pH of samples pooled by site were also measured. A modified Kelowna protocol (Soil and Crop Diagnostic Center, 1995) was used to extract phosphate (PO_4^{3-}), and a 2M KCl extraction

protocol (Maynard et al., 2007) to extract nitrate (NO^{3-}) and ammonium (NH^{4+}). Values were expressed as mg kg^{-1} after colorimetric quantification. Soil pH was measured in deionized water.

2.2.2 Fungal biomass quantification

To test how disturbances affected soil total fungal biomass, I extracted and quantified ergosterol from a subset of the 700 collected soil samples. I divided each plot into four equal parts and selected a random soil sample from each (4 samples x 28 sites = 112). I subsampled soils in a 1:3 organic to mineral ratio and later extracted ergosterol from 125 mg of freeze-dried soil using methanol, according to Sterkenburg et al. (2015). Extracts were filtered through glass wool, and a 20 µL injection volume analyzed using an Ultra High Performance Liquid Chromatograph (1290 Infinity, Agilent Tech., Santa Clara, CA, USA) fitted with a Poroshell 120 EC-C18 column (2.1 mm x 150 mm, 2.7 µm; Agilent Tech.). The mobile phase consisted of an isocratic binary system of 25% methanol (HPLC-grade) and 75% acetonitrile flowing at 0.4 mL min^{-1} for 10 min. The ergosterol peak was detected by UV/VIS at 282 nm, and its concentrations (µg g^{-1} soil) quantified using a concentration standard curve comprised of serial dilutions of an ergosterol standard (≥ 95%; Sigma-Aldrich, St. Louis, MO, USA).

2.2.3 DNA extraction and sequencing

I sampled each of the 700 collected soil cores to characterize fungal community composition. Cores were individually subsampled in a 1:3 organic to mineral soil ratio and further lyophilized at -45 °C for 72 h in a freeze-drier (Labconco, Kansas City, MO, USA). Subsamples were transferred to 2 mL Eppendorf tubes and ground on a TissueLyser II (Qiagen Inc., Mississauga, ON, CAN). I isolated total genomic DNA from 250 mg of each of the ground samples using the

E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) and quantified concentrations in ND-1000 Nanodrop (Thermo Fisher Sci, Waltham, MA, USA). The internal transcribed spacer 1 (ITS1) and small ribosomal subunit (SSU) regions of the fungal nuclear ribosomal DNA (rDNA) were amplified in a two-step PCR using primers ITS1F – ITS2 and WANDA – AML2, respectively (White et al., 1990; Lee et al., 2008; Dumbrell et al., 2011). The former primer pair serves as a Dikarya fungal barcode (Blaalid et al., 2013), while the latter is commonly used to characterize Glomeromycotina communities (Öpik et al., 2013). To sequence using the Illumina Miseq (Illumina Inc., San Diego, CA, USA) platform, forward and reverse primer oligonucleotides containing the Illumina overhang adapters were added to the locus-specific sequence.

I carried out all PCR amplifications in 25 μL volumes, according to Platinum™ SuperFi™ Green PCR Master Mix (Invitrogen, Carlsbad, CA, USA) specifications, containing 1 μL of DNA. Conditions for the first ITS1 PCR consisted of an initial denaturation at 95 °C for 2 min followed by 35 cycles of 95 °C for 30 s, 58 °C for 30 s, and 68 °C for 1 min, with a final extension of 68 °C for 7 min. For SSU, I used the same initial denaturation followed by 30 cycles of 95 °C, 54 °C, and 72 °C each for 1 min and a final extension at 72 °C for 10 min. I purified PCR products using Mag-Bind® TotalPure NGS (Omega Bio-tek) following the manufacturer's protocol, and 2.5 μL of each reaction was used for indexing PCR to allow a multiplexed sequencing. For this second PCR, I used the same polymerase enzyme in combination with Nextera XT Index Kit (Illumina, Inc.) with the following conditions: 95 °C for 3 min followed by 8 cycles of 95 °C, 55 °C, and 72 °C for 30 sec each, and 72 °C for 5 min. Again, I purified PCR products as previously described and used 5 μL of each sample pooled together for two separate sequencing runs, each containing 350 samples. Using an Agilent 2100

Bioanalyzer, I checked the size and concentration of the pooled second PCR product. Samples were divided into two amplicon libraries, each containing 350 samples, four positive controls, and four negative controls. Libraries were submitted to the Molecular Biology Facility at the University of Alberta for sequencing in Illumina MiSeq platform using 2 x 300 bp paired-end reads with v3 chemistry. Sequence data was deposited in NCBI (BioProject ID PRJNA594651).

2.2.4 Bioinformatic analysis, taxonomic classification, and guild membership

Demultiplexed reads were first checked for non-biological oligonucleotide content in FastQC and further imported to 'Quantitative insights into microbial ecology 2' (QIIME2) version 2018.6 (Bolyen et al., 2019), for trimming, quality control, and taxonomic classification. I trimmed Illumina adapters and primer complements using the 'cutadapt' plugin (Martin, 2001) to retain only biologically relevant sequences and used the 'DADA2' plugin (Callahan et al., 2016) for quality control of reads, to filter chimeras, and resolve amplicon sequence variants (ASVs) of forward reads. Although the use of operational taxonomic units (OTUs) is common in fungal metabarcoding studies, I employed ASVs given their better resolution on characterizing fungal communities and the similarity of ecological conclusions drawn from the two different methods (Glassman & Martiny, 2018; Pauvert et al., 2019). Paired-end reads were not possible as one of the sequencing runs was of low quality on reverse reads. I assigned taxonomy to ITS1 ASVs using the UNITE dynamic classifier, which considers individual lineages to assign taxonomy on a 97 to 98% threshold (Abarenkov et al., 2010). For the SSU dataset, I used the MaarjAM classifier, also assigning taxonomy based on ≥ 97% similarity (Öpik et al., 2010). Taxa not assigned to Kingdom Fungi were removed. Based on species rarefaction curves, a total of 2244 ITS1 and 2116 SSU sequences were subsampled per sample for further analyses. The presence-

absence of each ASV was calculated for each soil core, and these values were averaged to calculate the frequency of individual ASVs per site. I used the FUNGuild database to assign ecological guilds to ASVs (Nguyen et al., 2016) and only retained taxa assigned to a single guild and with confidence rankings of 'probable' or 'highly probable' from the EcM, all pathogenic, and all saprotrophic fungal guilds.

2.2.5 Data analysis

All statistical analyses were conducted in R version 3.5.1 (R Core Team 2018). Significance was set to $\alpha < 0.1$. I selected this value to balance type one and two errors. Specifically, high replication is difficult to achieve in landscape studies. In consequence, the power of statistical analyses is constrained, therefore increasing the probability of type two error.

2.2.6 Fungal biomass, soil nutrients, and organic layer depth

I tested whether soil ergosterol concentration differed between disturbances and control sites. Control sites were not specifically paired with their disturbed sites in the analysis because control groups (i.e., control sites paired to the specific disturbances) were homogeneous in their ergosterol concentrations. I performed a linear mixed effects model on log-transformed ergosterol concentrations using the 'lmer' function within the R 'lmer4' package (Bates et al., 2015). For this model, disturbance type was used as a fixed factor and site as a random effect to account for the nested sampling design. Furthermore, I tested the correlation between log-transformed ergosterol concentration and the ASV frequency of ectomycorrhizal (EcM), saprotrophic, pathogenic, and arbuscular mycorrhizal (AM) fungi using Pearson's correlation tests. I examined whether soil nutrients and the organic layer depth differed between disturbed

and pooled control sites using Kruskal-Wallis tests by ranks, including Holm adjustments for multiple comparisons. Additionally, I examined the correlation between the depth of the organic layer in the different sites and the ASV frequency of EcM and arbuscular AM fungi using Spearman's rank correlation.

2.2.7 Fungal community composition

For the whole fungal community and individual guilds, I evaluated differences in community composition between disturbed and control sites based on Bray-Curtis dissimilarities. To test the differences, I used permutational multivariate analysis of variance (perMANOVA) with 999 permutations on the dissimilarity matrices with the 'adonis' function of the 'vegan' package in R, followed by pairwise comparisons with Holm correction for multiple testing using the 'pairwise.perm.manova' function of the 'RVAideMemoire' package (Oksanen et al., 2019; Hervé, 2020). Differences in communities, based on Bray-Curtis dissimilarities, were then visualized using non-metric multidimensional scaling (NMDS) with 95% confidence ellipses, both from the 'vegan' package (Oksanen et al., 2019).

To test if disturbances affected guilds differently, I calculated the proportion of each ITS guild (EcM, saprotrophic, and pathogenic fungi), dividing their individual guild frequency by the total sum of frequencies from those same guilds. Arbuscular mycorrhizal fungi were not included in proportion calculations as they were characterized with a separate primer pair. Differences in the proportion of guilds were evaluated using Kruskal-Wallis tests, including a Holm adjustment for multiple comparisons. I calculated the diversity of individual fungal guilds per site by converting the Shannon diversity index into effective number of species, which is the number of equally abundant species required to produce an observed value of an index (Jost, 2006). I

decided to use this diversity metric because the non-linearity associated with standard diversity indices can lead to misinterpretation of the diversity in ecological communities. This limitation can be overcome using effective number of species. Additionally, I assessed differences in the effective number of species of all guilds, excluding AM fungi, using one-way ANOVA with Tukey's HSD adjustments. For AM fungal diversity, I used the Kruskal-Wallis test by ranks with Holm adjustments to test differences between disturbances and control sites.

To determine if disturbances changed the frequency of particular taxonomic groups, ASVs were collapsed to the genus level and averaged per disturbance type and control sites. I evaluated the significance of the changes in frequency using nonparametric *t*-tests with 999 Monte Carlo permutations. Furthermore, using the frequency for a given genus at paired control sites as a baseline, percentage change was calculated for the genera comprising the largest changes and graphed in a heatmap using the R 'pheatmap' package (Kolde, 2019).

2.3 Results

2.3.1 Forest site properties

My site selection encompassed pronounced differences in stand basal area of living lodgepole pine, along with differences in the understory structure (Appendix 2.4). Basal area of live lodgepole pine trees was greater in controls than in disturbed sites; however, MPB disturbed sites had at least half the living basal area of controls owing to insect attack. Control sites had a higher abundance of understory individuals, both conifers and non-conifers, compared to all other disturbances (Appendix 2.4). Among disturbances, clear-cut and salvage-logging had a higher abundance of non-conifer understory individuals compared to wildfire and MPB outbreak sites; however, conifer abundance was similar across disturbances (Appendix 2.4).

Depth of the soil organic layer differed by disturbances (Kruskal-Wallis: $\chi^2_4 = 13.81$, $P = 0.008$), while soil nutrients did not (Appendix 2.5). Control and MPB disturbed sites, had organic layer depths of 4.94 ± 0.4 cm (mean ± SE) and 5.8 ± 1.0 cm, respectively. The depth of the organic layer was shallower in all other disturbances with a 43% decline in wildfire, 47% in clear-cut logging, and 19% in salvage-logging relative to control forests.

2.3.2 Fungal biomass

Soil fungal biomass, measured by ergosterol concentration, differed between some disturbances and control forests (Fig. 2.1). Relative to controls, fungal biomass was, on average, 56% lower (Linear mixed model: $F_{4, 22.5} = 6.21$, $P = 0.002$) in sites where disturbances resulted in the loss or combustion of trees (clear-cut logging, salvage-logging, and wildfire). Wildfire had the most pronounced effect on fungal biomass in soils with a 75% decline, followed by clear-cut logging (50%), and salvage-logging (43%), although the latter disturbance was not statistically different from controls (Appendix 2.6, 2.7). However, in MPB disturbed sites, fungal biomass was 44% higher than that of controls. There was no difference in ergosterol concentration between MPB outbreak and salvage-logging, although the concentration in the latter was, on average, more similar to that of clear-cut logging than to control sites (Fig. 2.1).

2.3.3 Fungal community composition

Over two Illumina MiSeq runs, I obtained a total of 20.6 million DNA reads, averaging 26,384 and 33,520 per sample, respectively. Raw reads per sample ranged from 59 to 52,817, with only seven samples yielding less than 4,000 reads. After 'DADA2' quality control and filtering, 10.1 million DNA reads remained for downstream analysis, representing 27,879 amplicon sequence

variants (ASVs). From these ASVs, I recorded a total of 1,181 taxa, 1,144 from ITS1 and 37 from SSU rDNA. Furthermore, based on species rarefaction curves, a total of 2,244 ITS1 and 2,116 SSU sequences were randomly subsampled from each sample for comparison. Saprotrophs had the highest representation in the species pool with 311 taxa, followed by 270 EcM, 84 pathogens, and 37 AM fungi. In the ITS1 dataset, 83% of the ASVs were assigned to guilds, and the other 17% remained unclassified. From the ASVs with assigned guilds, 16% had dual roles or were from guilds not of interest for the study, and 9% had confidence rankings of 'possible' thus were not used. Sequence-wise, guilds ranked as 'possible' accounted for 43% of the ITS1 reads while taxa with unassigned guilds represented 18 to 23% in controls and disturbed sites, respectively. Guilds not used owing to their dual roles or not of interest for the study represented 7% of the sequences. EcM fungi accounted for 20% of the reads, saprotrophic fungi for 9%, and pathogenic fungi for 0.5%.

Whole community (ITS guilds and AM fungi) composition was significantly different from the controls following wildfire and salvage-logging disturbances (perMANOVA: $F_{4, 23} = 1.18$, $R^2 = 0.20$, $P = 0.014$); (Appendix 2.8, 2.9). To verify that time since disturbance was not a defining factor structuring the soil fungal community, I added the year in which the disturbance occurred to the Appendix 2.10A (Appendix 2.10B). I did not find any particular year clustering closer together, suggesting that disturbance age was not explaining the variation in community composition.

Ectomycorrhizal fungal community composition differed among disturbances from control sites (perMANOVA: $F_{4, 27} = 1.87$, $R^2 = 0.25$, $P < 0.001$). Specifically, wildfire, clear-cut logging, and salvage-logging disturbances had different EcM fungal communities compared to controls (Fig. 2.2A, Appendix 2.8, 2.9). Ectomycorrhizal fungal communities in MPB disturbed sites,

although not significantly different from the other disturbances, had communities more similar to controls than to other disturbances (Appendix 2.9). Soil pH also explained some of the variation in the EcM fungal community (perMANOVA: $F_{3, 27} = 1.33$, $R^2 = 0.18$, $P = 0.001$) (Appendix 2.8).

For the pathogenic fungi, wildfire was the only disturbance that changed the community composition (perMANOVA: $F_{4, 23} = 1.35$, $R^2 = 0.19$, $P = 0.038$) (Appendix 2.9); fungal communities from all other disturbances clustered together with controls (Fig. 2.2B). No differences in composition between controls and any of the disturbances were found for AM fungal community (perMANOVA: $F_{4, 23} = 1.18$, $R^2 = 0.18$, $P = 0.320$); (Fig. 2.2C; Appendix 2.8). In the saprotrophic community, despite the significant difference in community composition (perMANOVA: $F_{4, 23} = 1.29$, $R^2 = 0.18$, $P = 0.069$) (Appendix 2.8), I do not consider it biologically important given the lack of significance in the pairwise comparison (Fig. 2.2D, Appendix 2.9).

Disturbances shifted the dominance and altered the proportion and frequency of particular fungal guilds (Fig. 2.3). Community dominance shifted from EcM to saprotrophic fungi across all disturbances relative to controls. While EcM fungi in controls comprised 49% of the total proportion, significant declines of 53, 59, and 51% occurred in clear-cut logging, salvage-logging, and wildfire disturbances, respectively (Kruskal-Wallis: $\chi^2_4 = 18.63$, $P < 0.001$). However, in sites disturbed by MPB outbreak, the shift in dominance was less pronounced, showing a 35% decline in EcM fungal proportion. I confirmed these effects by comparing results from proportions to those of relative sequence abundance of EcM, saprotrophic, and pathogenic fungi as well as whole ITS1 sequences (Appendix 2.11).

Opposite to EcM, the saprotrophic fungal community in controls accounted for 44% of the proportion (Fig. 2.3). The proportion of saprotrophic fungi increased by 55, 62, and 45% in clear-cut logging, salvage-logging, and wildfire disturbances, respectively (Kruskal-Wallis: χ^2_4 = 17.45, P = 002) (Appendix 2.12). The increase, however, was less pronounced in MPB disturbed forests where saprotrophic fungi increased by 35%. Similarly, but not significantly, the proportion of pathogenic soil fungi increased by 65% after wildfire but only 21–23% after clear-cut logging, salvage-logging, and MPB disturbances (Kruskal-Wallis: χ^2_4 = 4.78, P = 0.310) (Appendix 2.12).

Although AM fungal frequency in disturbed sites was compared only to their controls and not to proportions of guilds profiled with the ITS1 dataset, AM fungal frequency showed opposite trends to the EcM fungal proportion (Kruskal-Wallis: χ^2_4 = 8.02, P = 0.091) (Fig. 2.3). Arbuscular mycorrhizal fungi increased in frequency, relative to controls, by 52, 31, and 36% in clear-cut logging, salvage-logging, and wildfire, respectively, but only 5% after MPB disturbance. However, the increase was significant following only wildfire disturbance (Appendix 2.12).

Ergosterol concentration was positively correlated to the EcM fungal frequency (Pearson: r^2 = 0.42, P = 0.024) but showed no correlation to saprotrophic (r^2 = -0.04, P = 0.827) and pathogenic fungal (r^2 = -0.04, P = 0.855) frequencies (Appendix 2.13). Compared to AM fungal frequency, ergosterol showed a negative correlation (r^2 = -0.35, P = 0.062). However, this is likely due to the indirect relationship between EcM and AM fungal frequencies measured as frequencies and not their direct contribution to ergosterol concentration. Furthermore, the decrease in the soil organic layer with disturbance was negatively correlated to EcM fungal frequency but positively correlated with AM fungal frequency (Spearman: r = 0.51, P = 0.006

and r = -0.48, P = 0.009; EcM and AM fungi, respectively) (Fig. 2.4). I found a positive correlation between the depth of the soil organic layer with the proportion of EcM fungi (Pearson: r = 0.44, P = 0.019); (Appendix 2.14).

The diversity of fungal guilds differed across the disturbances (Fig. 2.5). The effective number of EcM fungal species declined in all disturbances from that in controls (ANOVA: $F_{4, 23}$ = 6.24, P = 0.001). Although the decline was only significant for clear-cut logging and wildfire disturbances, MPB-disturbed forests and those salvage logged also declined in EcM fungal species diversity (Appendix 2.12). The diversity of saprotrophic fungi also varied among disturbances (ANOVA: $F_{4,23}$=3.584, P = 0.021). Salvage-logging significantly increased saprotrophic fungal diversity compared to controls, and a non-significant increase occurred in clear-cut logging and MPB-disturbed forests. No significant differences in diversity from controls were found in the AM and pathogenic fungal communities.

Particular taxa decreased or increased in frequency depending on the disturbance (Fig. 2.6); (Appendix 2.15). Although the frequency of ASVs of EcM fungi mostly decreased, some ASVs had up to a 16% increase. Both logging disturbances increased the frequency of the EcM genus *Tuber*, while in MPB-disturbed forests, *Tricholoma* increased. The genera *Piloderma*, *Cortinarius*, *Russula,* and *Lactarius*, had losses of 14 to 52% across all disturbances.

In the saprotrophic fungal community, some of the major gains and losses were in the genera *Mortierella*; it increased in frequency across all disturbances except for wildfires, and *Clavaria*, which increased in all disturbances, but MPB outbreak. Disturbances had minimal effects on pathogenic fungal frequency, but genera such as *Venturia* and *Galerina* increased with all disturbances, except for MPB outbreak. Lastly, the frequency of the AM fungal genera *Glomus*

and *Claroideoglomus* increased after all disturbances but MPB outbreak, where only *Glomus* decreased.

2.4 Discussion

Forest disturbances impacted the community composition and proportion or frequency of soil fungi. Specifically, stand replacing disturbances (wildfire, clear-cut, and salvage-logging) shifted the dominance from ectomycorrhizal (EcM) to saprotrophic fungi, and increased the frequency of arbuscular mycorrhizal (AM) fungi, although only significantly so for the latter guild following wildfire. However, these effects were less pronounced in beetle-killed sites, possibly because compared to the other disturbances, the aboveground forest structure and soil organic layer were not severely or rapidly disturbed. Combustion and physical disruption of the soil organic layer seems to drive both the decline of EcM and the increase of AM fungal ASV frequency.

Most disturbances decreased the fungal biomass in soils, an effect with implications in soil C storage. Using the correction factor from Montgomery et al. (2000), I estimated the fungal biomass–C per gram of dried soil from ergosterol concentration; c. 426 $\mu g\ g^{-1}$ to 738 $\mu g\ g^{-1}$ fungal biomass–C is being lost from soils after abiotic disturbances that disrupt the organic layer. This loss represents a 44 to 75% decline after salvage-logging, clear-cut logging, and wildfires when compared to the fungal biomass–C in controls. In contrast, the effect of MPB outbreak represented a 45% increase in fungal biomass–C. In a meta-analysis on the response of microbial biomass to forests disturbances, Holden & Treseder (2013) discussed how abiotic disturbances consistently reduce the biomass of both fungi and bacteria in soils while biotic disturbances have neutral or positive effects, likely due to differences in the amount and type of organic C

remaining in forest soils. They suggested that when abiotic disturbances remove the organic C contained in the forest floor, the microbial biomass declines due to C limitation, but the opposite occurs during and after biotic disturbances, such as insect outbreaks, where there may be an influx of dead plant litter and other labile organic C compounds to the soil (Holden & Treseder, 2013).

Similar to Holden & Treseder (2013), I found a decline in fungal biomass after abiotic disturbances that disrupted the soil organic layer and removed or combusted the overstory biomass. I also found a non-significant increase after MPB outbreak even when the ratio of organic to mineral soil was kept constant when analyzing samples of disturbances and controls. Despite not knowing the relative contribution of individual fungal guilds to soil fungal biomass, the only guild that mirrors the effect of disturbances on ergosterol concentration is EcM fungi. The parallel trends may suggest that EcM fungi are a significant contributor to fungal biomass in forest soils, especially in the organic layer where they dominate. In fact, the observed positive correlation between EcM fungal frequency and ergosterol concentration suggests that the decline in fungal biomass is driven by a loss in EcM fungi in soils. While only 25% of boreal forest C stocks is plant biomass, the largest proportion is found in soil organic matter (40%) (Kurz et al., 2013). It has been demonstrated that roots and their associated fungi drive soil C dynamics in the boreal forest biome as large portions of the C fixed aboveground is transferred to roots and further becomes immobilized in fungal mycelia (Heinemeyer et al., 2007; Clemmensen et al., 2013). Therefore, changes in the dominance of soil fungi potentially have broader implications in long term soil C storage, especially in the event of frequent disturbances that accelerate soil C turnover (Treseder & Allen, 2000; Köster et al., 2014; Wallander & Ekblad, 2015).

While disturbances that physically remove or combust overstory biomass—wildfire, clear-cut logging, and salvage-logging—changed the community composition of EcM fungi, MPB outbreak, which is the only disturbance where overstory biomass is removed through the long-term decomposition of dead standing trees, did not steer the community away from the controls. In contrast to my results, previous studies have shown that MPB and other insect outbreaks significantly change the community composition of EcM fungi (Saravesi et al., 2008; Štursová et al., 2014; Karst et al., 2015; Pec et al., 2017). However, in the current study, MPB-disturbed sites had a wider-range of tree mortality (25–90%), with an overall higher amount of living stand basal area than that of earlier studies (750–100% mortality). Thus, the less extreme disturbance associated with MPB appears to have lessened the effect on soil fungi. Previous research also supports my results on the directionality of changes in EcM fungal communities following disturbances. For instance, both wildfire and clear-cut logging led to declines in EcM fungal richness and abundance, also decreasing the extracellular enzymatic activity in soil (Holden et al., 2013; Walker et al., 2016; Kohout et al., 2018). I found that all abiotic disturbances resulted in declines in the proportion and diversity of EcM fungi. However, communities did not diverge in composition among disturbances, suggesting that filters imposed by various disturbances are not taxonomically selective. Instead, different operating filters among disturbances result in a similar fungal taxonomic assortment in soil.

I found that the dominance of fungal guilds shifted from EcM to saprotrophic fungi across all disturbances. The increase in saprotrophic proportion in both logging disturbances could be due to the input of root and aboveground litter resulting from tree harvesting practices (i.e., removal of the aboveground portion of trees) (Brazee et al., 2014). Another guild whose frequency increased after wildfire was AM fungi, with a non-significant increase after clear-cut and

salvage-logging. This is likely due, as discussed below, to their symbiosis with many plant species succeeding fire and the disruption of the soil organic layer (Xiang et al., 2015). Whereas many studies profiling AM fungal communities following disturbances have been conducted in agricultural systems, few have studied AM fungi in coniferous forest soils. The few existing studies report increased AM fungal abundance and infective propagules within the following decade of a fire (Korb et al., 2003; Treseder et al., 2004).

Impacts on root-symbiotic fungi appear to be largely mediated by the physical disruption or combustion of the forest floor. The disruption of the soil organic layer correlated with the increased frequency of AM fungi. As the forest floor and aboveground biomass are removed or combusted by disturbances, herbaceous plants that resprout or colonize the site mostly associate with and increase the frequency of AM fungi (Treseder et al., 2004; Öpik et al., 2008). Nonetheless, this might not affect the taxonomic composition of AM fungi, given their cosmopolitan distribution and low host specificity (Davison et al., 2015; Powell & Bennett, 2016). However, I did not identify the understory plant community to species level, which hinders the possibility of testing whether ground cover and understory plant communities increase the frequency of AM fungi following disturbances. Furthermore, the changes in the soil environment, as well as the shift from EcM to saprotrophic fungal dominance, may benefit AM fungi given their inorganic and saprotrophic dependant nutrient economy (Phillips et al., 2013). These patterns in mycorrhizal abundance reflect trends from various chronosequences where, after a few decades, the biomass of EcM fungi increases while that of AM fungi starts to decline (Treseder et al., 2004; Twieg et al., 2007; Fichtner et al., 2014). However, these trends are based on past and current disturbance regimes. With increasing intensity and frequency of disturbances with climate change, the disturbance-recovery cycles of forests and soil fungal communities may

be lengthened, potentially causing abrupt changes in the system (Wotton et al., 2010; Ratajczak et al., 2018; Hansen & Turner, 2019).

Contrary to AM fungi, the frequency of EcM fungi declined with increased disruption of the soil organic layer. Although the decline in EcM fungi can be caused by the loss of the autotrophic hosts allocating photosynthates belowground to fungal symbionts (Pec et al., 2017), my results suggest that the loss of the organic soil layer may also have negative effects on EcM fungi. Only disturbances that disrupted the soil organic layer had substantial declines in EcM fungal frequency in the current study. Others have reported similar impacts on fungal communities resulting from changes in soil abiotic conditions following organic layer removal (Kranabetter et al., 2017a). For instance, long-term monitoring of soil conditions and EcM fungi in harvested sites with and without removal of the upper organic layer showed an average of 60% increase in soil temperature and dryness in sites following removal, effects that also suppress microbial activity and C cycling (Allison & Treseder, 2008; Wilhelm et al., 2017). In the same studies, the difference in fungal community composition between harvested and reference control sites was larger when the forest floor was disrupted, especially for EcM fungi, which had the most drastic decline in diversity (Baker et al., 2013; Wilhelm et al., 2017; Kranabetter et al., 2017a). However, harvesting practices with soil organic matter retention can buffer the decline in EcM fungi by preventing consequential changes in organism propagules and soil properties such as temperature and moisture (Kranabetter et al., 2017a,b). Practices aiming to preserve forest legacies should be considered to maintain EcM fungal species diversity when the post-disturbance system organizes, thus preventing loss of this guild and its function.

Shifts in particular fungal communities and their biomass may impact multiple ecosystem functions, including nutrient and carbon dynamics. With the loss of EcM fungi, free-living

saprotrophs can dominate, potentially altering soil N and C cycles as decomposition is increased thus reducing soil C and locking N in the saprotrophic microbial pool (Orwin et al., 2011; Koide et al., 2013; Treseder & Lennon, 2015; Averill & Hawkes, 2016). Together with changes in nutrient dynamics, an altered soil biotic environment can affect seedling establishment, a critical step for forest regeneration that can be in part mediated by mycorrhizal fungi (Horton et al., 1999; Nara, 2006; Karst et al., 2015; Horton & Van Der Heijden, 2018). Future studies should focus on how altered soil fungal communities impact forest regeneration and whether I should amend these communities to enhance ecosystem resilience through the selection of particular fungal traits. In particular, regeneration studies should investigate the effects of cumulative abiotic stressors that could synergistically impact seedlings under the expected climatic conditions of the future. Important fungal traits to consider in post-disturbance studies may include nutrient mineralization and transfer to host as well as facilitation of host stress tolerance and enhancement of plant defenses (Koide et al., 2013; Crowther et al., 2014; Karst et al., 2015).

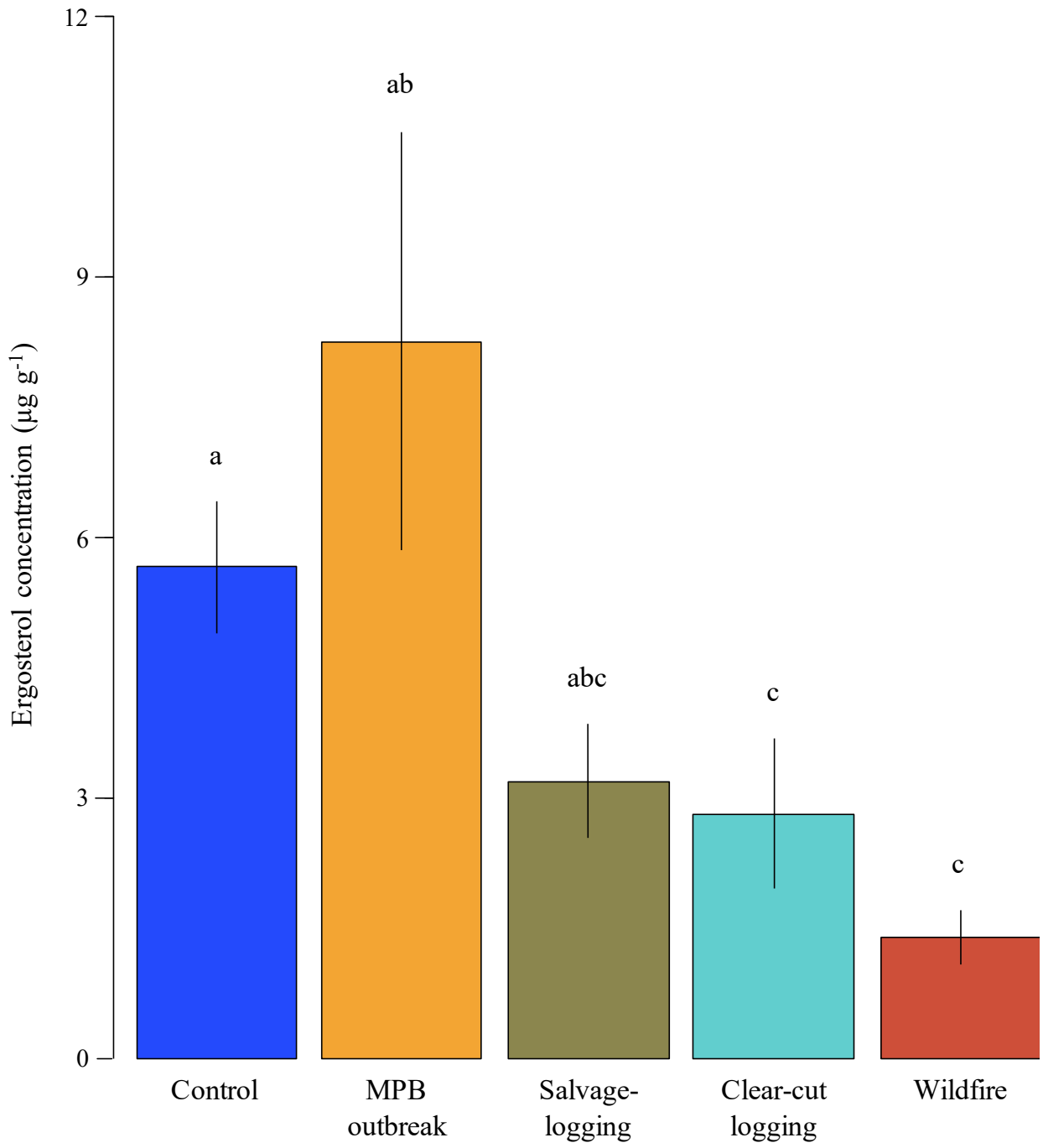


Figure 2.1. Mean ergosterol concentration (µg g^{-1}) of soils from control and disturbed sites. Data represent mean ± SE (n = 56 for control, n = 16 for salvage-logging and clear-cut logging, and n = 12 for MPB outbreak and wildfire). Letters indicate significant difference between groups at α = 0.1 tested with linear mixed-effects model followed by Tukey's HSD for multiple comparisons. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

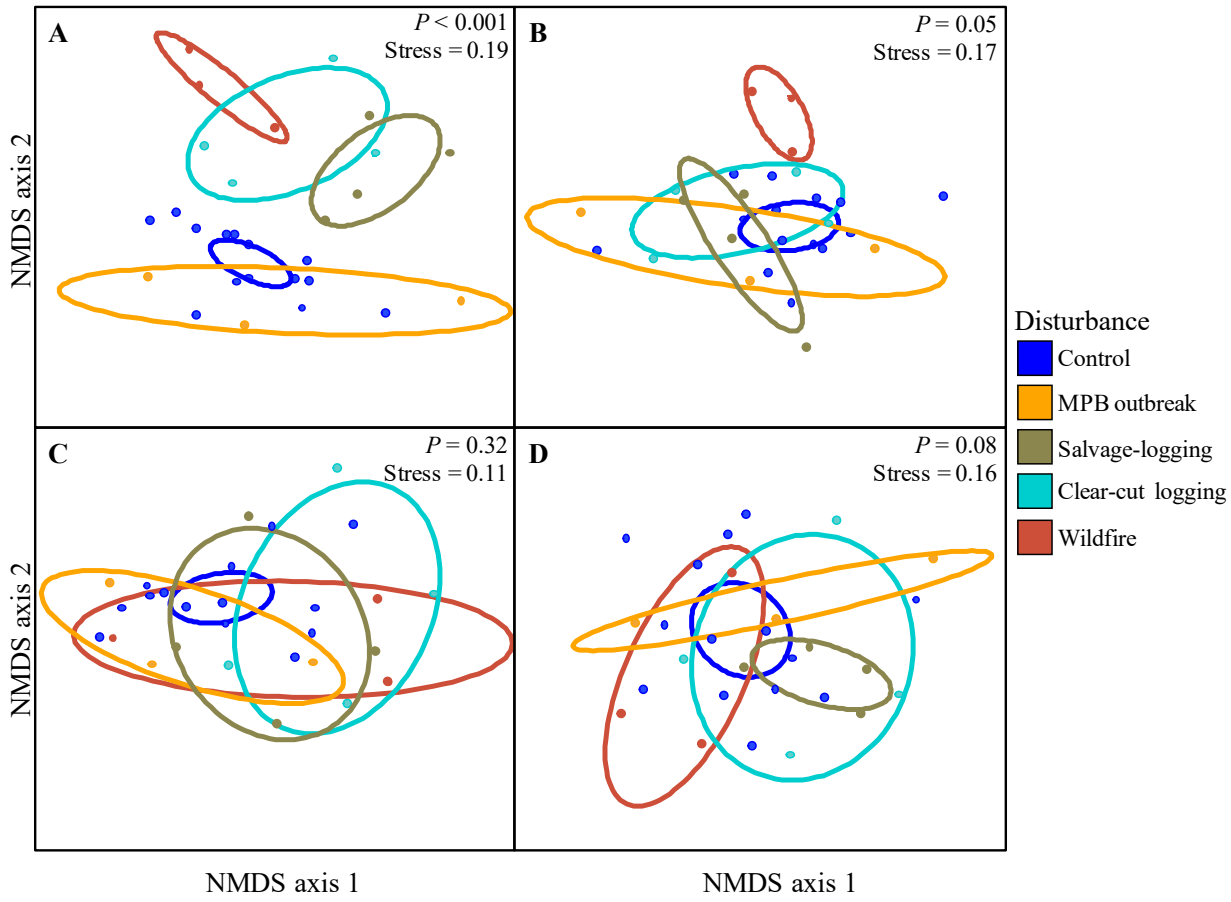


Figure 2.2. Non-metric multidimensional scaling plot of the effect of forest disturbances on ectomycorrhizal (A), pathogenic (B), arbuscular (C), and saprotrophic (D) fungal community composition. Points represent individual sites and ellipses 95% confidence intervals. Significance from control at $\alpha = 0.1$ was tested using perMANOVA. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

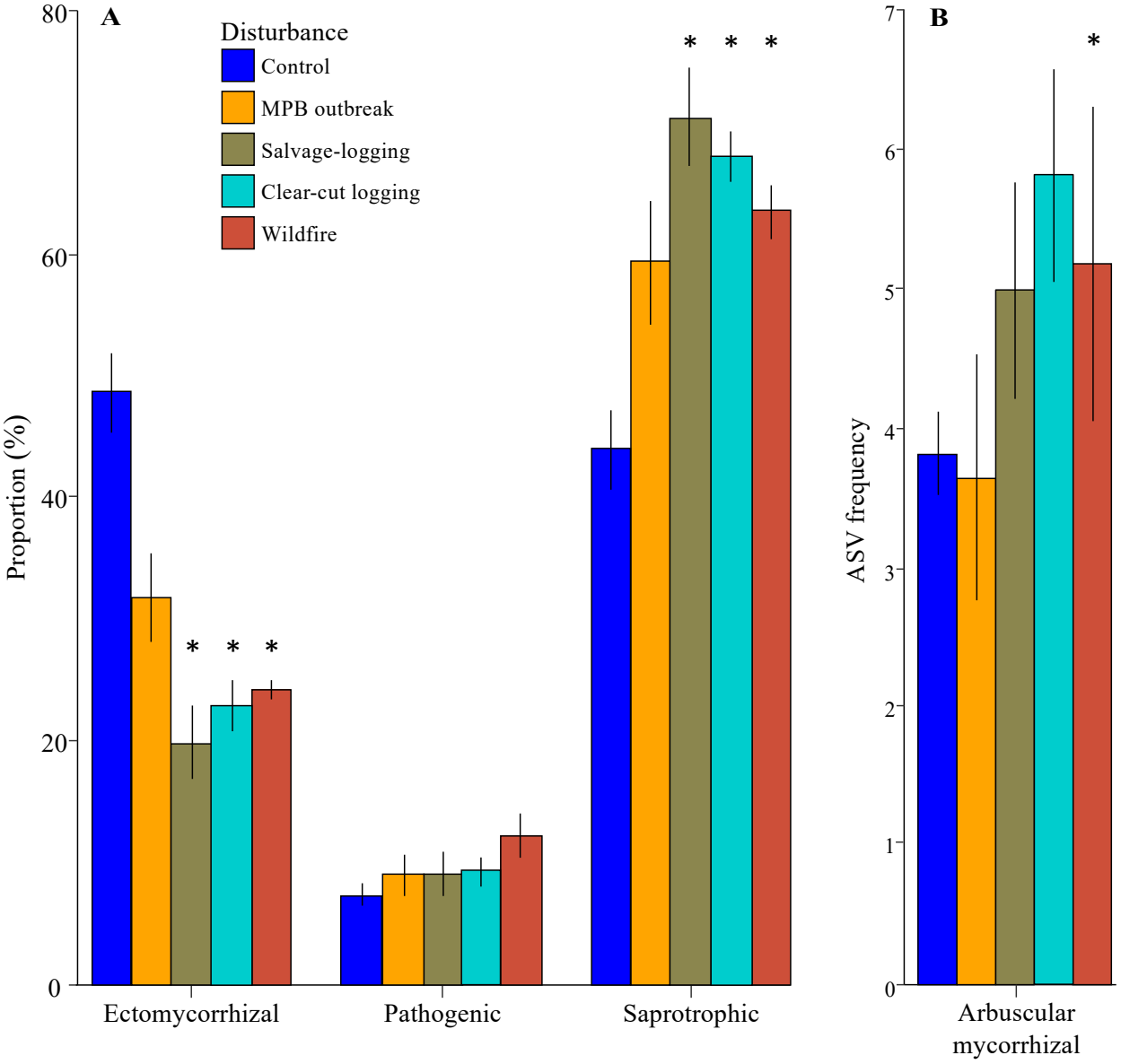


Figure 2.3. Proportion of guilds characterized using ITS1 (A) and ASV frequency of arbuscular mycorrhiza (SSU) (B) in soil from control and disturbed sites. Data represent mean ± SE (n = 14 for control, n = 4 for salvage-logging and clear-cut logging, and n = 3 for wildfire and MPB outbreak). Asterisks indicate statistical significance from control at $\alpha = 0.1$, tested with Kruskal-Wallis test of ranks. $\chi^2_4 = 18.63$, $P < 0.001$ in ectomycorrhizal guild, $\chi^2_4 = 17.45$, $P = 0.002$ for saprotrophic, and $\chi^2_4 = 8.02$, $P = 0.091$ for arbuscular mycorrhizal fungi. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

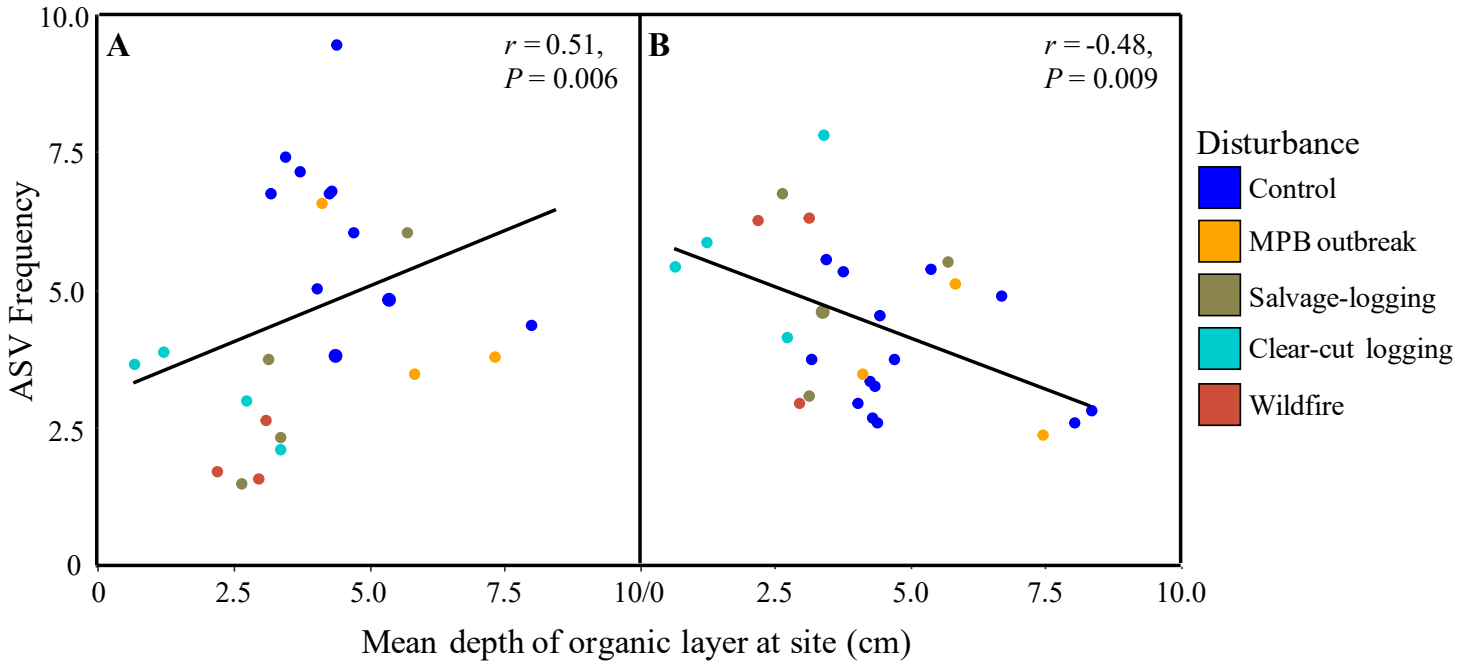


Figure 2.4. Relationship between the mean ASV frequency of ectomycorrhizal (A) and arbuscular mycorrhizal fungi (B) and the depth of the soil organic layer in control and disturbed sites.

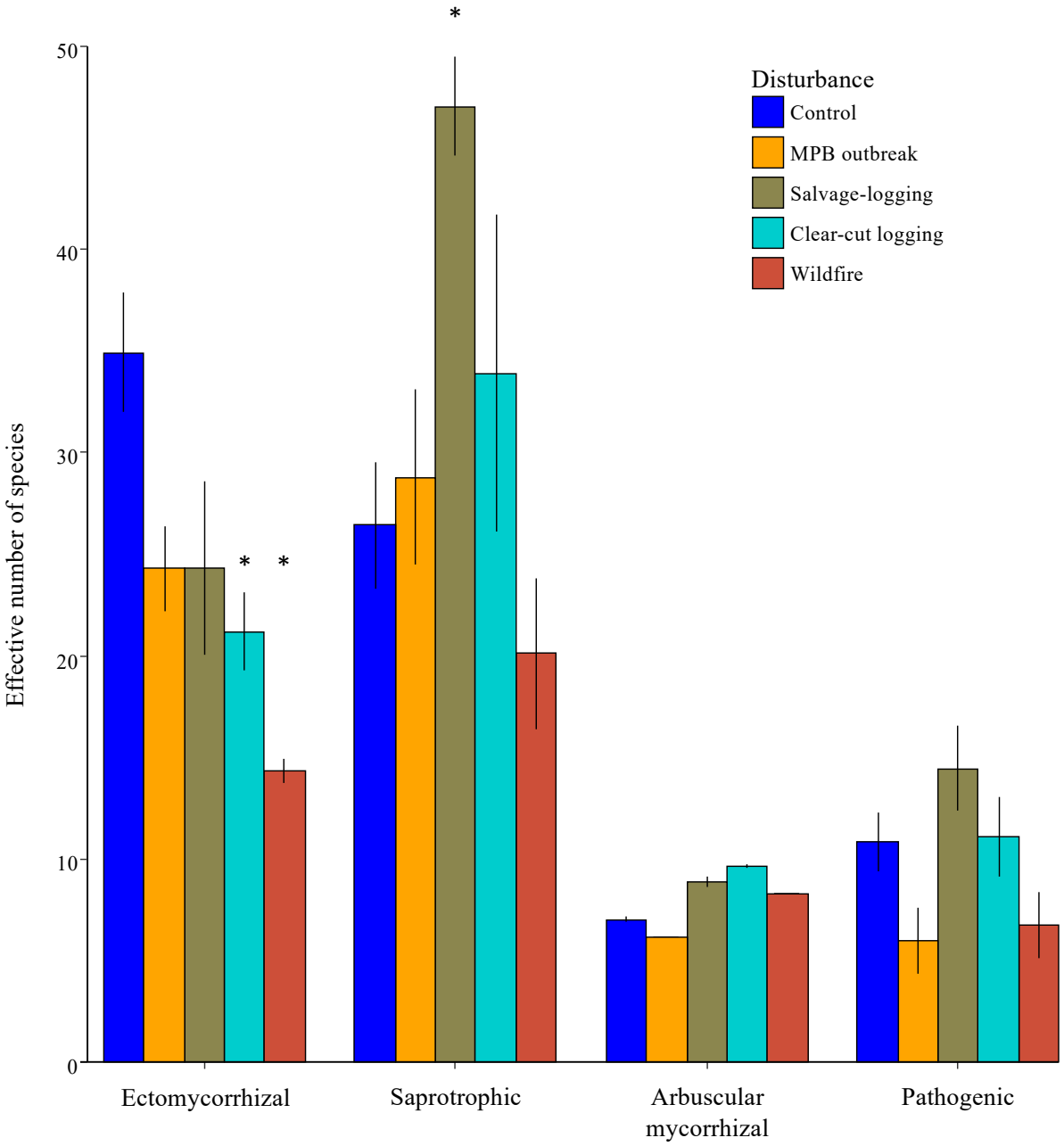


Figure 2.5. Diversity, as effective number of species, of fungal guilds from soil of control and disturbed sites. Data are mean ± SE (n = 14 for control, n = 4 for salvage-logging and clear-cut logging, and n = 3 for wildfire and MPB outbreak). Asterisks indicate statistical significance from Control at α = 0.1, tested with one-way ANOVA. $F_{4, 23} = 6.25$, $P = 0.001$ for ectomycorrhizal and $F_{4, 23} = 3.58$, $P = 0.021$ for saprotrophic. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

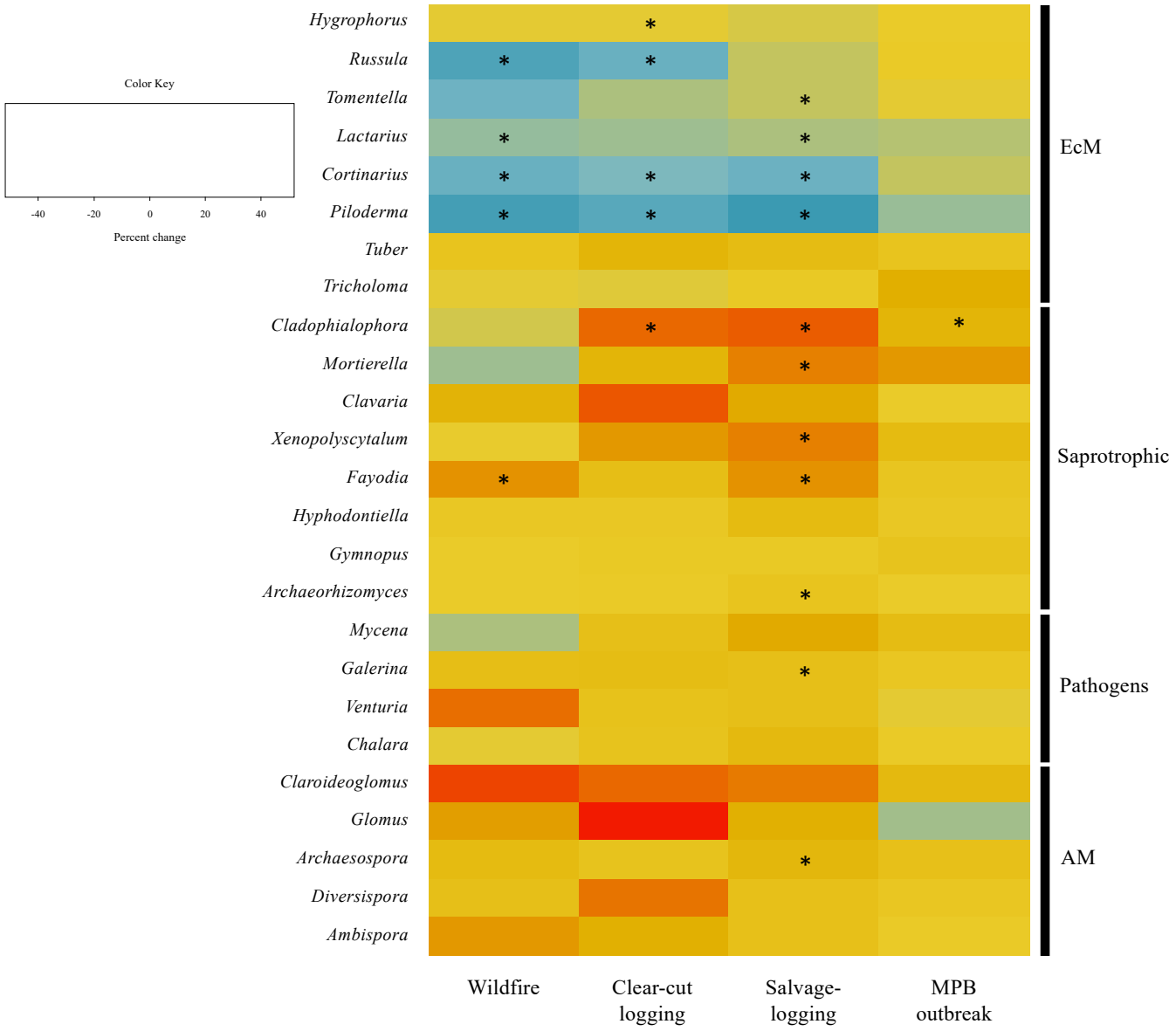


Figure 2.6. Percent change heatmap showing the ASV frequency gains and losses of genera, within guilds, in the different disturbed forests relative to controls. Data represent means and the different colors specify the magnitude of the change; blue indicates frequency losses whereas red indicates gains. Asterisks (*) represent a significant difference between the frequency of disturbance and control for individual genera tested with nonparametric t-tests with Monte Carlo permutations. EcM: ectomycorrhizal fungi. AM: arbuscular mycorrhizal fungi. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

Chapter 3

Disturbances override the restoration of fungi via soil transfers: impacts on pine seedling performance

3.1 Introduction

Forest resilience, recovery, and biodiversity are quickly changing with the current shifts in disturbance regimes driven by climate change (Johnstone et al., 2016; Newman, 2019). Such changes are particularly pertinent in North American coniferous forests, which are historically influenced by frequent natural and anthropogenic disturbances (Stevens-Rumann et al., 2018; Turner et al., 2019). Novel disturbance regimes involving fire, droughts, insects, and pathogen outbreaks for example, threaten the timber volumes that can be harvested and the forest ecosystem services that bolster local economies (Boucher et al., 2018). If shifts in disturbance regimes compromise forest resilience and recovery, active restoration may be necessary to return the species and ecosystem services on which humans depend. With forest recovery being dependent on seedling establishment, studying the underlying mechanisms of seedling establishment and performance is critical for management practices aiming to restore coniferous forests.

Manipulating mycorrhizal fungal communities in restoration has recently drawn attention as they influence plant community development by facilitating establishment and improving the performance of target species (Wubs et al., 2016; Policelli et al., 2020). Two main lines of research, controlled (i.e., greenhouse) and field experiments, have been used to study the influence of ectomycorrhizal (EcM) fungi on host plants following disturbances, with a few

using a combination of root inoculation followed by field out-planting (Teste et al., 2004; Menkis et al., 2007; Rincón et al., 2007; Beck et al., 2020). Overall, studies in controlled environments report that inoculation with mycorrhizal fungi increase the growth and/or biomass of seedlings compared to non-inoculated seedlings (Sousa et al., 2011; Karst et al., 2015; Sebastiana et al., 2018; Beck et al., 2020) and that these effects can depend on the complexity of the mycorrhizal fungal community (Baxter & Dighton, 2001; Sim & Eom, 2006; Hoeksema et al., 2010; Dalong et al., 2011). While controlled environments can isolate the effects of mycorrhizal fungal inoculum, field studies enable us to test the interactive effects of inoculation and site-specific conditions. In most restoration studies, seedling inoculation and performance have been assessed following disturbances that result in soil degradation or in novel ecosystems, as is the case of mining (Onwuchekwa et al., 2014; Sýkorová et al., 2016; Stefani et al., 2018; Policelli et al., 2020). However, few studies have manipulated EcM fungal communities *in-situ* (Grove et al., 2019) and across multiple disturbances, particularly in sites where fungal disturbance legacies are present.

Plant species in the *Pinaceae* family form mutualistic associations with EcM fungi, where plants exchange photosynthetically-fixed C for nutrients and water (Smith & Read, 2008). This obligate symbiosis is critical for the survival and growth of pine seedlings, as demonstrated by the limits on pine range expansion where compatible EcM fungi are absent (Horton et al., 1999; Nuñez et al., 2009; Horton & van der Heijden, 2018). However, disturbances where host trees die or are removed tend to decrease the abundance and richness of EcM fungi (Karst et al., 2014; Treu et al., 2014; Saravesi et al., 2015; Rodriguez-Ramos et al., 2021), potentially impacting pine regeneration. Since the beginning of the mountain pine beetle (*Dendroctonus ponderosae*; MPB) outbreak in western Canada, over 18 million hectares of lodgepole pine (*Pinus contorta*

var. *latifolia*) have been attacked while wildfire has impacted approximately 647,140 hectares in the last decade. In addition, in the same area, approximately 270,000 hectares are allocated for timber every year (Natural Resources Canada, 2019). In some cases, given the timing and intensity of fires, lodgepole pine is not predicted to recover (Hansen & Turner, 2019) on the landscape and in some areas following MPB attack, pine seedling regeneration has been limited (Mcintosh & Macdonald, 2013, Karst et al., 2015). Given the dependence of lodgepole pine on EcM fungi for establishment and growth, restoring EcM fungal communities following forest disturbances may be required to maintain this tree species on the landscape.

Despite the evident benefits of EcM fungi during early development of pine seedlings, knowledge gaps remain on when and how to use these fungi to restore boreal forests (Policelli et al., 2020). Plant community succession is tightly linked to the belowground microbial community, and it has been suggested that the introduction of soil inoculum from donor late-stage to recipient early-stage successional ecosystems can steer the plant community development in restoration projects (Wubs et al., 2016). Furthermore, the use of soil inoculum from late-successional conspecific forests in controlled environments has shown to enhance the performance of pine seedlings compared to soils from disturbances such as mountain pine beetle (MPB) outbreak, clear-cut, and salvage-logging (Karst et al., 2015; Beck et al., 2020). However, when EcM fungi from late-successional forests are introduced in disturbed sites with EcM fungal legacies and lacking mature host trees, 'late-stage' fungi might not establish due to C limitation and competition with the native fungal community (Kranabetter & Friesen, 2002).

Here, I transferred soil from late-successional lodgepole pine forests into sites recovering following different biotic (MPB outbreak) and abiotic disturbances (wildfire, clear-cut logging, and salvage-logging of MPB attacked sites) to examine the impact of soil transfers on seedling

germination, establishment, and biomass. In contrast to most restoration studies that use nursery-grown or pre-inoculated seedlings, I tested soil transfers on seedlings germinated and grown in the field, where EcM fungal legacies exist (Rodriguez-Ramos et al., 2020). The first objective of this study is to determine whether soil transfers improve the survival and performance of lodgepole pine seedlings growing in disturbed sites. I predict that, compared to non-inoculated plots, an increased volume of transferred soil will increase the establishment and performance of pine seedlings. Second, I examine the effects of soil transfers on the total and EcM fungal communities associated with soil and roots across the four different disturbances. Given the higher abundance of EcM fungi and the differing composition of EcM fungal communities in soils from late-successional forests, compared with disturbed sites, I predict that soil transfers will induce changes in both soil and root fungal community composition. Thus, an increase in seedling establishment and performance would be coupled with a shift in fungal community composition driven by the volume of soil transferred into disturbed forests should alterations of the EcM fungal community be important to restoration.

3.2 Materials and Methods

3.2.1 Field site selection

Between June and August of 2016, I selected 28 sites in west-central Alberta, Canada, that captured a range of disturbances (Appendix 1.1). Six years prior to conducting the experiment, 14 of these sites were disturbed by either clear-cut logging (n = 4), MPB outbreak (n = 3), wildfire (n = 3), or salvage-logging following MPB outbreak (n = 4). Prior to these disturbances, the sites were mature forests dominated by lodgepole pine with a minimum of 70% stand basal area. Stand characteristics are further described in Rodriguez-Ramos et al. (2021). For each

disturbed site, I selected a corresponding late-successional forest located within 10 km, which had similar stand age, basal area, and forest structure to the pre-disturbance conditions of its pair. Late-successional forests were located at least 100 m away from the edge of the disturbed patch and any roads. In the case of MPB disturbed sites, I established the experimental plots in a central location within the post-outbreak stands, given that disturbance edges were no longer visible.

3.2.2 Experimental design

In 2016, at each of the disturbed sites (30 m × 30 m), I established four 2 m × 2 m plots (with 1 m buffer between plots), where I created seedbeds by exposing the mineral layer and then sowed 250 lodgepole pine seeds into each plot (14,000 total). I obtained the seeds from the Alberta Tree Improvement & Seed Center (Government of Alberta Agriculture and Forestry, Smokey Lake, AB, Canada) and represent the genetic pool of lodgepole pine individuals from Alberta. Seeds were initially surface-sterilized using 5% sodium hypochlorite (bleach) for 15 min and then washed with distilled water. After sowing seeds, plots were covered in plastic screening (1.6 mm mesh size), to prevent predation, and left to stratify and germinate for 12 months. In June of 2017, I collected soil cores (2.5 cm diameter, 25.4 cm depth) in late-successional forests and used the soil as inoculum for plots in the respective disturbed sites. The four plots were randomly assigned to one of four levels of soil transfer: 0, 1, 2, or 4 cores, representing 0, 128.6, 257.3, and 514.6 mL of soil respectively. Small volumes of 150 ml have previously been used to enhance mycorrhizal formation in conifer seedlings (Amaranthus & Perry, 1987). In each plot, I removed the equivalent volume of soil assigned to be transferred at either the center or evenly distributed

within the plot when 2 or 4 soil transfer levels. Then, I filled the space with the soil cores collected in the late-successional forests paired to the individual disturbed site.

After transferring the soil, I sowed 175 additional surface-sterilized lodgepole pine seeds into each plot to ensure seedlings were present in the following year. The mesh was repositioned, and seeds/seedlings were left to germinate and/or grow until the third year of this study (2018), when I measured the proportion of seedling establishment from the 250 seeds added on year one and germination of the 175 seeds added on year two at each plot. In 2018, I then harvested seedlings at random within each plot, together with soil using a shovel and preserving roots as intact as possible. I placed seedlings and soil on dry ice for no longer than 72 h and stored them at 4 °C until processed. Growth of three randomly selected seedlings from each year cohort and plot was measured by the height—root collar to tip—of seedlings, and dry biomass of the shoots. I washed seedling roots free of soil and lyophilized shoots and roots at -50 °C for 72 h in a freeze-drier (Labconco, Kansas City, MO, USA). During harvesting, I also collected two soil cores (1.9 cm diam; 23 cm depth) from each of the plots, put on dry ice until stored at -20 °C, and further lyophilized.

3.2.3 DNA extraction and sequencing

From the lyophilized roots, I selected fine roots from seedlings of each year cohort per plot and cut them into 1.5–2.0 cm long fragments. From a 2.5-cm^2 grid plate, I randomly sampled roots in grid cells until 100 mg of root tissue were obtained. Soil (n = 112) and root subsamples (n = 103) were then separately ground on a TissueLyser II (Qiagen Inc., Mississauga, ON, CAN) and used for DNA extraction.

I isolated total genomic DNA from 250 mg of soil and 10 mg of roots, using E.Z.N.A.® Soil and Plant DNA Kits (Omega Bio-tek, Norcross, GA, USA), respectively, and quantified concentrations in ND-1000 Nanodrop (Thermo Fisher Sci, Waltham, MA, USA). The internal transcribed spacer 1 (ITS1) of the fungal nuclear ribosomal DNA was amplified in a two-step PCR using primers ITS1F – ITS2 containing overhang adapters to allow sequencing using the Illumina Miseq (Illumina Inc., San Diego, CA, USA) platform. I carried out all PCRs and prepared the sequencing library according to Rodriguez-Ramos et al. (2021). The library was sequenced by the Molecular Biology Facility at the University of Alberta with the Illumina MiSeq platform using 2 x 300 bp paired-end reads with v3 chemistry.

3.2.4 Bioinformatic analysis, taxonomic classification, and guild membership

Demultiplexed reads were imported into 'Quantitative insights into microbial ecology 2' (QIIME2) version 2019.7 (Bolyen et al., 2019), for trimming, quality control, and taxonomic classification. I trimmed Illumina adapters together with primer and adapter complements using the 'cutadapt' plugin (Martin, 2001). Trimmed reads were processed with the 'DADA2' pipeline (Callahan et al., 2016) for quality control of reads, to filter chimeras, and resolve amplicon sequence variants (ASVs) of paired-end reads. Specifically, forward sequences were trimmed at 250 bp and reverse sequences at 243 bp to remove low quality (Q score < 30) ends of the reads. Taxonomy was assigned to ASVs using the UNITE dynamic classifier, which considers individual lineages to assign taxonomy on a 97 to 98% threshold (Abarenkov et al., 2010). Based on rarefaction curves, a total of 3,052 and 10,592 sequences were subsampled for further analyses in soil and root samples, respectively. I used the FUNGuild database to assign ecological guilds to ASVs (Nguyen et al., 2016) and only retained taxa assigned to a single guild

and with confidence rankings of ‘probable’ or ‘highly probable’ from the EcM and saprotrophic fungal guilds.

3.2.5 Data analysis

All statistical analyses were conducted in R version 3.5.1 (R Core Team, 2018). Significance was set to $\alpha < 0.05$.

3.2.6 Seedling survival and growth

I assessed seedling responses to soil transfer volumes and disturbance type by quantifying seed germination and seedling establishment, as well as seedling biomass and height. Seedling establishment was calculated as the proportion of live seedlings, from seeds sowed in year one, in each of the plots out of a total of 250. Germination rate of seeds sown in the second year was calculated in the same way as establishment but using 175 as denominator. I tested whether survival and performance metrics differed between soil transfer volume and disturbance type with linear mixed-effects models or generalized linear mixed models with ‘Gamma’ specification in the ‘lmer’ function of the R ‘lmer4’ package (Bates et la., 2015). When normality of residuals was not met, I used non-linear mixed effects models with the ‘lme’ function from the ‘nlme’ package (Pinheiro et al. 2020). For these models, disturbance type and soil transfer volume were used as fixed factors and site as a random effect to account for plot-to-plot variation within sites. I followed significant results with Tukey’s HSD adjustments for multiple comparisons using the ‘glht’ function in the package ‘multcomp’ (Hothorn et al., 2008).

3.2.7 Fungal community composition

For each disturbance type, I calculated the proportion of reads belonging to each guild in soils and associated with pine seedling roots. Additionally, I evaluated differences in fungal community composition between the two sampled habitats—soils and pine roots—based on Bray-Curtis dissimilarities. To test the differences in community composition among disturbances, I used permutational multivariate analysis of variance (perMANOVA), in the 'adonis' function of the 'vegan' package, with 999 permutations on the dissimilarity matrices. For the perMANOVAs, habitat and disturbance type were treated as explanatory variables and site was included as a control variable given than the 'adonis' function does not allow for random effects. However, I considered it appropriate to account for the variation in community composition explained by soil and root samples nested within sites.

When disturbances were treated separately to test for differences in community composition, I used sampled habitat as an explanatory variable with site as a control variable. However, to test differences in the total fungal community and EcM and saprotrophic fungal communities across disturbances, I used soil transfer volume and disturbance type as explanatory variables and site as a control variable in perMANOVAs. The effects of disturbances and soil transfer volume were considered significant only if *post hoc* comparisons also deemed significant results. I performed the pairwise comparisons with Holm correction for multiple testing using the 'pairwise.perm.manova' function of the 'RVAideMemoire' package (Hervé, 2020). However, I am aware of the limitation of including a single factor for pairwise-comparison tests. This caveat hinders the possibility of taking into account the possible effects of site in the community composition. Lastly, indicator species analysis was performed for root EcM fungal communities among disturbances, using the 'multipatt' function in the 'indicspecies' package (De Cáceres & Legendre, 2009).

3.3 Results

3.3.1 Effects of soil transfers and disturbance on seedlings

Soil transfer volume and disturbances did not affect the germination of lodgepole pine seedlings (soil transfer: $F_{1,3} = 0.176$, $P = 0.677$; disturbance: $F_{3,3} = 0.945$, $P = 0.452$) or establishment (soil transfer: $F_{1,3} = 0.048$, $P = 0.828$; disturbance: $F_{3,3} = 0.185$, $P = 0.905$; Appendix 3.1). Though not significantly different, the largest proportion of established seedlings (0.18 ± 0.037, M ± SE) occurred in wildfire, followed by salvage-logging (0.12 ± 0.019), clear-cut logging (0.100 ± 0.015), and MPB outbreak (0.093 ± 0.027). Wildfire sites also had the largest proportion of germinating seedlings (0.38 ± 0.056), followed by clear-cut logging (0.30 ± 0.047), salvage-logging (0.24 ± 0.042), and MPB outbreak (0.20 ± 0.078). In one MPB outbreak site, seed germination and establishment was 0%.

Similar to seedling germination and establishment, soil transfer volume and disturbance type did not affect shoot mass or height of any of the seedling cohorts (Figs. 3.1 & 3.2). Despite the biomass of second year cohorts significantly differing among disturbances, comparisons of medians among disturbances as well as site specific sample distributions within salvage-logging disturbance type suggest that a site effect instead of disturbance type drives the difference (Fig 3.1A); biomass (soil transfer: $F_{1,3} = 0.397$, $P = 0.5287$; disturbance: $F_{3,3} = 22.775$, $P < 0.001$) and height (soil transfer: $F_{1,3} = 3.606$, $P = 0.057$; disturbance: $F_{3,3} = 7.348$, $P = 0.062$). For the first year cohort, I found no effects of disturbance type and soil transfer volume on seedling performance measured as biomass (soil transfer: $F_{1,3} = 0.968$, $P = 0.325$; disturbance: $F_{3,3} = 4.686$, $P = 0.196$) or height (soil transfer: $F_{1,3} = 0.376$, $P = 0.540$; disturbance: $F_{3,3} = 2.711$, $P = 0.438$).

3.3.2 Sequencing output and read proportion

I obtained a total of 12.0 million rDNA reads from the Illumina MiSeq sequencing run, averaging 54,209 sequence reads per sample. After 'DADA2' quality control and filtering, 6.3 million DNA reads remained for downstream analysis, representing 6,249 amplicon sequence variants (ASVs). Based on species rarefaction curves, a total of 3,052 and 10,596 sequences for soils and roots respectively, were randomly subsampled from each sample for analysis. From the remaining sequence reads, I recorded a total of 578 different taxa; 175 saprotrophic fungi, 75 EcM fungi, 89 with dual roles, 106 without guild assignment, 82 with guild assignments of 'possible' confidence, and 51 from guilds that I did not consider for the study, hereafter others.

Across disturbances, the proportion of reads from the different guilds differed between soils and roots (Fig. 3.3). Overall, in soils, EcM fungi and saprotrophic fungi accounted for 10.5% and 13.9% of the sequence reads, respectively. Taxa with multiple roles accounted for 32.9% of the reads, within which 99.1% were saprotrophic fungi, 16.7% EcM fungi, and 16.5% both. Taxa with an unassigned guild represented 29.8% of the sequence reads in soils while those classified to a guild with 'possible' as confidence level accounted for 10.9% of the reads. The remaining sequence reads (2.1%) were classified to other guilds. In roots, the majority of sequence reads belonged to EcM fungi, 39.4%, while saprotrophic fungi accounted for 5.0% of reads. Taxa with dual roles accounted for 25.0% of the reads, within which 97.5% were EcM fungi, 99.7% saprotrophic fungi, and 76.3% both. Unassigned taxa accounted for 2.0% of the sequence reads in roots while those classified to a guild with 'possible' as confidence level 27.0% of reads. The remaining sequence reads (1.6%) were classified to other guilds.

3.3.3 Effects of soil transfers and disturbance on soil and root fungi

Fungal community composition differed between habitats (roots versus soils) and disturbances (Fig. 3.4). For the total fungal community of soils and roots, habitat types ($R^2 = 0.12$, $P < 0.001$), disturbances ($R^2 = 0.05$, $P < 0.001$), and sites ($R^2 = 0.12$, $P < 0.001$) but not soil transfer volume ($R^2 = 0.11$, $P = 0.147$) influenced community composition. Fungal communities differed between soils and roots across all disturbances; wildfire ($R^2 = 0.29$, $P < 0.001$; Fig. 3.4A), MPB outbreak ($R^2 = 0.19$, $P < 0.001$; Fig. 3.4C), clear-cut logging ($R^2 = 0.13$, $P < 0.001$; Fig. 3.4D), and salvage-logging ($R^2 = 0.23$, $P < 0.001$; Fig. 3.4B).

When total fungal communities of soils and roots were considered separately, disturbance type but not soil transfer volume affected fungal composition (Fig 3.5). In soils, while disturbances affected the total community composition ($R^2 = 0.13$, $P < 0.001$; Fig. 3.5A; Appendix 3.3), soil transfers had no effect ($R^2 = 0.02$, $P = 0.983$; Appendix 3.3). Site also significantly influenced the soil fungal community composition ($R^2 = 0.48$, $P < 0.001$; Appendix 3.3). Similarly, disturbance type but not soil transfer volume explained variation in total fungal community composition of pine roots (disturbance: $R^2 = 0.17$, $P < 0.001$; volume: $R^2 = 0.02$, $P = 0.823$; Fig. 2.5B, Appendix 3.3). Site also significantly influenced the composition of fungi associated with roots ($R^2 = 0.39$, $P < 0.001$; Appendix 3.3). Based on pairwise comparisons, total fungal communities differed between all disturbance types.

Differences in composition of EcM fungal communities varied between roots and soils (Fig. 3.6). While differences in soil EcM fungi were mostly influenced by site ($R^2 = 0.34$, $P < 0.001$; Appendix 3.3), disturbance type had a relatively small but significant effect ($R^2 = 0.08$, $P < 0.001$; Appendix 3.3) on composition; only communities in wildfire and MPB outbreak disturbances significantly differed from each other ($P = 0.018$) (Fig. 3.6A). However, compared

to roots, the variation among EcM fungal communities in soils were not as visually evident. In roots, the effect of disturbance type on the EcM fungal community was stronger ($R^2 = 0.16$, $P < 0.001$; Appendix 3.3) with all disturbances but clear-cut and salvage-logging differing from each other. Site also explained some of the compositional variation of root-associated EcM fungal communities ($R^2 = 0.27$, $P < 0.001$; Fig. 3.6B, Appendix 3.3). However, soil transfer volume did not have an effect on the EcM fungal communities of soil or roots (soil: $R^2 = 0.05$, $P = 0.105$; root: $R^2 = 0.03$, $P = 0.217$). The community composition of saprotrophic fungi in soils was explained to some extent by site ($R^2 = 0.23$, $P = 0.003$) (Appendix 3.2); disturbance type ($R^2 = 0.06$, $P = 0.122$) and soil transfer volume ($R^2 = 0.06$, $P = 0.117$) did not significantly influence composition of this fungal guild. In roots, however, both disturbance type ($R^2 = 0.10$, $P < 0.001$) and site ($R^2 = 0.38$, $P < 0.001$), explained differences in saprotrophic fungal composition. Similar to soil saprotrophs, soil transfer volume did not have an effect on the root saprotrophic community composition ($R^2 = 0.02$, $P = 0.226$).

3.3.4 Indicator taxa analysis of disturbances

In total, 20 EcM fungal taxa were indicator species for single disturbance types: five for wildfire, one for clear-cut logging, 14 for MPB outbreak, and none for salvage-logging (Appendix 3.4). Indicators of wildfire included *Tomentella badia* as well as an unidentified species from the same genus. Additionally, *Sphaerosporella brunnea* of the Pyronemataceae family, *Mallocybe* sp., and *Clavulina cinereal* were also found to be wildfire indicator taxa. In clear-cut logging, *Wilcoxina rehmii* was the only indicator species. Ectomycorrhizal fungal indicator taxa in seedling roots of MPB outbreak sites were three species from the genus *Russula* (*caerulean, densiflora*, and an unidentified species), two *Lactarius* (*helvus* and an unidentified species), three *Tylospora*

(*fibrillose* and two unidentified species), two *Hygrophorus* (*hypothejus* and an unidentified species), *Suillus glandulosipes*, *Inocybe leptophylla*, *Cortinarius casimiri*, and *Tricholoma equestre*.

3.4 Discussion

Here, I show that soil transfers, at the volumes tested, do not influence pine seedling establishment and performance. Furthermore, my results demonstrate that roughly seven years after disturbances, *in-situ* fungal communities are resistant to the introduction of fungal propagules from late-successional forests. The community composition of root-colonizing fungi and, to some extent of soil fungi, differed across disturbances but was functionally redundant with respect to pine seedling performance.

3.4.1 Effects of soil transfers and disturbance on seedlings

Contrary to my predictions, soil transfers from late-successional forests to disturbed sites at the volumes tested did not impact germination, establishment, or performance of lodgepole pine seedlings in the field. The absence of an effect is likely due to the resistance of the legacy fungal community to the introduction of fungal propagules from the late-successional forest soils and the relative insensitivity of pine seedlings to variation in fungal community composition. Although my previous work showed that late-successional forests had a higher proportion of EcM fungi compared to disturbed sites, the disturbances did not fully decimate EcM fungi (Rodriguez-Ramos et al., 2021). Therefore, though different in composition across disturbance types, the availability of EcM fungal propagules did not limit or differentially affect seedling

establishment or performance in the disturbed sites. This latter finding suggests that stronger biotic/abiotic environmental gradients would be required to affect seedling outcomes in the field.

Wubs et al. (2016) suggested that soil transfers from late- to early-successional ecosystems can benefit target plant species in restoration given the higher proportion of microbial mutualists in soils from late-stage ecosystems. However, most studies testing this approach, regardless of mycorrhizal group and host species, have been carried out in controlled settings devoid of the abiotic variation present in the field (Sebastiana et al., 2018; Beck et al., 2020), in highly degraded soils that have low mutualist propagule density (Berman & Bledsoe, 1998; White et al., 2008; Onwuchekwa et al., 2014; Maltz & Treseder, 2015; Sýkorová et al., 2016; Wubs et al., 2016; Stefani et al., 2018; Pec et al., 2019; Vahter et al., 2020), or outside of the native range of the focal plant species, where compatible mycorrhizal fungi might not be present (Nuñez et al., 2009; Dickie et al., 2010). My study contrasts those above in that I used soil transfers from late-successional forests to restore native species *in situ*, following disturbances not resulting in novel ecosystems. In line with Grove et al. (2019), my study does not support the use of soil transfers from late- to early successional forests as method to steer the composition of EcM fungi and enhance seedling performance.

Greenhouse inoculation studies using soils from geographically similar locations produced different results from what I observed. Karst et al. (2015) and Beck et al. (2020) reported that although pine seedling biomass increased when using soil transfer with active fungal propagules, seedling performance was lower when inoculated with soils from logging disturbances and MPB-outbreak origin, respectively, compared to that in sterilized soils. The lack of pine seedling response to soil transfers *in*-situ suggests that abiotic factors associated with particular disturbances and individual sites play a crucial role in the assembly of EcM fungal communities.

Furthermore, the different outcomes between controlled vs. field studies demonstrate that, although soil transfer affects seedling performance in control studies, these effects can disappear when tested in the field. The efficacy of using inoculated seedlings in forest restoration should thus be demonstrated before committing to this technique.

3.4.2 Effects of soil transfers and disturbances on soil and root-associated fungi

Disturbance type and site explained differences in EcM fungal community composition associated with pine seedling roots. Though soils from late-successional forests harboured a different EcM fungal community than that of all abiotic disturbances (Rodriguez-Ramos et al., 2020), fungal communities of recipient sites were resistant to soil transfers in the current study. My results indicate that the composition of EcM fungi associated with roots of pine seedlings is mostly determined by disturbance type. Competitive interactions between EcM fungi adapted to disturbance and those from late-successional forests, together with disturbance-specific abiotic changes in soil environments, may hamper the establishment of fungi from soil transfers (Ortega et al., 2004). Alternatively, it is also possible that time between the disturbance events and introduction of soil transfers lessened the effects of the inoculum, and thus intervention immediately after the disturbances could steer the EcM fungal composition in soils and pine roots.

The effect of disturbances on root-associated EcM fungal communities contrasted that of soil, as disturbances explained only a minimal portion of community beta-diversity in soil fungi. Specifically, composition differed between EcM fungal communities in soils of wildfire and MPB outbreak disturbances. Nonetheless, it is possible that the soil transfer volume was not enough to generate changes in the fungal communities of disturbed sites despite the presence of

live propagules in soil transfers (Beck et al., 2020). When higher soil transfer volumes in the field have been used, the soil microbial community composition resembles that of donor sites (Wubs et al., 2016; Pec et al., 2019). For example, soil transfers of 2.51 L m^{-2} were necessary to observe a shift in fungal community composition in grassland restoration (Wubs et al., 2016). This rate would require adding 40.16 L of soil to the four plots and 2,259 L in total to individual sites; this amount would be impractical and logistically unfeasible at a stand level restoration project.

That EcM fungal communities of roots diverge across disturbances with no effect on seedlings, suggests that species in the different communities have overlapping niches and carry out similar ecological functions in supporting pine performance (Dahlberg, 2001; Jones et al., 2010). Similar performance outcomes have been observed in greenhouse experiments where mock EcM fungal communities did not affect pine seedling performance, even in the presence of competitive interactions between EcM fungal taxa (Kennedy et al., 2007). Furthermore, performance effects depend on the complexity of the associated fungal community, as seedling survival and growth in greenhouse inoculation experiments is positively correlated with the complexity of root symbiotic community (Menkis et al., 2007). However, once seedlings are transplanted into soils with existing EcM fungal propagules, natural re-colonization can govern the community assembly, sometimes outweighing greenhouse inoculation effects (Teste et al., 2004; Menkis et al., 2007; Rincón et al., 2007; Sýkorová et al., 2016). It has been suggested that despite individual fungal species influencing functional traits such as enzyme secretion (Jones et al., 2012; Kipfer et al., 2012), EcM fungal communities can become functionally redundant with increasing taxonomic diversity (Rineau & Courty, 2011; Talbot et al., 2014). In this study, the high diversity of EcM fungi in boreal forests may ensure that, despite disturbances inducing

changes in environmental conditions that filter fungal taxa, traits regarding seedlings establishment and performance during regeneration are maintained. Overall, my study suggests that upon germination of lodgepole pine seed in forest soils, the subsequent seedlings are resilient to current disturbance regimes in the first few years of their life.

3.4.3 Indicator taxa analysis of disturbances

Indicator fungal taxa differed among the disturbance types, results likely related to how each disturbance affects aboveground forest structure. Biotic and abiotic disturbances result in different degrees of tree mortality and disruptions to the soil organic layer, properties known to influence EcM fungal communities (Pec et al., 2017; Rodriguez-Ramos et al., 2020). In the current study, indicator taxa for the different disturbance types differed in their ecological successional traits. While logging and wildfires rapidly remove or combust the forest overstory, biotic disturbances such as MPB outbreak, are characterized by a long and gradual decomposition of standing dead trees, an intact understory, and a few live residual trees (Erbilgin et al., 2017; Six et al., 2018; Zhao & Erbilgin, 2019). Correspondingly, many of the indicator taxa from wildfire and clear-cut logging disturbances were typical 'early-stage' fungi such as *Wilcoxina* spp., *Tomentella* spp., and *Sphaerosporella* spp. These particular genera have been previously reported as 'early-stage' colonizers and a primary inoculum source for seedlings following wildfires and other disturbances (Egger & Paden, 1986; Egger et al., 1991; Baar, et al., 1999; De Román & De Miguel, 2005; Buscardo et al., 2010; Barker et al., 2013). These results fit well with the current understanding of EcM fungal succession in natural areas following disturbances where 'early-stage' fungi colonization via spores dominates over mycelial strands (Fleming, 1983, 1984; Peay et al., 2011).

In contrast to abiotic disturbances, many of the indicator taxa for MPB outbreak disturbance belonged to genera commonly associated with late-successional forests. This included fungi from the genera *Cortinarius, Russula, Suillus, Hygrophorus, Tricholoma* (Visser, 1995; Peay et al., 2007; Liao et al., 2016) and suggests that residual, mature conspecific pines may be source of fungal colonization to seedling roots (Haskins & Gehring, 2005). As demand for plant-derived carbon tends to be lower for early-stage than for late-stage EcM fungi (Dighton & Mason, 1985; Newton, 1992), it is likely that seedlings regenerating in sites affected with the MPB outbreak become highly colonized via mycelial strands already connected to roots of live residual pine trees capable of sustaining sufficient belowground C transfer to maintain mycelial systems of late-stage EcM fungi (Fleming, 1983, 1984; Kranabetter & Friesen, 2002; Karst et al., 2014). In this case, root-associated EcM fungal community composition of regenerating seedlings would resemble that of seedlings growing in late-successional forests. However, for regeneration following abiotic disturbances, where little or no live residual pine trees are accessible, seedlings might be more dependent on 'early-stage' fungi with lower photosynthate demand. Similarly, Kranabetter & Friesen, (2002) reported that roots of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) failed to maintain colonization by 'late-stage' EcM fungi after seedlings were transplanted from late-successional forests into harvested forests.

3.4.4 Management implications

With a six-fold increase in the probability of all Canadian timber volume being impacted by natural and anthropogenic disturbances within this century (Boucher et al., 2018), reforestation strategies that increase forest resilience should be considered (Policelli et al., 2020). This study sheds light on how to triage restoration and reforestation efforts from a belowground perspective.

As one of the first field studies aiming to restore fungal communities following disturbances, my results suggest that EcM fungal inoculum for regenerating pines should be assessed prior to planting and attempting to restore microbial communities. Although mycorrhizal fungal inoculum in the form of soil transfers or spore may be justified when initial EcM fungal propagules are absent, as in the case of novel ecosystems or species introductions outside of their range (Dickie et al., 2010), legacy fungal communities resulting from current disturbance regimes might be sufficient to ensure pine establishment. Therefore, germination and establishment of lodgepole pine in this particular system are not constrained by a lack of EcM fungi or by the different legacy communities. Furthermore, if an objective is to induce EcM symbiosis between seedlings and fungal taxa typical of late-successional forests, we should prepare the sites in a way that emulates a biotic disturbance, as in the case of retention harvest (Jones et al., 2017). Monitoring ecosystem resilience in the onset of short interval successive disturbances should be considered to ensure that the critical ecological functions of EcM fungi endure with forest regeneration.

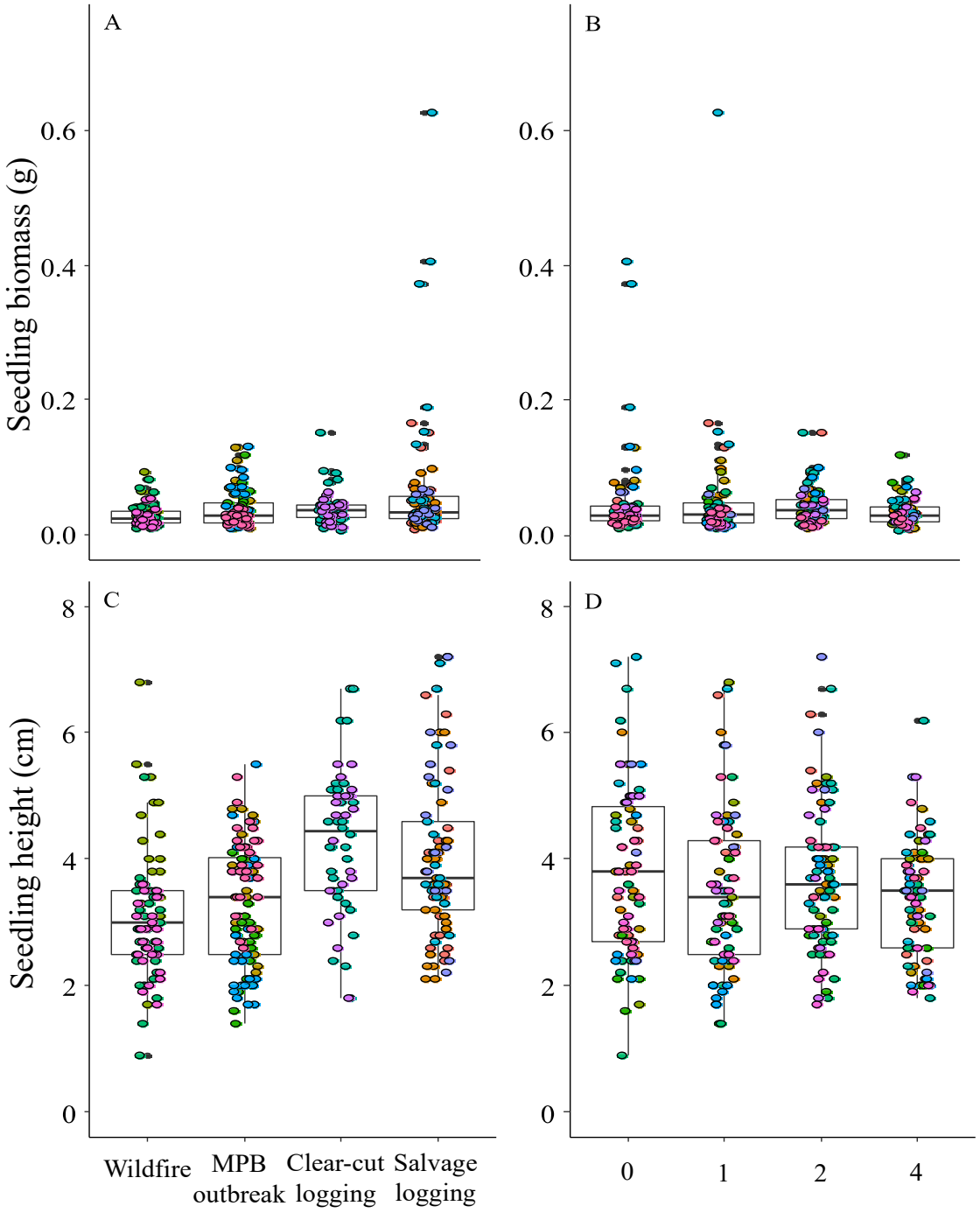


Figure 3.1. Biomass (A, B) and height (C, D) of pine (*Pinus contorta* var. *latifolia*) germinants (second-year cohort) across disturbance types (A, C) and soil transfer volumes (B, D). Points represent individual seedlings and colors represent sites within the disturbance or transfer volume levels as 0, 1, 2, or 4 soil cores. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

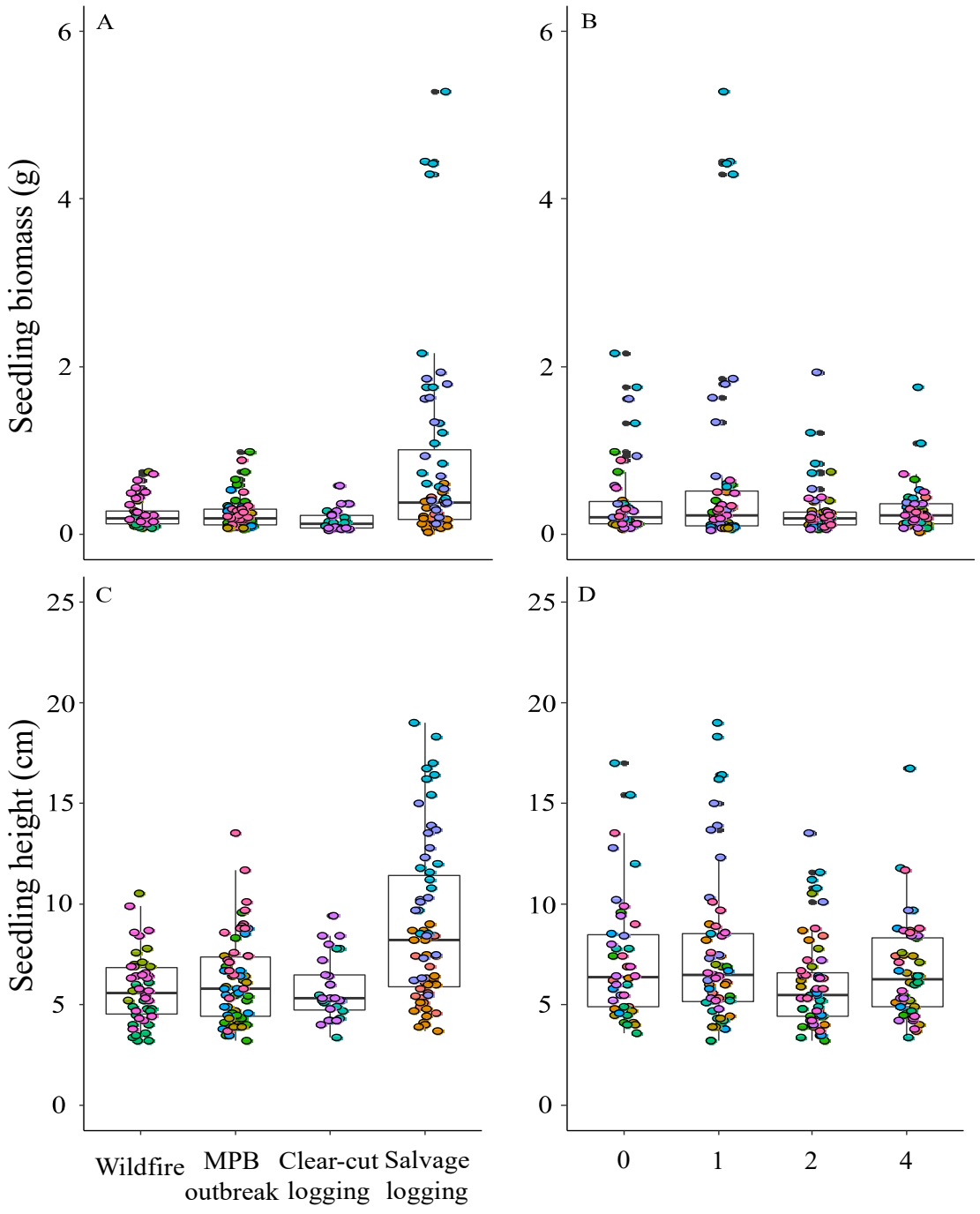


Figure 3.2. Biomass (A, B) and height (C, D) of pine (*Pinus contorta* var. *latifolia*) established seedlings (first-year cohort) across disturbance types (A, C) and soil transfer volumes (B, D). Points represent individual seedlings and colors represent sites within the disturbance or transfer volume levels as 0, 1, 2, or 4 soil cores. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

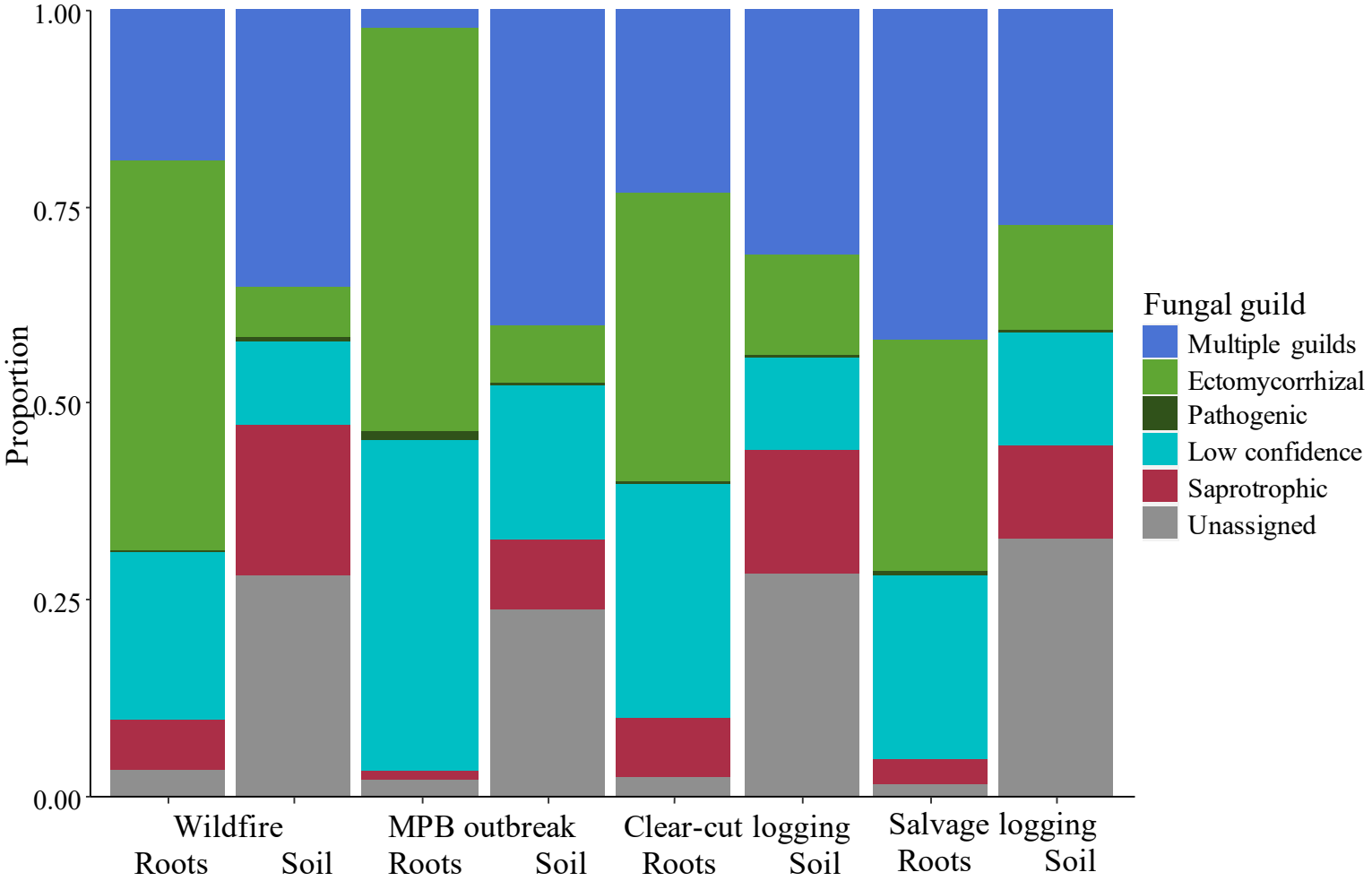


Figure 3.3. Read proportion of fungal guilds identified in sampled pine (*Pinus contorta* var. *latifolia*) seedling roots and surrounding soils from the different disturbances. 'Possible and others' represent fungal taxa with guild membership classified with low confidence based on FUNGuild database (Nguyen et al., 2016), and guilds not considered for this study. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

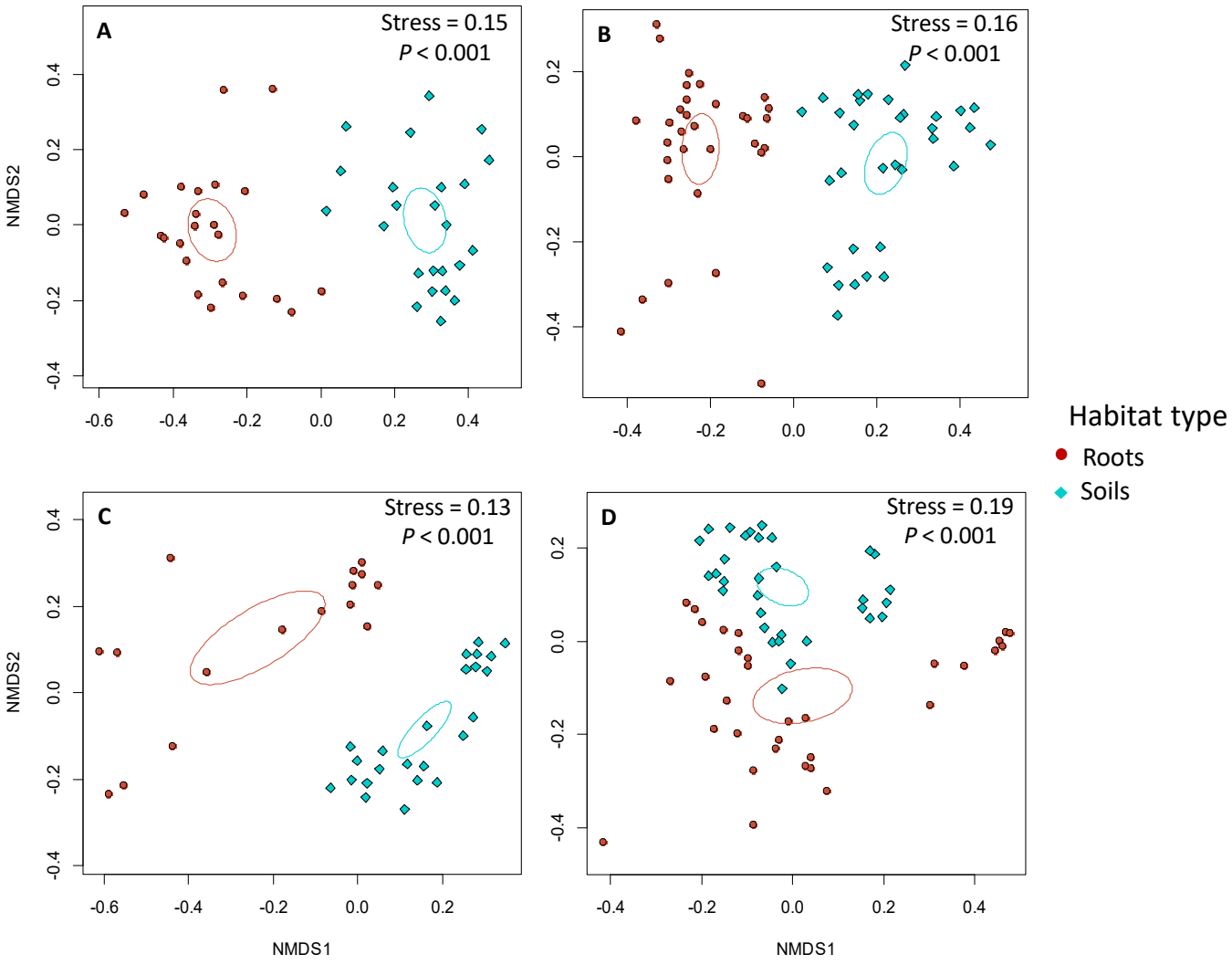


Figure 3.4. Non-metric multidimensional scaling plot using Bray-Curtis distance of total fungal communities in soil and root samples across forest disturbances; wildfire (A), salvage-logging (B), mountain pine beetle (*Dendroctonus ponderosae*) outbreak (C), and clear-cut logging (D). Points represent individual samples and ellipses standard error. Significant differences between habitats at $\alpha = 0.05$ was tested using perMANOVA.

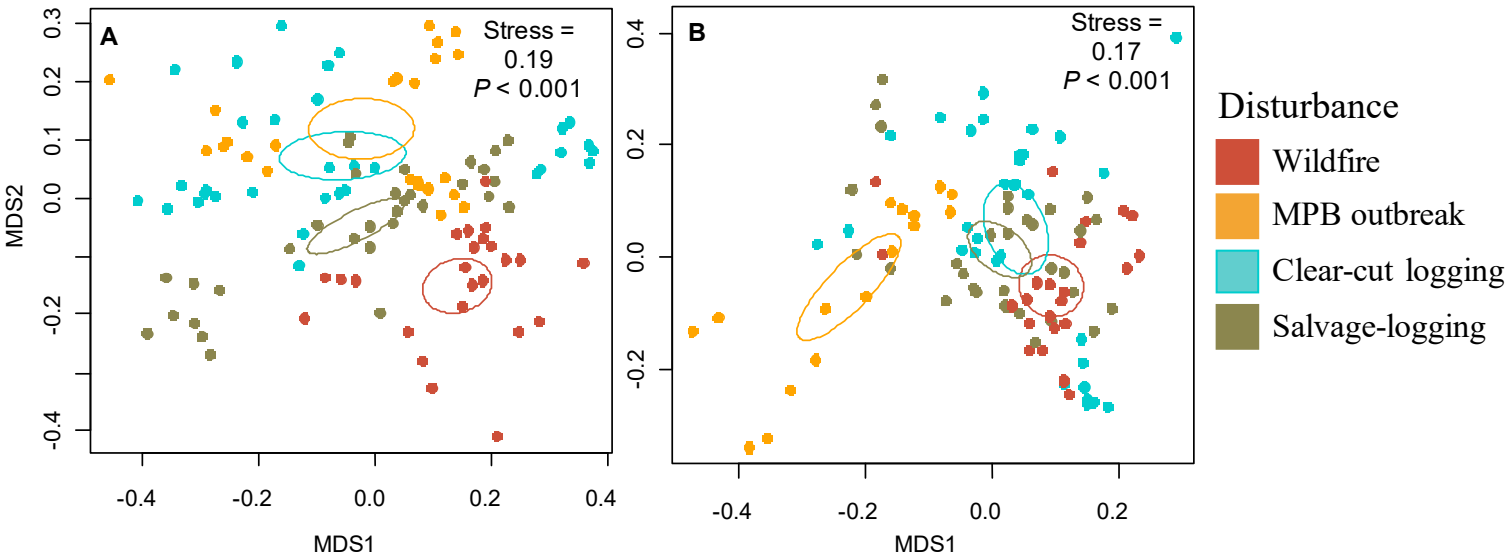


Figure 3.5. Non-metric multidimensional scaling plot using Bray-Curtis distance of soil (A), and root (B) total fungal communities. Points represent individual samples colored by disturbance type, and ellipses standard errors. Significant differences in communities at $\alpha = 0.05$ was tested using perMANOVA. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

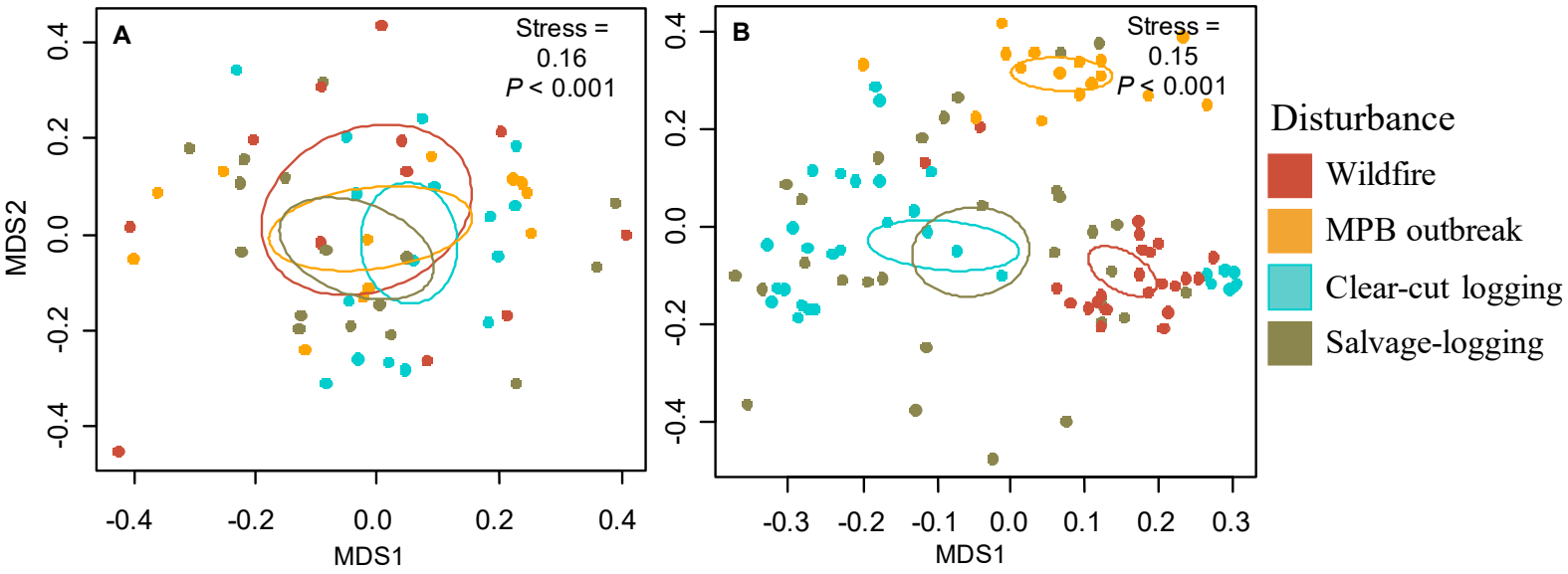


Figure 3.6. Non-metric multidimensional scaling plot using Bray-Curtis distance on ectomycorrhizal fungal communities in soil (A) and roots (B). Points represent individual samples colored by disturbance type, and ellipses standard errors. Significant differences in communities at $\alpha = 0.05$ was tested using perMANOVA. MPB: mountain pine beetle (*Dendroctonus ponderosae*

Chapter 4

Relative roles of neutral and deterministic processes in the fine-scale assembly of root-associated ectomycorrhizal fungi across disturbances

4.1 Introduction

Plant roots continuously interact with soil microbes, including fungi (Mercado-Blanco et al., 2018). A ubiquitous interaction between plant roots and fungi is the mycorrhizal symbiosis, where fungi colonize roots and provide plants with nutrients and water in exchange for C fixed through photosynthesis (Smith & Read, 2008). For many ectomycorrhizal (EcM) tree species, this type of symbiosis is an obligate mutualism, as they rely on the fungi for their establishment and growth (Smith & Read, 2008; Horton & van der Heijden, 2018). It is well established that EcM fungi exhibit biogeographical patterns at global and regional spatial scales (> 1 km), where environmental gradients in soil pH and host presence and identity can determine the diversity and composition of EcM fungi (Talbot et al., 2014; Tedersoo et al., 2014; Van Der Linde et al., 2018; Wang et al., 2020). However, the processes underlying EcM fungal community assembly at finer spatial scales (i.e., within 1 m), such as colonization of plant roots by fungi in the surrounding soil, remain unknown, despite the increasing interest by scientists and land managers to manipulate this soil microbial group to enhance plant performance (Field et al., 2020).

At fine spatial scales, two contrasting but not mutually exclusive processes may underlie the community assembly of EcM fungi: determinism and neutrality (Bell, 2001; HilleRisLambers, Adler, Harpole, Levine, & Mayfield, 2012; Hubbell, 2001; Wennekes,

Rosindell, & Etienne, 2012). If determinism underlies the assembly of root-associated EcM fungal community, the environment filters the taxonomic composition of species colonizing roots. Filters may include host-specificity and soil edaphic factors (i.e., pH, nutrients) acting on EcM fungal species traits (i.e., growth rates, enzymatic activity), and interspecific interactions (Kennedy & Bruns, 2005; Genney et al., 2006; Pickles et al., 2012; Glassman et al., 2017; Wang et al., 2020). In this case, each EcM fungal species occupies and maintains a niche that is determined by its competitive advantage in soil and/or roots (Peay et al., 2008). In contrast, neutrality deems all species to be ecologically equivalent, and thus all EcM fungal species in the surrounding soil are equally fit colonizers of roots. In other words, a species abundance in the soil metacommunity solely determines its likelihood to colonize plant roots (Chave, 2004; Zhou & Ning, 2017). If neutrality underlies the assembly of EcM fungi, differences in community composition are explained by processes such as dispersal and ecological drift (Hubbell, 2001; Vellend, 2010).

While most studies focus on EcM fungal assembly processes at global and regional scales, the few studies at local scales have been limited to tree islands and have not incorporated how disturbances impact assembly processes (Peay et al., 2007; Peay et al., 2010; Glassman et al., 2017). Disturbances can lead to major shifts in the environment that can impact the relative abundance, diversity, and interactions of soil microbes, and promote or restrain particular assembly processes (Chase, 2007), which in turn affect the relationship between biodiversity and ecosystem function at local scales (Knelman & Nemergut, 2014). For example, Ferrenberg et al. (2013) found that forest wildfires can promote neutral assembly processes of soil bacteria shortly after the disturbance, while deterministic processes became more important upon recovery. Furthermore, Zhang et al. (2016) showed that different anthropogenic disturbances can promote

or restrict the relative importance of neutral processes on the assembly of soil bacteria. In boreal forests, disturbances such as wildfires, insect outbreaks, and tree harvesting are frequent events that alter biodiversity at both regional and local scales by modifying the environment and acting as ecological filters of community structure and composition (Sun et al., 2015; Day et al., 2019; Rodriguez-Ramos et al., 2021). However, it is unknown how these disturbances modulate the relative contribution of neutral and deterministic processes on the assembly of root-associated EcM fungi.

Neutral community models (NCMs) use random sampling from a known community to simulate communities expected in the absence of particular ecological mechanisms (Gotelli & Graves, 1996). Previous NCMs included Bell (2001) and Hubbell (2001), but these early models fall short for use with high-throughput amplicon sequencing of microbial communities mainly because they are not capable of dealing with the very large populations that characterize microbial communities (Sloan et al., 2006). The recent development of NCMs adapted for microbial communities allows us to quantify the contribution of neutral and deterministic processes in their assembly (Sloan et al., 2006). Specifically, the Sloan NCM uses a source community found in the immediate environment to determine whether neutral processes alone are sufficient to explain the local, host-associated community. To date, this model has been successfully applied to understand assembly mechanisms of microbial communities in diverse studies, including human lungs and skin (Venkataraman et al., 2015; Tong et al., 2019), fish guts (Burns et al., 2016; Heys et al., 2020), subtropical rivers (Chen et al., 2019), and arbuscular mycorrhizal plants (Verbruggen *et al.*, 2012) and have elucidated the relative roles of neutral and deterministic assembly in microbe-host interactions.

In this study, I use the Sloan NCM in combination with high throughput sequencing of the fungal ITS1 rDNA to examine the community assembly of EcM fungi in roots of lodgepole pine (*Pinus contorta* var. *latifolia*) seedlings planted in forests recently disturbed by wildfire, mountain pine beetle (MPB, *Dendroctonus ponderosae*) outbreak, clear-cut logging, or salvage-logging (i.e., tree harvesting following a MPB outbreak). Considering soils surrounding pine seedling roots (< 1 m) as the primary source, I test whether neutral processes predict the root-associated EcM fungal community composition found in seedlings across the four disturbance types. In fungal communities assembled by neutral processes, abundant species in the surrounding soil would have a high probability of dispersal to roots whereas the opposite would be expected for species with low abundance in the source community. I predict that if different EcM fungal taxa are not ecologically different, as assumed by NCMs, neutral processes rather than deterministic would be more important in the fine-scale assembly of EcM fungi. Moreover, since Chapter 3 showed that the community composition of root-associated fungi differed among disturbances, I predict that communities of taxa assigned as 'neutral' and 'deterministic' by the NCM would not be constant across the disturbance types.

4.2 Materials and Methods

The experimental design of this study is reported in Chapter 3. Briefly, in 2016, I selected 14 sites in west-central Alberta, capturing a range of disturbances: clear-cut logging (n = 4), MPB outbreak (n = 3), wildfire (n = 3), or salvage-logging following MPB outbreak (n = 4). At each site, in an area of 6 m^2, I established four 2 m × 2 m plots (with 1 m buffers among plots), where I exposed the mineral layer and then sowed 250 lodgepole pine seeds in June to July of that same year, and another 175 seeds into each plot the following year. In 2017, I transferred to the plots 0, 1, 2, or 4

soil cores collected from paired mature conspecific sites located within 10 km of the disturbed site. In 2018, I then harvested multiple seedlings from each cohort together with two soil cores (25 cm depth) at each of the plots. Seedling roots were washed of soil while soil samples were subsampled vertically to constitute a single sample; both roots and soils were freeze-dried. Once dry, I selected fine roots from seedlings from each of the two cohorts per plot and cut them into 1.5–2.0 cm long fragments. From a 2.5-cm^2 grid plate, I randomly sampled roots in grid cells until 100 mg of root tissue were obtained. Soil (n = 112; 14 sites × 4 plots × 2 samples) and root samples (n = 103; 14 sites × 4 plots × 2 cohorts; germination in one MPB outbreak site and a single plot in other site equaled 0) were then separately ground and genomic DNA of soil and roots were extracted and fungal rDNA sequenced. Methods of extraction, amplification and sequencing are described in Chapter 3. For each root sample, I considered EcM fungal taxa in the surrounding sampled soil to be the 'source community', and those present on roots of the harvested seedlings to be the 'local-pool'. Samples belonging to different cohorts and soil transfer volumes were kept separate; however, cohort and soil transfer volume were not used as explanatory variables in the current study as they did not affect the fungal community composition (see p.71 Results, Chapter 3).

Fungal taxonomy was assigned to amplicon sequence variants (ASVs) using the UNITE dynamic classifier (Abarenkov et al., 2010). In contrast to Chapter 3, I did not rarefy sequence counts for soil and root samples since a step of the NCM analysis includes normalization of the community. I used FUNGuild (Nguyen et al., 2016) to assign ecological guilds to ASVs and only retained taxa assigned to the EcM fungal guild with confidence rankings of 'probable' or 'highly probable,' regardless of whether they were assigned to a single or multiple guilds. I obtained a total of 12.0 million rDNA reads from the Illumina MiSeq sequencing run, averaging 54,209 sequence reads per sample. After 'DADA2' quality control and filtering, 6.3 million DNA reads

remained for downstream analysis, representing 6,249 amplicon sequence variants (ASVs). From these ASVs, I recorded a total of 638 EcM fungal taxa occurring in soils and roots; 478 in soils, 333 in roots, and 173 in both. Soils and plant tissues can differ in extraction efficacy and/or sequencing depth (Glassman et al., 2015); however, alpha diversity species accumulation curves showed a majority of the ASVs detected in both roots and soils (Appendix 4.1) indicating that sequencing depth recovered the majority of extractable ASVs.

4.2.1 Neutral model of community assembly

To examine the relative contribution of neutral and non-neutral assembly processes in the fine-scale assembly of root-associated EcM fungi, I assessed the fit of the Sloan NCM (Sloan et al., 2006) to the structure of EcM fungal communities for each disturbance type, separately. Custom scripts of the model were implemented in R version 3.5.1 (R Core Team, 2018). The Sloan NMC has been adapted from Hubbell (2001) to allow for large population sizes typical of microbial communities by transforming the model into a continuous form (Sloan *et al*., 2006). In the case of my study, this model predicts a neutral distribution of the fungal community based on the observed abundance of EcM fungal taxa in the source community. It further creates a relationship between the frequency of individual fungal ASVs in roots, based on their relative abundance in the source community, allowing the identification of EcM fungal ASVs that fit within the neutral assembly prediction as well as those that deviate. The frequency of root-associated EcM fungal ASVs was calculated as the number of root samples from a particular disturbance in which an individual ASV was detected in the community, divided by the total amount of root samples of the same disturbance. The relative abundance of individual ASVs in the soil community was calculated as the number of sequences for the particular ASV divided by

the total number of sequences in the soil community of a particular disturbance. Based on the unified neutral theory of biodiversity (Hubbell, 2001), highly abundant taxa occurring in the source community will lead to an increased likelihood of dispersal and random sampling by seedling roots. On the contrary, taxa with low abundance in the soil EcM fungal community are less likely to occur in the root-associated community.

As adapted from Burns et al. (2016), the Sloan NCM was fitted to the observed frequency of ASVs (proportion of roots in which the ASV was found) and the mean relative abundance of the ASVs across soils of each disturbance type, using the estimated migration rate (m) that describes the probability that a random loss of an individual in the root community will be replaced by dispersal from another individual from the soil community. The distribution of ASVs for each disturbance type was fitted using nonlinear least-squares in the 'minpack.lm' package (Elzhov et al., 2013) with a binomial proportion 95% confidence intervals around the model predictions calculated with the 'HMisc' package (Harrell Jr., 2006). I further examined the goodness of fit of the neutral community models on the data using the coefficient of determination, R^2, with $R^2 \geq 0.5$ considered a good fit of the model.

The Sloan NCM constructs a local community by random sampling from a source community, therefore, identifying taxa with frequencies predicted by neutral assembly processes and those that deviate from the model presumably due to deterministic assembly processes. To test whether ASVs assigned as neutral or deterministic comprising communities differed across disturbances, I compared their composition using a multivariate statistical analysis. For this analysis, the community composition of root samples was pooled by disturbance type and rarefied to 447,948 reads to enable comparisons across disturbances. Once rarefied, the ASVs of each disturbance were grouped into three separate communities based on their fit in the model:

taxa fitting above (i.e., overrepresented in the local community), below (i.e., underrepresented in the local community), or within the 95% confidence intervals of the neutral distribution model (neutrally assembled). Therefore, each of the three groups of each disturbance represented a different community for the subsequent analysis. However, due to the lack of model fit for the MPB outbreak disturbance (see Results; Figure 4.1), only a total of nine communities were used for the following test (three community types (overrepresented, underrepresented and neutrally assembled) × three disturbances). To test for differences in community composition among neutrally and deterministically assembled communities, I used permutational multivariate analysis of variance (perMANOVA), in the 'adonis' function of the 'vegan' package, with 999 permutations on the dissimilarity matrices based on Bray-Curtis distances (Oksanen et al., 2012). Non-metric multidimensional scaling was used to visualize the differences among neutrally and deterministically assembled communities

4.3 Results

Across disturbances, the Sloan NCM predicted the frequency of most ASVs on roots by the mean relative abundance of ASVs in soil indicating the importance of neutral processes in shaping EcM fungal communities found on seedlings (Fig. 4.1).The fit of the NCM (R^2) varied among disturbances with the EcM fungal community from MPB outbreak disturbance being a poor fit (R^2=0.19) and below the threshold of $R^2 \geq 0.50$ (Fig. 4.1A-D). The poor fit was likely due to the lower sample size for roots (n=16 from only two sites) obtained from this disturbance type, as suggested by the overall decline and lack of model fit across the rest of the disturbances, except for wildfire, when using the same sample size as MPB outbreak (wildfire, $R^2 = 0.53$; clear-cut logging, $R^2 = -0.45$; salvage-logging, $R^2 = 0.20$). Despite clear-cut logging disturbance

having a R^2 slightly lower than the threshold, I considered it a good fit since the root-associated EcM fungal community of this disturbance and salvage-logging do not differ in composition (Chapter 3, Fig. 3.6), and the neutrally and deterministically assembled taxa of these two disturbances showed a large overlap (Table 4.1).

The frequency at which most fungal ASVs occurred on roots across disturbances was largely predicted by their abundance in the soil community, and therefore assigned 'neutral'. A large portion (77%) of EcM fungal ASVs were predicted by the neutral model for MPB outbreak, 64% in salvage-logging, 60% in clear-cut logging, and 58% in wildfire. The percentage of taxa whose frequencies were not predicted by the neutral model, and therefore assembled through deterministic processes, represent those ASVs whose abundances were over- or underrepresented in roots compared to soils. The ASVs that did not fit the neutral model for each disturbance type are shown in Table 4.1. Furthermore, across disturbances, communities differed and clustered based on whether and how they deviated from the model—above, below, within the neutral prediction—(perMANOVA $F_{2,6} = 2.11$, $R^2 = 0.41$, $P = 0.037$; Fig. 4.2). Therefore, the taxa making up the communities diverging or fitting the neutral prediction remain relatively similar across disturbances.

4.4 Discussion

This study examined the ecological processes driving the assembly of EcM fungal communities on roots of pine seedlings growing in disturbed forests. Specifically, I examined the relative contribution of neutral and deterministic processes in shaping the community structure of EcM fungi at fine spatial scales. The NCM predicted the abundances of a large percentage of root-associated EcM fungal taxa based on their relative abundances in the soil community, suggesting

that most of the ASVs found in soils appear to be probabilistically dispersed to roots, while a smaller but sizeable portion is assembled through deterministic processes influencing their colonization rates. Furthermore, similar local root-associated EcM fungal communities were assigned as deterministic and neutral, despite the differences seen in communities at the regional spatial scale (Fig. 3.6, Chapter 3).

The abundance of most EcM fungal taxa on roots was predicted by their abundance in the source soil community, suggesting that neutral processes, notably dispersal, dominate the community assembly. Studies have previously pointed at the importance of neutral processes in the assembly of root-associated EcM fungi after exhaustively measuring edaphic factors which often explained only a limited amount of the variation in community composition (Peay et al., 2010; Gao et al., 2015). In the current study, probabilistic dispersal rather than ecological drift at a fine spatial scale seems to be the main neutral process operating on the assembly of the root-associated EcM fungal community, as indicated by the strong correlation between abundance in soils and roots for most taxa. Similar patterns of neutral assembly in fungi colonizing roots of arbuscular mycorrhizal plants have been observed across land management types (Verbruggen et al., 2012). Specifically, the abundance of individual arbuscular mycorrhizal fungal taxa in roots across 40 sites were predicted by that of soils regardless of whether fields were managed organically or conventionally (Verbruggen et al., 2012). In EcM fungal dominated systems, studies analyzing their composition in tree islands have suggested that ecological drift might be an important assembly process in communities that experience dispersal limitation (Peay et al., 2010). In the current study, however, ecological drift is unlikely to have occurred as root-associated EcM fungal communities were similar among geographically distant plots (c. 125.3 km) sharing a history of disturbance (Fig. 3.6, Chapter 3). This result is in contrast to what would

be expected among communities experiencing ecological drift; a high compositional turnover and stochastic fluctuations in species abundances resulting from isolation from a metacommunity (Vellend, 2016).

My results show that deterministic processes also notably shape the local assembly of root-associated EcM fungi. Previous fine-scale studies comparing the EcM fungal community colonizing root tips to that of extramatrical mycelium in soil found that the frequency of detecting individual species differed based on whether sampling occurred in soils or root habitats (Koide et al., 2005; Genney et al., 2006) mainly due to priority effects resulting from interspecific competition (Pickles et al., 2012). Within a same soil profile and root system, different co-occurring EcM fungal species can have different enzymatic activities for nutrient uptake (Buée et al., 2007, Jones et al., 2010), indicating that trait differentiation between taxa can impact their growth and root colonization success (Kennedy & Bruns, 2005). In this study, those EcM fungal ASVs positioned above or below the neutral prediction suggest that the over- and underrepresented root-associated EcM fungal taxa have different traits that increase or decrease their competitive abilities to colonize roots. An unexpected but interesting result was the similarity in the taxa making up these over- and underrepresented communities across disturbances, suggesting that similar deterministic processes act across different disturbances. For example, it is possible that across disturbances, EcM fungal host-specificity filters taxa (Goldmann et al., 2016) or that particular species traits from these communities determine their colonization rates and relative abundance in seedling roots (Glassman et al., 2015; Kennedy & Bruns, 2005).

Taxa occurring less frequently than expected by their abundance in the source community may be potential pathogens selected against by the host (Burns et al. 2016). In the current study,

taxa from the family Helotiaceae, such as *Acephala applanata* and *Meliniomyces* spp. that were classified by FUNGuild as EcM fungi, were consistently underrepresented in roots, compared to their relative abundance in soils across all disturbances. Tedersoo *et al.* (2009) indicated that many EcM-associated ascomycete fungi (i.e., Helotiaceae family) represent root endophytes and rhizoplane colonists, with some having kinship to plant and fungal pathogens and parasites. Therefore, despite their relatively high abundance and rapid mycelial growth in soils that would lead to high root colonization rates under neutral processes, it is possible that pine seedlings disfavor this taxonomic group. Other taxa found to be underrepresented in roots and across disturbances were ASVs classified in the Thelephoraceae family; however, more taxonomic resolution is required to resolve the traits influencing the mechanisms underlying their low abundance in roots relative to soils.

Some of the overrepresented taxa likely indicate host preference or specificity for particular EcM fungal groups. These overrepresented taxa comprised genera often found to colonize conifer seedlings at remarkably high abundances regardless of their level of fungal specificity towards conifer hosts. For example, *Suillus, Rhizopogon, Piloderma, Lactarius, Russula,* and *Amphinema* spp. have previously been found to colonize lodgepole pine seedlings in regenerating stands and mature trees of Alberta (Bradbury, 1998; Bradbury et al., 1998; Douglas et al., 2005; Berch et al., 2006). Furthermore, generalists such as *Cenococcum geophilum* and *Wilcoxina* spp. commonly dominate seedling root systems following tree harvesting disturbance (Dahlberg & Stenström, 1991; Jones et al., 2003).

Together with host-preference and specificity, species reproductive traits can also influence their competitive advantage for root colonization. A majority of the observed overrepresented EcM fungal taxa in roots have been previously described as dominant species in

the soil spore bank, a trait that could increase their competitiveness. For example, Glassman et al. (2015) studied resistant spore banks across North American pine forests and found that species from the genera *Wilcoxina, Cenococcum, Rhizopogon, Piloderma* were among those dominating soil spore banks and colonizing pine roots. Furthermore, Policelli et al. (2019) suggested that the large production of resistant spores by suilloid fungi promote rapid colonization of pine seedlings. While many *Suillus* species produce spores in epigeous structures, most of the other overrepresented taxa sporulate within the soil. For example, *Wilcoxina* spp, *Cenococcum geophilum* sporulate within the soil, *Rhizopogon* spp. produce hypogeous fruiting structures, and *Amphinema* spp. and *Piloderma* spp. from resupinate crusts in soils (Glassman et al., 2015; Policelli et al., 2019). Based on the outcome of the current study and others (Baar et al., 1999; Bruns et al., 2009), I suggest that the physiological capacity to produce high density of resistant spores by taxa overrepresented on seedling roots can act as the primary inoculum for seedlings. This trait is possibly most important for regeneration of seedlings following stand replacing disturbances such as wildfires, clear-cut logging, and salvage-logging, where colonization mediated by resistant spore banks in soils or dispersed by wind might dominate following the disruption of EcM networks form overstory tree mortality and disruption of the soil organic layer (Fleming, 1984).

The dominant role of neutral processes on the fine-scale assembly of root EcM fungi indicates that these communities could be influenced by ecological dynamics occurring at larger spatial scales (Shi et al., 2018). While at a global spatial scale, richness of EcM fungi follows a unimodal distribution that peaks at temperate and boreal latitudes, at a regional scale, deterministic factors such as host identity and availability, soil edaphic factors, and disturbance history result in spatial heterogeneity of EcM fungal communities (Tedersoo et al., 2012; Barker

et al., 2013; Glassman et al., 2015; Sterkenburg et al., 2015; Lamit et al., 2016; Pec et al., 2017; Kohout et al., 2018; Van Der Linde et al., 2018; Day et al., 2019). Therefore, hierarchical assembly dynamics likely act on EcM fungal communities across spatial scales, where deterministic processes take place at large scales and determine the regional species pool, and mainly neutral processes underlie the fine-scale EcM fungal colonization of roots from the surrounding soil.

In summary, I provide evidence supporting the hypothesis that, at fine spatial scales, EcM fungi assemble by both neutral and deterministic processes, and that the relative contribution of each of these processes do not vary across different disturbances. However, as the NCM used focuses only on processes occurring at the fine spatial scale, mainly dispersal from surrounding soils to roots, it is unable to test the influence of neutral and deterministic processes occurring only in soils, which can affect the resulting relative abundance of root-associated EcM fungal taxa. Comparing the outcome of this study to the assembly of EcM fungi associating with mature lodgepole pine at the same spatial scale would elucidate whether disturbances modulate the relative roles of neutrality and determinism compared to intact forests. Overall, my results demonstrate that the local species pool of EcM fungi in disturbed forests is likely the result of host-specificity of EcM fungi, large production of resistant spores, and potential uncertain ecological roles of taxa commonly identified as EcM fungi specific, in combination with dispersal from the soil source pool. At regional spatial scales, the EcM fungal community composition is defined by disturbance history with a filtered community that, at fine local scales, largely assembles through neutral processes. Overall, these results highlight the importance of processes occurring at both regional and local scales, which should be considered when managing and manipulating a host's associated fungal community *in-situ*. If dominant abundance

of the spore bank following disturbances is the main mechanism of pine root colonization, then root-associated EcM fungal communities could be manipulated by adding spores of target species to soils while taking into account the legacy *in-situ* soil EcM fungal community that will also colonize roots through dispersal.

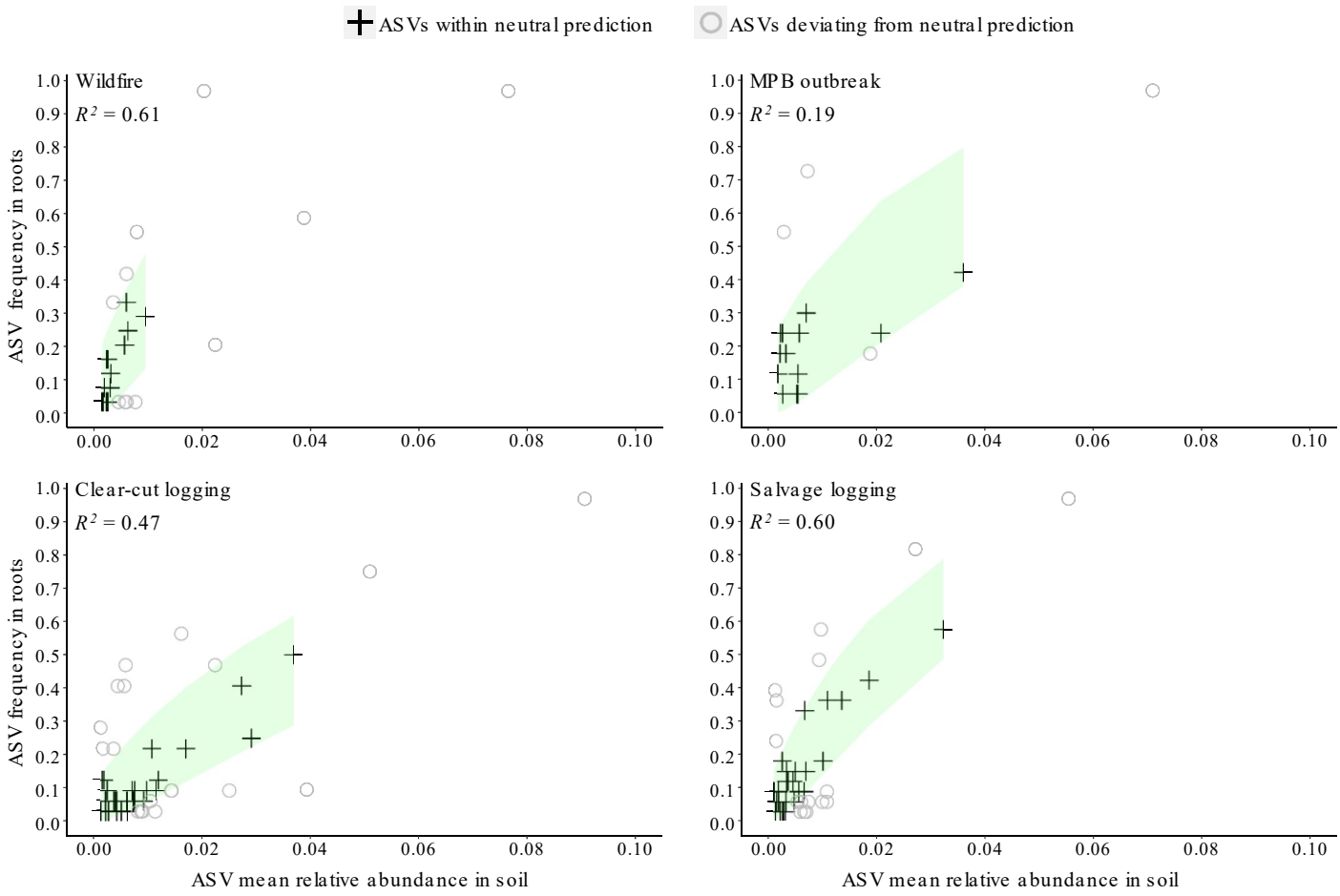


Figure 4.1 Fit of the Sloan neutral community model across disturbances. Amplicon sequence variants (ASVs) that occur more or less frequently than predicted by the model are shown as circles, and those whose abundance conforms model predictions, crosses. Green shade represents 95% confidence intervals around the neutral model prediction. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

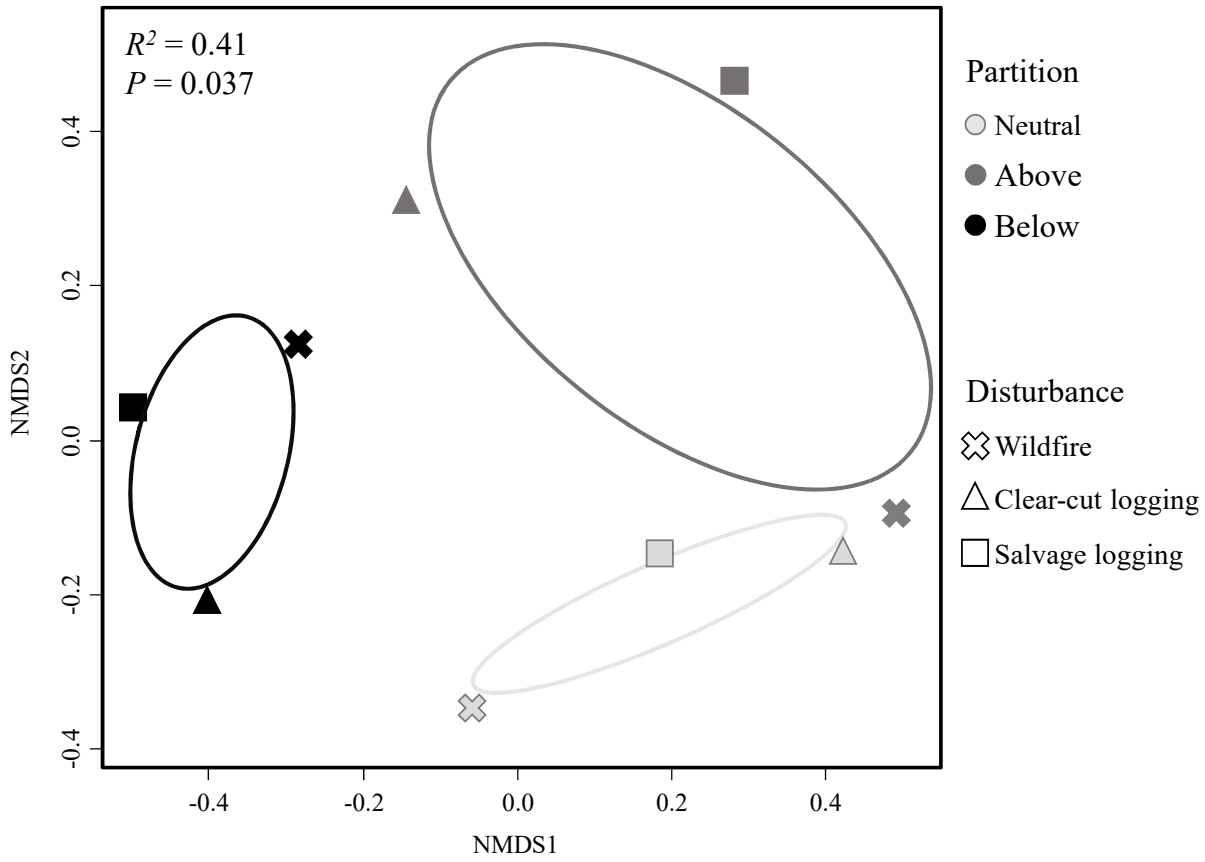


Figure 4.2 Non-metric multidimensional scaling plot using Bray-Curtis distance among root-associated ectomycorrhizal fungal communities of amplicon sequence variants (ASVs) assigned as neutral or non-neutral across disturbances. Neutral is defined as ASVs with frequencies conforming to Sloan neutral community model predictions, and non-neutral as those fitting above or below the model prediction. Mountain pine beetle (MPB, *Dendroctonus ponderosae*) outbreak communities not shown due to low neutral model fit on data. Significant difference of communities at $\alpha = 0.05$ was tested using perMANOVA.

Table 4.1. Fit of the Sloan neutral community model and amplicon sequence variants (ASVs) that deviated from neutral predictions across disturbances. Overrepresented ASVs are those more abundant, and underrepresented ASVs are those less abundant than the neutral model prediction, given their abundance in the surrounding soil. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

Disturbance	Model fit (R^2)	Overrepresented ASVs	Underrepresented ASVs
Wildfire	0.609	*Wilcoxina mikolae* *Wilcoxina rehmii* *Amphinema* sp 1 *Amphinema* sp 2 *Piloderma* sp 1	*Acephala applanata* Helotiaceae sp 1 *Caliptrozyma* sp 1 *Meliniomyces variabilis* Thelephoraceae sp 1 Thelephoraceae sp 2
MPB outbreak	0.194	*Cenococcum geophilum* *Wilcoxina mikolae* *Piloderma* sp 1	*Acephala applanata*
Clear-cut logging	0.446	*Cenococcum geophilum* *Hygrophorus hypothejus* *Piloderma* sp 1 *Rhizopogon abietis* *Suillus* sp 1 *Suillus flavidus* *Lactarius helvus* *Tomentella badia* *Acephala applanata* Helotiaceae sp 1	*Caliptrozyma* sp 1 *Meliniomyces* sp 1 *Meliniomyces* sp 2 *Meliniomyces* sp 3 *Meliniomyces* sp 4 *Inocybe rufoalba* Thelephoraceae sp 1 Thelephoraceae sp 2 Thelephoraceae sp 3
Salvage-logging	0.597	*Cenococcum geophilum* *Wilcoxina rehmii* *Piloderma* sp 1 *Suillus flavidus* *Suillus plorans* *Lactarius helvus* *Russula* sp 1	*Acephala applanata* Helotiaceae sp 1 *Meliniomyces* sp 1 *Meliniomyces* sp 2 *Inocybe* sp1 *Inocybe rufoalba* Thelephoraceae sp 1 Thelephoraceae sp 2 Thelephoraceae sp 3

Chapter 5

General discussion and conclusion

The response of forest communities to disturbance events is an area of great interest in light of altered disturbance regimes set by climate change and human demands for resources (Newman, 2019). In this thesis, I mainly investigated the impacts of forest disturbances on soil fungal communities and the potential cascading effects that alterations to soil fungi can have on the establishment and performance of seedlings of a major reforestation tree species, lodgepole pine. Furthermore, I investigated how root-associated fungi assemble through recruitment from the surrounding soil fungal community. My research highlights how soil fungal response to disturbances depends on the disturbance type and the fungal guilds studied. Moreover, my research indicates that fungal disturbance legacies are detected in lodgepole pine roots approximately seven years following disturbances, and that such fungal legacies are resistant to the introduction of a different fungal community. Lastly, I determined that despite neutral processes dominating the assembly of lodgepole root-associated EcM fungi across disturbances, deterministic processes also influence the structure of the fungal taxa colonizing the roots. Overall, this thesis contributes to the current understanding of forest disturbance, recovery, and community ecology from a belowground perspective.

The first experimental part of my dissertation (Chapter 2) examined how overall soil fungal biomass and the community composition and abundance of individual fungal guilds compared between control and disturbed forests. Disturbances decreased the soil fungal biomass by 44 to 75%, compared to control forests; however, MPB outbreak sites had a 45% increase in fungal biomass. Along with changes in fungal biomass, disturbances shifted the community

composition and abundance of particular soil fungal guilds. For example, the most notable disturbance impacts on a guild were observed on EcM fungi, where community composition in wildfire, clear-cut, and salvage-logging differed from that of controls. However, the composition of EcM fungal communities did not diverge among disturbances, suggesting that the different filters that each disturbance type represents are not taxonomically selective; instead, different disturbance filters result in similar EcM fungal communities. These changes in soil EcM fungal composition were likely driven by the overall relative abundance of this fungal guild, which sharply declined following the same disturbances that changed their composition compared to controls. With a decline of EcM fungal abundance, the saprotrophic fungi became the dominant guild in disturbed forests, while the frequency of arbuscular mycorrhizal fungi also increased. Furthermore, my research indicated that one of the factors mediating the decline of EcM fungi and increase in AM fungi is the disruption of the soil organic layer, where EcM fungi was negatively correlated but AM fungi positively correlated with thinning of the organic layer caused by disturbances.

Changes in the soil fungal biomass and relative abundance of guilds may impact ecosystem functions such as nutrient and carbon dynamics of boreal forests. With 40% of the boreal forest C stocks found in the soil organic matter, to which EcM fungi accounts for a significant fraction and helps stabilize (Averill et al., 2014; Clemmensen et al., 2013), soil C turnover rates might be increased. With a loss of EcM fungi and a shift in dominance to saprotrophic and AM fungi, a decrease in competition between EcM and saprotrophic fungi can increase decomposition rates of organic matter (Averill & Hawkes, 2016; Orwin et al., 2011; Phillips et al., 2013). Shifts in nutrient economies, from organic to inorganic, and related C dynamics could be exacerbated with more frequent and intense disturbance regimes where forest

and soil fungal recovery might be successively impacted, processes that can affect the ecosystem resilience (Johnstone et al., 2016).

As introduced in Chapter 1, one of the functions of EcM fungi is that they facilitate the establishment and growth of plants; therefore, the decline of soil EcM fungi following disturbances seen in Chapter 2 potentially posits a problem for pine seedling regeneration. It has been suggested that soil microbial communities from late-successional ecosystems can benefit target plant species in early-successional ecosystems (Wubs et al., 2016). Thus, in Chapter 3, I investigated whether soil transfers from late-successional lodgepole pine forests into disturbed forests affected pine seedling establishment and performance. Soil transfers did not impact seedling establishment or performance and did not change the soil or root-associated fungal community composition at the disturbed sites. Instead, fungal disturbance legacies shaped the EcM fungal composition at both soils and pine roots.

The absence of a compositional effect from soil transfers in disturbed sites is likely due to a soil fungal legacy resistant to the introduction of a fungal community from late-successional forests. Such fungal legacies did not affect any of the tested seedling variables suggesting that seedlings were insensitive to the variations in fungal communities across disturbance. Furthermore, it suggests that, under the studied disturbance types and intensities, the establishment of lodgepole pine seedling is not limited by EcM fungal availability in these forest types. Despite studies in controlled settings showing the effects that soil amendments can have on pine seedling performance (Beck et al., 2020; Sebastiana et al., 2018), my results suggest that these effects can disappear when tested in the field. Boreal forests harbor a high diversity of EcM fungi (Tedersoo et al., 2012), which may ensure that, despite the filters that disturbances impose on fungal communities, seedling establishment and performance is maintained by the diversity

and/or functional redundancy among soil fungal communities (Jones et al., 2010; Rineau & Courty, 2011; Talbot et al., 2014).

In Chapter 3, I also found that EcM fungal indicator taxa associated with seedling roots growing in sites impacted by wildfire and logging disturbances, differed in ecological successional traits compared to those of MPB outbreak sites, likely due to how these two types of disturbances impact the aboveground forest structure. Root-associated EcM indicator taxa in wildfire and clear-cut sites were typical 'early-stage' fungi that tend to colonize seedlings via spore germination. In contrast, in the MPB outbreak disturbance, where the perturbation does not disrupt soils, the EcM indicator taxa tended to be 'late-stage' fungi that colonize via mycelial strands. It is notable to mention that following MPB outbreak, residual trees remained and thus seedlings may have connected to EcM networks, since my experimental plots were not trenched. Therefore, across the first two studies, it is notable that the fungal community composition of soils and lodgepole pine roots was determined by the disturbance type. However, the decline in EcM fungal abundance and diversity in soils of disturbed forests did not impact the establishment of lodgepole pine seedlings, suggesting that approximately seven years following disturbances, resilience remains regarding lodgepole pine establishment.

Little is known about how the root-associated EcM fungal community assembles from the community in the surrounding soil. In Chapter 4, I examined the roles of neutral and deterministic processes on the assembly of root-associated EcM fungal communities and found that neutral processes dominated over deterministic across all disturbances. However, deterministic assembly processes had a sizeable contribution on the assembly of communities comprising taxa with particular traits. The majority of taxa found to occur less frequently than expected, based on their abundance in soil, represented fungi from the taxonomic family

Helotiaceae, which has been suggested to be root endophytes with kinship to plant and fungal parasites and pathogens. This result could suggest that taxa from this family is disfavored by the host or that taxa within this family have a large degree of unresolved ecological function, despite being present in the roots and currently categorized as EcM fungi. The taxa found to be more abundant than expected in roots, largely comprised those which dominate the spore banks in forest soils and are considered either conifer-specific EcM fungi, or able to colonize pine roots of both seedlings and mature pine trees. Furthermore, it possible that following disturbances that remove or combust the overstory pine trees, resistant spore banks are the source of EcM fungi for establishing seedlings, therefore, fungal species that dominate the spore bank at the time of seed germination have competitive advantages compared to those whose main mechanism of colonization is through mycelial strands.

Aside from the genus *Wilcoxina*, indicator species of stand-replacing disturbances observed in Chapter 3 were taxa not overrepresented in pine roots. I found this particularly interesting as it may suggest that, despite the high abundance of indicator taxa in specific disturbance types, such indicator taxa seem to assemble mainly though neutral processes. Therefore, their high abundance in roots likely reflect their probabilistic dispersal from the surrounding soils. Overall, this suggests that while deterministic factors, such as disturbance type, shape the soil and root-associated EcM fungal community in soils at a regional spatial scale, colonization of roots by EcM fungi in disturbed forests is influenced mostly by dispersal from the surrounding soil.

Altogether, my research contributes new knowledge regarding the ecological filters that disturbances impose on soil and root-associated fungal communities, establishment of lodgepole pine seedlings, and assembly of root-associated EcM fungi. Future research should focus on the

function of these fungal communities. For example, despite different root-associated EcM fungal communities not having an impact on the establishment and performance of lodgepole pine seedlings, studying how the transcript profiles of these fungal communities may diverge would greatly contribute to the overall understanding of their functions, not only in roots but also in soils. It is important to monitor both community composition and functionality in forests impacted by cumulative disturbances other than salvage-logging, such as wildfires followed by droughts as multifactor environmental changes can show 'ecological surprises' that only become apparent with the interaction of multiple factors (Rillig et al., 2019). Furthermore, testing the root-associated EcM fungal community assembly using mock communities with known traits may highlight more in depth the individual traits that could be selected for or against by plant hosts.

Comprehensive bibliography

Abarenkov K, Henrik Nilsson R, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, et al. 2010. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **186**: 281–285.

Agee JK. **1993**. *Fire ecology of Pacific Northwest forests*. Washington, DC : Island Press, 113–150.

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, et al. **2010**. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**: 660–684.

Allison SD, Treseder KK. **2008**. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology* **14**: 2898–2909.

Amaranthu, MP, & Perry DA 1987. Effect of soil transfer on ectomycorrhiza formation and the survival and growth of conifer seedlings on old, nonreforested clear-cuts. *Canadian Journal of Forest Research*, **17**(8): 944–950.

Anderson IC, Genney DR, Alexander IJ. **2014**. Fine-scale diversity and distribution of ectomycorrhizal fungal mycelium in a Scots pine forest. *New Phytologist* **201**: 1423–1430.

Averill C, Turner BL, Finzi AC. **2014**. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* **505**: 543–545.

Averill C, Hawkes C V. **2016**. Ectomycorrhizal fungi slow soil carbon cycling. *Ecology Letters* **19**: 937–947.

Baar J, Horton TR, Kretzer AM, & Bruns TD. 1999. Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist* **143**(2): 409–418.

Baldrian P. **2016**. Forest microbiome: diversity, complexity and dynamics (E Banin, Ed.). *FEMS Microbiology Reviews* **41**: 109–130.

Bale JS, Masters GJ, Hodkinson ID, Awmack C, Bezemer TM, Brown VK, Butterfield J, Buse A, Coulson JC, Farrar J, et al. **2002**. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology* **8**: 1–16.

Barker JS, Simard SW, Jones MD, Durall DM. **2013**. Ectomycorrhizal fungal community assembly on regenerating Douglas-fir after wildfire and clearcut harvesting. *Oecologia* **172**: 1179–1189.

Baldrian P. **2016**. Forest microbiome: diversity, complexity and dynamics (E Banin, Ed.). *FEMS Microbiology Reviews* **41**: 109–130.

Bates D, Mächler M, Bolker B, Walker S. **2015**. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Baum C, El-Tohamy W, Gruda N. **2015**. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. *Scientia Horticulturae* **187**: 131–141.

Baxter JW & Dighton J 2001. Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch *(Betula populifolia)* seedlings in host-symbiont culture conditions. *New Phytologist*, **152**(1): 139–149.

Beck JL., Cale JA, Rodriguez-Ramos JC, Kanekar SS, Karst J, Cahill JF, Simard SW, Erbilgin, N. 2020. Changes in soil fungal communities following anthropogenic

disturbance are linked to decreased lodgepole pine seedling performance. *Journal of Applied Ecology*, **57**(7): 1292–1302.

Belillas CM, Feller MC. **1998**. Relationships between fire severity and atmospheric and leaching nutrient losses in British Columbia's coastal western hemlock zone forests. *International Journal of Wildland Fire* **8**: 87–101.

Bell G. **2001**. Neutral macroecology. *Science* **293**: 2413–2418.

Berch SM, Brockley RP, Battigelli JP, Hagerman S, Holl B. **2006**. Impacts of repeated fertilization on components of the soil biota under a young lodgepole pine stand in the interior of British Columbia. *Canadian Journal of Forest Research* **36**: 1415–1426.

Berman JT, & Bledsoe CS. 1998. Soil transfers from valley oak (*Quercus lobata* Nee) stands increase ectomycorrhizal diversity and alter root and shoot growth on valley oak seedlings. In *Mycorrhiza* **7**(5): 223-235.

Blaalid R, Kumar S, Nilsson RH, Abarenkov K, Kirk PM. **2013**. ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources* **13**: 218–224.

Bödeker ITM, Lindahl BD, Olson Å, Clemmensen KE. **2016**. Mycorrhizal and saprotrophic fungal guilds compete for the same organic substrates but affect decomposition differently. *Functional Ecology* **30**: 1967–1978.

Bogar LM, Peay KG. **2017**. Processes maintaining the coexistence of ectomycorrhizal fungi at a fine spatial scale. In: Tedersoo L. eds. *Biogeography of Mycorrhizal Symbiosis.* Berlin, Germany: Springer, 79–105.

Bolton DK, Coops NC, Wulder MA. **2015**. Characterizing residual structure and forest recovery following high-severity fire in the western boreal of Canada using Landsat time-series and airborne lidar data. *Remote Sensing of Environment* **163**: 48–60.

Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**: 852–857.

Boucher D, Boulanger Y, Aubin I, Bernier PY, Beaudoin A, Guindon L, Gauthier S. **2018**. Current and projected cumulative impacts of fire, drought, and insects on timber volumes across Canada. *Ecological Applications* **28**: 1245–1259.

Bradbury SM. **1998**. Ectomycorrhizas of lodgepole pine (*Pinus contorta*) seedlings originating from seed in southwestern Alberta cut blocks. *Canadian Journal of Botany* **76**: 213–217.

Bradbury SM, Danielson RM, Visser S. **1998**. Ectomycorrhizas of regenerating stands of lodgepole pine (*Pinus contorta*). *Canadian Journal of Botany* **76**: 218–227.

Brandt JP, Flannigan MD, Maynard DG, Thompson ID, Volney WJA. **2013**. An introduction to Canada's boreal zone: Ecosystem processes, health, sustainability, and environmental issues1. *Environmental Reviews* **21**: 207–226.

Brazee NJ, Lindner DL, D'Amato AW, Fraver S, Forrester JA, Mladenoff DJ. **2014**. Disturbance and diversity of wood-inhabiting fungi: effects of canopy gaps and downed woody debris. *Biodiversity and Conservation* **23**: 2155–2172.

Bruns TD, Peay KG, Boynton PJ, Grubisha LC, Hynson NA, Nguyen NH, Rosenstock NP. **2009**. Inoculum potential of *Rhizopogon* spores increases with time over the first 4 yr of a 99-yr spore burial experiment. *New Phytologist* **181**: 463–470.

Buée M, Courty PE, Mignot D, Garbaye J. **2007**. Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry* **39**: 1947–1955.

Buma B, Wessman CA. **2011**. Disturbance interactions can impact resilience mechanisms of

forests. *Ecosphere* **2**: 1–15.

Buma B, Wessman CA. **2012**. Differential species responses to compounded perturbations and implications for landscape heterogeneity and resilience. *Forest Ecology and Management* **266**: 25–33.

Buma B. **2015**. Disturbance interactions: Characterization, prediction, and the potential for cascading effects. *Ecosphere* **6**: 1–15.

Burns AR, Stephens WZ, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannan BJM. **2016**. Contribution of neutral processes to the assembly of gut microbial communities in the zebrafish over host development. *ISME Journal* **10**: 655–664.

Burton PJ, Messier C, Puettmann KJ, Coates KD. **2013**. Exploring complexity in boreal forests. *Managing forests as complex adaptive systems. Building resilience to the challenge of global change. Routledge*. London. Pp 79–109

Buscardo E, Rodríguez-Echeverría S, Martín MP, De Angelis P, Pereira JS, Freitas H. 2010. Impact of wildfire return interval on the ectomycorrhizal resistant propagules communities of a Mediterranean open forest. *Fungal Biology*, **114**(8): 628–636.

Byrd KB, Parker VT, Vogler DR, Cullings KW. **2000**. The influence of clear-cutting on ectomycorrhizal fungus diversity in a lodgepole pine (*Pinus contorta*) stand, Yellowstone National Park, Wyoming, and Gallatin National Forest, Montana. *Canadian Journal of Botany* **78**: 149–156.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Chase JM. **2007**. Drought mediates the importance of stochastic community assembly.

Proceedings of the National Academy of Sciences of the United States of America **104**: 17430–17434.

Chave J. **2004**. Neutral theory and community ecology. *Ecology Letters* **7**: 241–253.

Chen W, Ren K, Isabwe A, Chen H, Liu M, Yang J. **2019**. Stochastic processes shape microeukaryotic community assembly in a subtropical river across wet and dry seasons. *Microbiome* **7**:138.

Cigan PW, Karst J, Cahill JF, Sywenky AN, Pec GJ, Erbilgin N. **2015**. Influence of bark beetle outbreaks on nutrient cycling in native pine stands in western Canada. *Plant and Soil* **390**: 29–47.

Clemmensen KE, Bahr A, Ovaskainen O, Dahlberg A, Ekblad A, Wallander H, Stenlid J, Finlay RD, Wardle DA, Lindahl BD. **2013**. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science* **339**: 1615–8.

Clements FE. **1936**. Nature and Structure of the Climax. *The Journal of Ecology* **24**: 252–284.

Colinas C, Molina R, Trappe J, Perry D. **1994**. Ectomycorrhizas and rhizosphere microorganisms of seedlings of *Pseudotsuga menziesii* (Mirb.) Franco planted on a degraded site and inoculated with forest soils pretreated with selective biocides. *New Phytologist* **127**: 529–537.

Crowther TW, Maynard DS, Crowther TR, Peccia J, Smith JR, Bradford MA. **2014**. Untangling the fungal niche: the trait-based approach. *Frontiers in microbiology* **5**: 579.

Crowther TW, van den Hoogen J, Wan J, Mayes MA, Keiser AD, Mo L, Averill C, Maynard DS. **2019**. The global soil community and its influence on biogeochemistry. *Science* **365**: eaav0550.

Cudmore, T. J., Björklund, N., Carroll, A. L., & Staffan Lindgren, B. 2010. Climate change

and range expansion of an aggressive bark beetle: Evidence of higher beetle reproduction in naïve host tree populations. *Journal of Applied Ecology* **47**: 1036–1043.

Cullingham CI, Cooke JEK, Dang S, Davis CS, Cooke BJ, Coltman DW. **2011**. Mountain pine beetle host-range expansion threatens the boreal forest. *Molecular Ecology* **20**: 2157–2171.

Dahlberg A, Stenström E. **1991**. Dynamic changes in nursery and indigenous mycorrhiza of *Pinus sylvestris* seedlings planted out in forest and clearcuts. *Plant and Soil* **136**: 73–86.

Dahlberg A. 2001. Community ecology of ectomycorrhizal fungi: an advancing interdisciplinary field. *New Phytologist*, **150**: 555–562.

Dahlberg A, Schimmel J, Taylor AFS, Johannesson H. **2001**. Post-fire legacy of ectomycorrhizal fungal communities in the Swedish boreal forest in relation to fire severity and logging intensity. *Biological Conservation* **100**: 151–161.

Dalong, M, Luhe W, Guoting Y, Liqiang M, Chun L. 2011. Growth response of *Pinus densiflora* seedlings inoculated with three indigenous ectomycorrhizal fungi in combination. *Brazilian Journal of Microbiology*, **42**(3): 1197–1204.

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, et al. **2015**. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**: 970–973.

Day NJ, Dunfield KE, Johnstone JF, Mack MC, Turetsky MR, Walker XJ, White AL, Baltzer JL. **2019**. Wildfire severity reduces richness and alters composition of soil fungal communities in boreal forests of western Canada. *Global Change Biology* **25**: 2310–2324.

De Cáceres M, & Legendre P. 2009. Associations between species and groups of sites: Indices

and statistical inference. *Ecology*, **90**(12): 3566–3574.

DeLuca TH, Boisvenue C. **2012**. Boreal forest soil carbon: Distribution, function and modelling. *Forestry* **85**: 161–184.

De Román, M, & De Miguel AM. 2005. Post-fire, seasonal and annual dynamics of the ectomycorrhizal community in a *Quercus ilex* L. forest over a 3-year period. *Mycorrhiza*, **15**(6): 471–482.

Dhar A, Parrott L, Heckbert S. **2016**. Consequences of mountain pine beetle outbreak on forest ecosystem services in western Canada. *Canadian Journal of Forest Research* **46**: 987–999.

Dickie IA, Bolstridge N, Cooper JA, Peltzer DA. 2010. Co-invasion by *Pinus* and its mycorrhizal fungi. *New Phytologist*, **187**(2): 475–484.

Dighton J, Mason PA. 1985. Mycorrhizal dynamics during forest tree development. In: Moore D, Casselton LA, Wood DA, Frankland JC. eds. *Developmental biology of higher fungi*. Retrieved from https://books.google.ca/books?hl=en&lr=&id=vYeZVwpb8t4C&oi=fnd&pg=PA117&ots=J4uUaLcfIC&sig=lVCmjm84juRcC_uR_DXQ_g1HWWw&redir_esc=y#v=onepage&q&f=false

Douglas RB, Parker VT, Cullings KW. **2005**. Belowground ectomycorrhizal community structure of mature lodgepole pine and mixed conifer stands in Yellowstone National Park. *Forest Ecology and Management* **208**: 303–317.

Dumbrell AJ, Nelson M, Helgason T, Dytham C, Fitter AH. **2010**. Relative roles of niche and neutral processes in structuring a soil microbial community. *ISME Journal* **4**: 337–345.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T.

2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. *New Phytologist* **190**: 794–804.

Egerton-Warburton L, Allen MF. **2001**. Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): Patterns of root colonization and effects on seedling growth. *Mycorrhiza* **11**: 283–290.

Egger KN, Danielson RM, Fortin JA. 1991. Taxonomy and population structure of E-strain mycorrhizal fungi inferred from ribosomal and mitochondrial DNA polymorphisms. *Mycological Research*, **95**(7): 866–872.

Egger, KN, Paden JW. 1986. Biotrophic associations between lodgepole pine seedlings and postfire ascomycetes (Pezizales) in monoxenic culture. *Canadian Journal of Botany*, **64**(11): 2719–2725.

Ekblad A, Mikusinska A, Ågren GI, Menichetti L, Wallander H, Vilgalys R, Bahr A, Eriksson U. **2016**. Production and turnover of ectomycorrhizal extramatrical mycelial biomass and necromass under elevated CO_2 and nitrogen fertilization. *New Phytologist* **211**: 874–885.

El-Komy MH, Saleh AA, Eranthodi A, Molan YY. **2015**. Characterization of novel *Trichoderma asperellum* isolates to select effective biocontrol agents against tomato fusarium wilt. *Plant Pathology Journal* **31**: 50–60.

Elzhov T V., Mullen KM, Spiess A-N, Iker B bo. **2013**. minpack.lm: R interface to the Levenberg-Marquardt nonlinear least-squares algorithm found in MINPACK, plus support for bounds. R package version 1.1-8. [WWW document] URL http://CRAN.R-project.org/package=minpack.lm. [accessed 5 September 2020]

Erbilgin N, Cale JA, Hussain A, Ishangulyyeva G, Klutsch JG, Najar A, & Zhao S. 2017.

Weathering the storm: how lodgepole pine trees survive mountain pine beetle outbreaks. *Oecologia* **184**, 469–478.

Erbilgin N. **2019**. Phytochemicals as mediators for host range expansion of a native invasive forest insect herbivore. *New Phytologist* **221**: 1268–1278.

Ferrenberg S, O'neill SP, Knelman JE, Todd B, Duggan S, Bradley D, Robinson T, Schmidt SK, Townsend AR, Williams MW, et al. 2013. Changes in assembly processes in soil bacterial communities following a wildfire disturbance. *ISME Journal* **7**: 1102–1111.

Fichtner A, von Oheimb G, Härdtle W, Wilken C, Gutknecht JLM. **2014**. Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years. *Soil Biology and Biochemistry* **70**: 79–87.

Field KJ, Daniell T, Johnson D, Helgason T. **2020**. Mycorrhizas for a changing world: Sustainability, conservation, and society. *Plants, People, Planet* **2**: 98–103.

Fierer N, Strickland MS, Liptzin D, Bradford MA, Cleveland CC. **2009**. Global patterns in belowground communities. *Ecology Letters* **12**: 1238–1249.

Fleming LV. 1983. Succession of mycorrhizal fungi on birch: infection of seedlings planted around mature trees. In *Tree Root Systems and Their Mycorrhizas* **71:** 263–267.

Fleming LV. 1984. Effects of soil trenching and coring on the formation of etomycorrhizas on birch seedlings grown around mature trees. *New Phytologist*, **98**(1): 143–153.

Frac M, Hannula SE, Belka M, Jędryczka M. **2018**. Fungal biodiversity and their role in soil health. *Frontiers in Microbiology* **9**: 707.

Frey SD. **2019**. Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics* **50**: 237–259.

Gao C, Zhang Y, Shi N-N, Zheng Y, Chen L, Wubet T, Bruelheide H, Both S, Buscot F, Ding Q, et al. 2015. Community assembly of ectomycorrhizal fungi along a subtropical secondary forest succession. *New Phytologist* **205**: 771–785.

Genney DR, Anderson IC, Alexander IJ. **2006**. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist* **170**: 381–390.

Glassman SI, Peay KG, Talbot JM, Smith DP, Chung JA, Taylor JW, Vilgalys R, Bruns TD. **2015**. A continental view of pine-associated ectomycorrhizal fungal spore banks: a quiescent functional guild with a strong biogeographic pattern. *New Phytologist* **205**: 1619–1631.

Glassman SI, Wang IJ, Bruns TD. **2017**. Environmental filtering by pH and soil nutrients drives community assembly in fungi at fine spatial scales. *Molecular Ecology* **26**: 6960–6973.

Glassman SI, Martiny JBH. **2018**. Broadscale ecological patterns are robust to use of exact sequence variants versus operational taxonomic units. *mSphere* **3**: e00148–18.

Genney, D. R., Anderson, I. C., & Alexander, I. J. 2006. Fine-scale distribution of pine ectomycorrhizas and their extramatrical mycelium. *New Phytologist*, *170*(2), 381–390.

Gleason HA. **1939**. The individualistic concept of the plant association. *American Midland Naturalist* **21**: 92–110.

Gotelli NJ, Graves GR. **1996**. *Null models in ecology*. Washington, D.C.: Smithsonian Institution Press.

Grove, S., Saarman NP, Gilbert GS, Faircloth B, Haubensak KA, Parker IM. 2019. Ectomycorrhizas and tree seedling establishment are strongly influenced by forest edge proximity but not soil inoculum. *Ecological Applications*, **29**(3): e01867.

Hansen WD, Turner MG. **2019**. Origins of abrupt change? Postfire subalpine conifer regeneration declines nonlinearly with warming and drying. *Ecological Monographs* **89**: e01340.

Harrell FE. 2014. Hmisc: Harrell Miscellaneous. R Package Version 3.0-12. [WWW document] URL https://cran.r-project.org/web/packages/Hmisc/Hmisc.pdf [accessed 5 September 2020]

Hartmann M, Howes CG, VanInsberghe D, Yu H, Bachar D, Christen R, Henrik Nilsson R, Hallam SJ, Mohn WW. **2012**. Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. *The ISME Journal* **6**: 2199–2218.

Hartmann M, Niklaus PA, Zimmermann S, Schmutz S, Kremer J, Abarenkov K, Lüscher P, Widmer F, Frey B. **2014**. Resistance and resilience of the forest soil microbiome to logging-associated compaction. *The ISME Journal* **8**: 226–244.

Haskins KE, & Gehring CA. 2005. Evidence for mutualist limitation: The impacts of conspecific density on the mycorrhizal inoculum potential of woodland soils. *Oecologia*, **145**(1): 123–131.

Heinemeyer A, Hartley IP, Evans SP, Carreira de la Fuente JA, Ineson P. **2007**. Forest soil CO_2 flux: uncovering the contribution and environmental responses of ectomycorrhizas. *Global Change Biology* **13**: 1786–1797.

Hervé M. **2018**. *RVAideMemoire: testing and plotting procedures for biostatistics.* [WWW document] URL https://CRAN.R-project.org/package=RVAideMemoire/. [accessed 5 July 2019].

Van der Heyde M, Ohsowski B, Abbott LK, Hart M. **2017**. Arbuscular mycorrhizal fungus

responses to disturbance are context-dependent. *Mycorrhiza* **27**: 431–440.

Heys C, Cheaib B, Busetti A, Kazlauskaite R, Maier L, Sloan WT, Ijaz UZ, Kaufmann J, McGinnity P, Llewellyn MS. **2020**. Neutral processes dominate microbial community assembly in Atlantic Salmon, *Salmo salar*. *Applied and Environmental Microbiology* **86**: e02283-19.

HilleRisLambers J, Adler PB, Harpole WS, Levine JM, Mayfield MM. **2012**. Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* **43**: 227–248.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst, J, Koide RT, Pringle A, Zabinksi C, Bever JD, Moore JC. et al. 2010. A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecology Letters*, **13**: 394–407.

Holden SR, Gutierrez A, Treseder KK. **2013**. Changes in Soil Fungal Communities, Extracellular Enzyme Activities, and Litter Decomposition Across a Fire Chronosequence in Alaskan Boreal Forests. *Ecosystems* **16**: 34–46.

Holden SR, Treseder KK. **2013**. A meta-analysis of soil microbial biomass responses to forest disturbances. *Frontiers in Microbiology* **4**: 163.

Holden SR, Rogers BM, Treseder KK, Randerson JT. **2016**. Fire severity influences the response of soil microbes to a boreal forest fire. *Environmental Research Letters* **11**(3): 035004.

Horton TR, Bruns TD, Parker VT. **1999**. Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. *Canadian Journal of Botany* **77**: 93–102.

Horton TR, van der Heijden MGA. **2018**. The role of symbioses in seedling establishment and

survival. In: Leck MA, Parker VT, Simpson RL, eds. *Seedling Ecology and Evolution*. Cambridge: Cambridge University Press, 189–214.

Hothorn T, Bretz F, Westfal, P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal*, **50:** 346–363.

Hubbell SP. **2001**. *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press.

Jacobs LM, Sulman BN, Brzostek ER, Feighery JJ, Phillips RP, Richard Phillips CP. **2018**. Interactions among decaying leaf litter, root litter and soil organic matter vary with mycorrhizal type. *Journal of Ecology* **106**: 502–513.

Johnstone JF, Allen CD, Franklin JF, Frelich LE, Harvey BJ, Higuera PE, Mack MC, Meentemeyer RK, Metz MR, Perry GLW, et al. **2016**. Changing disturbance regimes, ecological memory, and forest resilience. *Frontiers in Ecology and the Environment* **14**: 369–378.

Jones MD, Durall DM, Cairney JWG. **2003**. Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist* **157**: 399–422.

Jones MD, Twieg BD, Ward V, Barker J, Durall DM, Simard SW. 2010. Functional complementarity of Douglas-fir ectomycorrhizas for extracellular enzyme activity after wildfire or clearcut logging. *Functional Ecology*, **24**(5): 1139–1151.

Jones MD, Phillips LA, Treu R, Ward V, Berch S. 2012. Functional responses of ectomycorrhizal fungal communities to long-term fertilization of lodgepole pine (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) stands in central British Columbia. Applied Soil Ecology **60**: 29-40.

Jones MD. 2017. Integrating ectomycorrhizas into sustainable management of temperate forests.

In: Johnson N, Gehring C, Jansa J. eds. *Mycorrhizal Mediation of Soil*, 1st ed.,. Elsevier, Amsterdam, pp 187-211.

Jost L. 2006. Entropy and diversity. *Oikos* **113**: 363–375.

Karst J, Erbilgin N, Pec GJ, Cigan PW, Najar A, Simard SW, Cahill JF. 2015. Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. *New Phytologist* **208**: 904–914.

Karst J, Randall MJ, Gehring CA. 2014. Consequences for ectomycorrhizal fungi of the selective loss or gain of pine across landscapes. *Botany*, **92**(12): 855–865.

Kasischke ES, Verbyla DL, Rupp TS, Mcguire AD, Murphy KA, Jandt R, Barnes JL, Hoy EE, Duffy PA, Calef M, et al. 2010. Alaska's changing fire regime-implications for the vulnerability of its boreal forests. *Canadian Journal of Forest Research* **40:** 1313–1324.

Kennedy PG, Bruns TD. 2005. Priority effects determine the outcome of ectomycorrhizal competition between two *Rhizopogon* species colonizing *Pinus muricata* seedlings. *New Phytologist* **166**: 631–638.

Kennedy PG, Hortal S, Bergemann SE, Bruns TD. 2007. Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. *Journal of Ecology*, **95**(6): 1338–1345.

Kipfer T, Moser B, Egli S, Wohlgemuth T, Ghazoul J. 2011. Ectomycorrhizal succession patterns in *Pinus sylvestris* forests after stand-replacing fire in the Central Alps. *Oecologia* **167**: 219–228.

Kipfer T, Wohlgemuth T, van der Heijden MGA, Ghazoul J, Egli S. 2012. Growth response of drought-stressed *Pinus sylvestris* seedlings to single- and multi-species inoculation with ectomycorrhizal fungi. *PloS ONE*, **7**(4): e35275.

Klinka K, Worrall J, Skoda L, Varga P. **1999**. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia's forests. *Sientia Silvica* **10**: 171–172.

Knelman, JE, & Nemergut, DR. 2014. Changes in community assembly may shift the relationship between biodiversity and ecosystem function. *Frontiers in Microbiology*, **5**: 424. doi: 10.3389/fmicb.2014.00424

Kohout P, Charvátová M, Štursová M, Mašínová T, Tomšovský M, Baldrian P. **2018**. Clearcutting alters decomposition processes and initiates complex restructuring of fungal communities in soil and tree roots. *The ISME Journal* **12**: 692–703.

Koide RT, Xu B, Sharda J. **2005**. Contrasting below-ground views of an ectomycorrhizal fungal community. *New Phytologist* **166**: 251–262.

Koide RT, Fernandez C, Malcolm G. **2013**. Determining place and process: functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist* **201**: 433–439.

Kolde R. **2019**. *Pheatmap: Pretty Heatmaps*. [WWW document] URL https://CRAN.R-project.org/package=pheatmap/. [accessed 7 September 2019].

Korb JE, Johnson NC, Covington WW. **2003**. Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments. *Journal of Applied Ecology* **40**: 101–110.

Köster K, Berninger F, Lindén A, Köster E, Pumpanen J. **2014**. Recovery in fungal biomass is related to decrease in soil organic matter turnover time in a boreal fire chronosequence. *Geoderma* **235**–**236**: 74–82.

Kranabetter JM, Friesen J. 2002. Ectomycorrhizal community structure on western hemlock

(*Tsuga heterophylla*) seedlings transplanted from forests into openings. *Canadian Journal of Botany*, **80**(8): 861–868.

Kranabetter JM, Dube S, Lilles EB. **2017b**. An investigation into the contrasting growth response of lodgepole pine and white spruce to harvest-related soil disturbance. *Canadian Journal of Forest Research* **47**: 340–348.

Kranabetter JM, Haeussler S, Wood C. **2017a**. Vulnerability of boreal indicators (ground-dwelling beetles, understory plants and ectomycorrhizal fungi) to severe forest soil disturbance. *Forest Ecology and Management* **402**: 213–222.

Kurz WA, Shaw CH, Boisvenue C, Stinson G, Metsaranta J, Leckie D, Dyk A, Smyth C, Neilson ET. **2013**. Carbon in Canada's boreal forest-A synthesis. *Environmental Reviews* **21**: 260–292.

Lamit LJ, Holeski LM, Flores-Rentería L, Whitham TG, Gehring CA. **2016**. Tree genotype influences ectomycorrhizal fungal community structure: Ecological and evolutionary implications. *Fungal Ecology* **24**: 124–134.

Landres PB, Morgan P, Swanson FJ. **1999**. Overview of the use of natural variability concepts in managing ecological systems. *Ecological Applications* **9**: 1179–1188.

Lee J, Lee S, Young JPW. **2008**. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbiology Ecology* **65**: 339–349.

Leibold MA, McPeek MA. **2006**. Coexistence of the niche and neutral perspectives in community ecology. *Ecology* **87**: 1399–1410.

Liao, HL, Chen Y, Vilgalys R. 2016. Metatranscriptomic study of common and host-specific patterns of gene expression between pines and their symbiotic ectomycorrhizal fungi in the genus *Suillus*. *PLOS Genetics*, **12**(10): e1006348.

Lindahl BD, Clemmensen KE. **2016**. Fungal ecology in boreal forest ecosystems. In: Martin F, ed. *Molecular Mycorrhizal Symbiosis*. Wiley Blackwell, 387–404.

Van Der Linde S, Suz LM, Orme CDL, Cox F, Andreae H, Asi E, Atkinson B, Benham S, Carroll C, Cools N, et al. 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. *Nature* **558**: 243–248.

MacArthur RH. **1965**. Patterns of species diversity. *Biological Reviews* **40**: 510–533.

Maltz MR, Treseder KK. 2015. Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. *Restoration Ecology*, **23**(5): 625–634.

Marín C, Godoy R, Valenzuela E, Schloter M, Wubet T, Boy J, Gschwendtner S. **2017**. Functional land-use change effects on soil fungal communities in Chilean temperate rainforests. *Journal of Soil Science and Plant Nutrition* **17**: 985–1002.

Martin M. **2001**. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**: 10–12.

Maynard D, Kalra Y, Crumbaugh. 2007. Nitrate and exchangeable ammonium nitrogen. In: Carter MR, Gregorich EG, eds. *Soil Sampling and Methods of Analysis*, 2nd ed. Boca Raton, FL, USA: CRC Press, 71-80.

Mcintosh ACS, Macdonald SE. **2013**. Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands. *Forest Ecology and Management* **295**: 11–19.

Menkis A, Vasiliauskas R, Taylor AFS, Stenlid J, Finlay R. 2007. Afforestation of abandoned farmland with conifer seedlings inoculated with three ectomycorrhizal fungi – Impact on plant performance and ectomycorrhizal community. *Mycorrhiza*, **17**(4): 337–348.

Mercado-Blanco J, Abrantes I, Caracciolo AB, Bevivino A, Ciancio A, Grenni P,

Hrynkiewicz K, Kredics L, Proença DN. **2018**. Belowground microbiota and the health of tree crops. *Frontiers in Microbiology* **9**: 1006.

Montgomery H., Monreal C., Young J., Seifert K. 2000. Determinination of soil fungal biomass from soil ergosterol analyses. *Soil Biology and Biochemistry* **32**: 1207–1217.

Nara K. **2006**. Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist* **169**: 169–178.

Natural Resources Canada. **2019**. The State of Canada's forests. https://www.nrcan.gc.ca/our-natural-resources/forests-forestry/state-canadas-forests-report/16496 (accessed September 10, 2020).

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, et al. **2013**. Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Newman EA. **2019**. Disturbance ecology in the Anthropocene. *Frontiers in Ecology and Evolution* **7**: 147.

Newton AC. 1992. Towards a functional classification of ectomycorrhizal fungi. *Mycorrhiza*, **2**(2): 75–79.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2016**. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nicholson BA, Jones MD. 2017. Early-successional ectomycorrhizal fungi demonstrate phenotypic plasticity and effectively support extracellular enzyme activities and seedling nitrogen accumulation in mature forests. *Mycorrhiza* **27**(3): 247-260.

Nuñez MA, Horton TR, Simberloff D. 2009. Lack of belowground mutualisms hinders

Pinaceae invasions. *Ecology*, **90**(9): 2352–2359.

Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson GL, Solymos P, M. Henry H. S, Wagner H. **2019**. Vegan: community ecology package. [WWW document] URL https://CRAN.R-project.org/web/packages/vegan/index.html [accessed 5 May 2018].

Onwuchekwa NE, Zwiazek JJ, Quoreshi A, Khasa DP. 2014. Growth of mycorrhizal jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) seedlings planted in oil sands reclaimed areas. *Mycorrhiza*, **24**(6): 431–441.

Öpik M, Moora M, Zobel M, Saks L, Wheatley R, Wright F, Daniell T. **2008**. High diversity of arbuscular mycorrhizal fungi in a boreal herb-rich coniferous forest. *New Phytologist* **179**: 867–876.

Öpik M, Vanatoa A, Vanatoa E, Moora M, Davison J, Kalwij JM, Reier Ü, Zobel M. **2010**. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytologist* **188**: 223–241.

Öpik M, Zobel M, Cantero JJ, Davison J, Facelli JM, Hiiesalu I, Jairus T, Kalwij JM, Koorem K, Leal ME, et al. **2013**. Global sampling of plant roots expands the described molecular diversity of arbuscular mycorrhizal fungi. *Mycorrhiza* **23**: 411–430.

Ortega U, Duñabeitia M, Menendez S, Gonzalez-Murua C, Majada J. 2004. Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of *Pinus radiata* in different water regimes. *Tree Physiology*, **24**(1): 65–73.

Orwin KH, Kirschbaum MUF, St John MG, Dickie IA. **2011**. Organic nutrient uptake by mycorrhizal fungi enhances ecosystem carbon storage: a model-based assessment. *Ecology Letters* **14**: 493–502.

Paine RT, Tegner MJ, Johnson EA. **1998**. Compounded perturbations yield ecological

surprises. *Ecosystems* **1**: 535–545.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C. **2019**. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Peay KG, Bruns TD, Kennedy PG, Bergemann SE, Garbelotto M. 2007. A strong species-area relationship for eukaryotic soil microbes: Island size matters for ectomycorrhizal fungi. *Ecology Letters*, **10**(6): 470–480.

Peay KG, Kennedy PG, Bruns TD. **2008**. Fungal community ecology: A hybrid beast with a molecular master. *BioScience* **58**: 799–810.

Peay KG, Garbelotto M, Bruns TD. **2010**. Evidence of dispersal limitation in soil microorganisms: Isolation reduces species richness on mycorrhizal tree islands. *Ecology* **91**: 3631–3640.

Peay KG, Kennedy PG, Bruns TD. 2011. Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology*, **4**(3): 233–240.

Pec GJ, Karst J, Taylor DL, Cigan PW, Erbilgin N, Cooke JEK, Simard SW, Cahill JF. **2017**. Change in soil fungal community structure driven by a decline in ectomycorrhizal fungi following a mountain pine beetle (*Dendroctonus ponderosae*) outbreak. *New Phytologist* **213**: 864–873.

Pec GJ, Scott NM, Hupperts SF, Hankin SL, Landhäusser SM, Karst J. 2019. Restoration of belowground fungal communities in reclaimed landscapes of the Canadian boreal forest. *Restoration Ecology*, **27**(6): 1369-1380.

Peng C, Ma Z, Lei X, Zhu Q, Chen H, Wang W, Liu S, Li W, Fang X, Zhou X. **2011**. A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature Climate Change* **1**: 467–471.

Peterson DL, Arbaugh MJ. **1986**. Postfire survival in Douglas-fir and lodgepole pine: comparing the effects of crown and bole damage. *Canadian Journal of Forest Research* **16**: 1175–1179.

Phillips RP, Brzostek E, Midgley MG. **2013**. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* **199**: 41–51.

Pickett STA, White PS. **1985**. *The ecology of natural disturbance and patch dynamics*.. London, United Kingdom: Academic Press.

Pickles BJ, Genney DR, Anderson IC, Alexander IJ. **2012**. Spatial analysis of ectomycorrhizal fungi reveals that root tip communities are structured by competitive interactions. *Molecular Ecology* **21**: 5110–5123.

Pinheiro J, Bates D, DebRoy S, Sarkar D, 2020. *Nlme: Linear and nonlinear mixed effects models*. R package version 3.1-149, https://CRAN.R-project.org/package=nlme.

Policelli N, Horton TR, Hudon AT, Patterson TR, Bhatnagar JM. **2020**. Back to roots: The role of ectomycorrhizal fungi in boreal and temperate forest restoration. *Frontiers in Forests and Global Change* **3**: 97.

Powell JR, Bennett AE. **2016**. Unpredictable assembly of arbuscular mycorrhizal fungal communities. *Pedobiologia* **59**: 11–15.

Pureswaran DS, De Grandpré L, Paré D, Taylor A, Barrette M, Morin H, Régnière J, Kneeshaw DD. **2015**. Climate-induced changes in host tree-insect phenology may drive

ecological state-shift in boreal forests. *Ecology* **96**: 1480–1491.

R Core Team. 2018. R v.3.5.1: a language andenvironment for statisticalcomputing. Vienna, Austria: R Foundation for Statistical Computing. [WWW document] URL http://www.r-project.org/.

Raffa KF, Mason CJ, Bonello P, Cook S, Erbilgin N, Keefover-Ring K, Klutsch JG, Villari C, Townsend PA. **2017**. Defence syndromes in lodgepole – whitebark pine ecosystems relate to degree of historical exposure to mountain pine beetles. *Plant, Cell & Environment* **40**: 1791–1806.

Ratajczak Z, Carpenter SR, Ives AR, Kucharik CJ, Ramiadantsoa T, Stegner MA, Williams JW, Zhang J, Turner MG. **2018**. Abrupt change in ecological systems: Inference and diagnosis. *Trends in Ecology & Evolution* **33**: 513–526.

Rillig MC, Ryo M, Lehmann A, Aguilar-Trigueros CA, Buchert S, Wulf A, Iwasaki A, Roy J, Yang G. 2019. The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science* **366**: 886–890.

Rincón A, de Felipe, MR, Fernández-Pascual M. 2007. Inoculation of *Pinus halepensis* Mill. with selected ectomycorrhizal fungi improves seedling establishment 2 years after planting in a degraded gypsum soil. *Mycorrhiza*, **18**(1): 23–32.

Rineau F, Courty PE. 2011. Secreted enzymatic activities of ectomycorrhizal fungi as a case study of functional diversity and functional redundancy. *Annals of Forest Science*, **68**(1): 69–80.

Rodriguez-Ramos JC, Cale JA, Cahill JF, Simard SW, Karst J, Erbilgin N. **2021**. Changes in soil fungal community composition depend on functional group and forest disturbance type. *New Phytologist* **229**(2): 1105–1117.

Roe AL, Alexander LL, Andrews MD. 1970. Engleman spruce regeneration practices in the Rocky Mountains. *U.S. For. Serv. Prod. Res. Rpt.* 115.

Safranyik L, Carroll AL, Regniere J, Langor DW, Riel WG, Shore TL, Peter B, Cooke BJ, Nealis VG, Taylor SW. **2010**. Potential for range expansion of mountain pine beetle into the boreal forest of North America. *Canadian Entomologist* **142**: 415–442.

Saravesi K, Markkola A, Rautio P, Roitto M, Tuomi J. **2008**. Defoliation causes parallel temporal responses in a host tree and its fungal symbionts. *Oecologia* **156**: 117–123.

Saravesi K, Aikio S, Wäli PR, Ruotsalainen AL, Kaukonen M, Huusko K, Suokas M, Brown SP, Jumpponen A, Tuomi J, et al. **2015**. Moth outbreaks alter root-associated fungal communities in subarctic mountain birch forests. *Microbial Ecology* **69**: 788–797.

Schneider RR. **2013**. *Alberta's Natural Subregions Under a Changing Climate: Past, Present, and Future*. Edmonton, Canada. [WWWdocument] URL http://biodiversityandclimate.abmi.ca/wp-content/uploads/2015/01/. [accessed 15 August 2019]

Siddiky MRK, Kohler J, Cosme M, Rillig MC. **2012**. Soil biota effects on soil structure: Interactions between arbuscular mycorrhizal fungal mycelium and collembola. *Soil Biology and Biochemistry* **50**: 33–39.

Sebastiana M, da Silva AB, Matos AR, Alcântara A, Silvestre S, Malhó R. 2018. Ectomycorrhizal inoculation with *Pisolithus tinctorius* reduces stress induced by drought in cork oak. *Mycorrhiza*, **28**(3): 247–258.

Shi Y, Li Y, Xiang X, Sun R, Yang T, He D, Zhang K, Ni Y, Zhu YG, Adams JM, et al. 2018. Spatial scale affects the relative role of stochasticity versus determinism in soil bacterial communities in wheat fields across the North China Plain. *Microbiome* **6**: 27.

Shi G, Liu Y, Mao L, Jiang S, Zhang Q, Cheng G, An L, Du G, Feng H. **2014**. Relative importance of deterministic and stochastic processes in driving arbuscular mycorrhizal fungal assemblage during the spreading of a toxic plant. *PLoS ONE* **9**: e95672.

Sim MY, Eom AH. 2006. Effects of ectomycorrhizal fungi on growth of seedlings of *Pinus densiflora*. *Mycobiology*, **34**(4): 191–195.

Six DL, Vergobbi C, Cutter M. 2018. Are survivors different? Genetic-based selection of trees by mountain pine beetle during a climate change-driven outbreak in a high-elevation pine forest. *Frontiers in Plant Science*, **9**: 993.

Sloan WT, Lunn M, Woodcock S, Head IM, Nee S, Curtis TP. **2006**. Quantifying the roles of immigration and chance in shaping prokaryote community structure. *Environmental Microbiology* **8**: 732–740.

Smith SE, Read DJ. **2008**. *Mycorrhizal symbiosis*. Amsterdam, Netherlands: Academic Press.

Soil and Crop Diagnostics Center. **1995**. Method SO23 determination of soil test nitrogen, phosphorus, sodium and potassium using Norwest Extractant. standard operating procedures manual. Alberta Agriculture, Edmonton, Canada.

Sousa WP. **1984**. The role of disturbance in natural communities. *Annual Review of Ecology and Systematics* **15**: 353–391.

Sousa, N. R., Franco, A. R., Ramos, M. A., Oliveira, R. S., & Castro, P. M. L. 2011. Reforestation of burned stands: The effect of ectomycorrhizal fungi on *Pinus pinaster* establishment. *Soil Biology and Biochemistry*, **43**(10): 2115–2120.

Stefani F, Isabel N, Morency MJ, Lamothe M, Nadeau S, Lachance D, Li EHY, Greer C, Yergeau E, Pinno B, Seguín A. 2018. The impact of reconstructed soils following oil sands exploitation on aspen and its associated belowground microbiome. *Scientific Reports*,

8: 2761.

Sterkenburg E, Bahr A, Brandström Durling M, Clemmensen KE, Lindahl BD. **2015**. Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist* **207**: 1145–1158.

Stevens-Rumann CS, Kemp KB, Higuera PE, Harvey BJ, Rother MT, Donato DC, Morgan P, Veblen TT. 2018. Evidence for declining forest resilience to wildfires under climate change. *Ecology Letters*, **21**: 243–252.

Sturrock RN, Frankel SJ, Brown A V., Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ. **2011**. Climate change and forest diseases. *Plant Pathology* **60**: 133–149.

Štursová M, Šnajdr J, Cajthaml T, Bárta J, Šantrů C ˇková H, Baldrian P. **2014**. When the forest dies: the response of forest soil fungi to a bark beetle-induced tree dieback. *The ISME Journal* **8**: 1920–1931.

Sun H, Santalahti M, Pumpanen J, Köster K, Berninger F, Raffaello T, Jumpponen A, Asiegbu FO, Heinonsalo J. **2015**. Fungal community shifts in structure and function across a boreal forest fire chronosequence. *Applied and Environmental Microbiology* **81**: 7869–7880.

Sýkorová Z, Rydlová J, Slavíková R, Ness T, Kohout P, Püschel D. 2016. Forest reclamation of fly ash deposit: A field study on appraisal of mycorrhizal inoculation. *Restoration Ecology*, **24**(2): 184–193.

Talbot JM, Bruns TD, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Peay KG. **2013**. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry* **57**: 282–291.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S,

Vylgalis R, Liao HL, Smith ME, Peay KG. **2014**. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences*, **111**(17): 6341–6346.

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S. **2009**. Soil organic carbon pools in the northern circumpolar permafrost region. *Global Biogeochemical Cycles* **23**: doi:10.1029/2008gb003327.

Taylor SW, Carroll AL, Alfaro RI, Safranyik L. **2006**. *Forest, climate and mountain pine beetle outbreak dynamics in western Canada* (L. Safranyik and W.R. Wilson, Ed.). Victoria BC: Canadian Forest Service, Pacific Forestry Centre, 67–94.

Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H. **2009**. Ascomycetes associated with ectomycorrhizas: Molecular diversity and ecology with particular reference to the Helotiales. *Environmental Microbiology* **11**: 3166–3178.

Tedersoo L, Bahram M, Toots M, Diédhiou AG, Henkel TW, Kjoller R, Morris MH, Nara K, Nouhra E, Peay KG, et al. 2012. Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology* **21**: 4160–4170.

Tedersoo L, Bahram M, Põlme S, Kõljalg U, Yorou NS, Wijesundera R, Ruiz LV, Vasco-Palacios AM, Thu PQ, Suija A, et al. 2014. Global diversity and geography of soil fungi. *Science* **346**: 1256688–1256688.

Teste FP, Schmidt MG, Berch SM, Bulmer C, & Egger KN. 2004. Effects of ectomycorrhizal inoculants on survival and growth of interior Douglas-fir seedlings on reforestation sites and partially rehabilitated landings. *Canadian Journal of Forest Research*, **34**(10): 2074–2088.

Teste FP, Lieffers VJ, Landhäuser SM. **2011**. Seed release in serotinous lodgepole pine forests after mountain pine beetle outbreak. *Ecological Applications* **21**: 150–162.

Tong X, Leung MHY, Wilkins D, Cheung HHL, Lee PKH. **2019**. Neutral processes drive seasonal sssembly of the skin mycobiome. *mSystems* **4**: e00004-19.

Treseder KK, Allen MF. **2000**. Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. *New Phytologist* **147**: 189–200.

Treseder KK, Mack MC, Cross A. **2004**. Relationships among fires, fungi, and soil dynamics in Alaskan boreal forests. *Ecological Applications* **14**: 1826–1838.

Treseder KK, Lennon JT. **2015**. Fungal traits that drive ecosystem dynamics on land. *Microbiology and Molecular Biology Reviews* **79**: 243–262.

Treu R, Karst J, Randall M, Pec GJ, Cigan P, Simard S, Cooke J, Erbilgin N, Cahill JFJ. **2014**. Decline of ectomycorrhizal fungi following a mountain pine beetle epidemic. *Ecology* **95**: 1096–1103.

Trivedi P, Singh BP, Singh BK. **2018**. Soil carbon: Introduction, importance, status, threat, and mitigation. In: *Soil Carbon Storage: Modulators, Mechanisms and Modeling*. Elsevier, 1–28.

Turner MG, Romme WH, Gardner RH. **1999**. Prefire heterogeneity, fire severity, and early postfire plant reestablishment in subalpine forests of Yellowstone National Park, Wyoming. *International Journal of Wildland Fire* **9**: 21-36.

Turner MG, Braziunas KH, Hansen WD, Harvey BJ. 2019. Short-interval severe fire erodes the resilience of subalpine lodgepole pine forests. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(23): 11319–11328.

Twieg BD, Durall DM, Simard SW. **2007**. Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist* **176**: 437–447.

Vahter T, Bueno CG, Davison J, Herodes K, Hiiesalu I, Kasari-Toussaint L, Oja J, Olsson

PA, Sepp SK, Zobel M, et al. 2020. Co-introduction of native mycorrhizal fungi and plant seeds accelerates restoration of post-mining landscapes. *Journal of Applied Ecology*, **57**(9):1741–1751.

Vellend M. **2010**. Conceptual synthesis in community ecology. *Quarterly Review of Biology* **85**: 183–206.

Vellend M. **2016**. *The Theory of Ecological Communities*. Princeton,USA: Princeton University Press.

Venkataraman A, Bassis CM, Beck JM, Young VB, Curtis JL, Huffnagle GB, Schmidt TM. **2015**. Application of a neutral community model to assess structuring of the human lung microbiome. *mBio* **6**: e02284-14.

Verbruggen E, Van Der Heijden MGA, Weedon JT, Kowalchuk GA, Rö-Ling WFM. **2012**. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Molecular Ecology* **21**: 2341–2353.

Visser S. 1995. Ectomycorrhizal fungal succession in jack pine stands following wildfire. *New Phytologist*, **129**(3): 389–401.

Volney WJA, Fleming RA. **2000**. Climate change and impacts of boreal forest insects. *Agriculture, Ecosystems and Environment* **82**: 283–294.

Walker JKM, Ward V, Jones MD. 2016. Ectomycorrhizal fungal exoenzyme activity differs on spruce seedlings planted in forests versus clearcuts. *Trees*, **30**(2): 497–508. doi: 10.1007/s00468-015-1239-7

Wallander H, Göransson H, Rosengren U. **2004**. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. *Oecologia* **139**: 89–97.

Wallander H, Ekblad A. **2015**. The Importance of Ectomycorrhizal Networks for Nutrient Retention and Carbon Sequestration in Forest Ecosystems. In: Horton T., ed. *Mycorrhizal Networks. Ecological Studies (Analysis and Synthesis)*. Springer, Dordrecht, 69–90.

Wang X, Parisien MA, Taylor SW, Candau JN, Stralberg D, Marshall GA, Little JM, Flannigan MD. **2017**. Projected changes in daily fire spread across Canada over the next century. *Environmental Research Letters* **12**: 25005.

Wang YL, Gao C, Chen L, Ji NN, Wu BW, Lü PP, Li XC, Qian X, Maitra P, Babalola BJ, et al. 2020. Community assembly of endophytic fungi in ectomycorrhizae of Betulaceae plants at a regional scale. *Frontiers in Microbiology* **10**: 3105. doi: 10.3389/fmicb.2019.03105

Wennekes PL, Rosindell J, Etienne RS. **2012**. The neutral-niche debate: a philosophical perspective. *Acta Biotheoretica* **60**: 257–271.

Wheeler NC, Guries RP. **1982**. Biogeography of lodgepole pine. *Canadian Journal of Botany* **60**: 1805–1814.

White T, Bruns T, Lee T. **1990**. *PCR Protocols: a guide to methods and applications*. New York, USA: Academic Press.

White JA, Tallaksen J, Charvat I. 2008. The effects of arbuscular mycorrhizal fungal inoculation at a roadside prairie restoration site. *Mycologia*, **100**(1): 6–11.

Whittaker RH. **1960**. Vegetation of the Siskiyou Mountains, Oregon and California. *Ecological Monographs* **30**: 279–338.

Wilhelm RC, Cardenas E, Maas KR, Leung H, Mcneil L, Berch S, Chapman W, Hope G, Wotton BM, Nock CA, Flannigan MD. **2010**. Forest fire occurrence and climate change in Canada. *International Journal of Wildland Fire* **19**: 253-271.

Wotton BM, Flannigan MD, Marshall GA. **2017**. Potential climate change impacts on fire intensity and key wildfire suppression thresholds in Canada. *Environmental Research Letters* **12**: 095003.

Wotton BM, Nock CA, Flannigan MD. **2010**. Forest fire occurrence and climate change in Canada. *International Journal of Wildland Fire* **19**: 253–271.

Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM. 2016. Soil inoculation steers restoration of terrestrial ecosystems. *Nature Plants*, **2**(8): 16107.

Xiang X, Gibbons SM, Yang J, Kong J, Sun R, Chu H. **2015**. Arbuscular mycorrhizal fungal communities show low resistance and high resilience to wildfire disturbance. *Plant and Soil* **397**: 347–356.

Zhang X, Johnston ER, Liu W, Li L, Han X. **2016**. Environmental changes affect the assembly of soil bacterial community primarily by mediating stochastic processes. *Global Change Biology* **22**: 198–207.

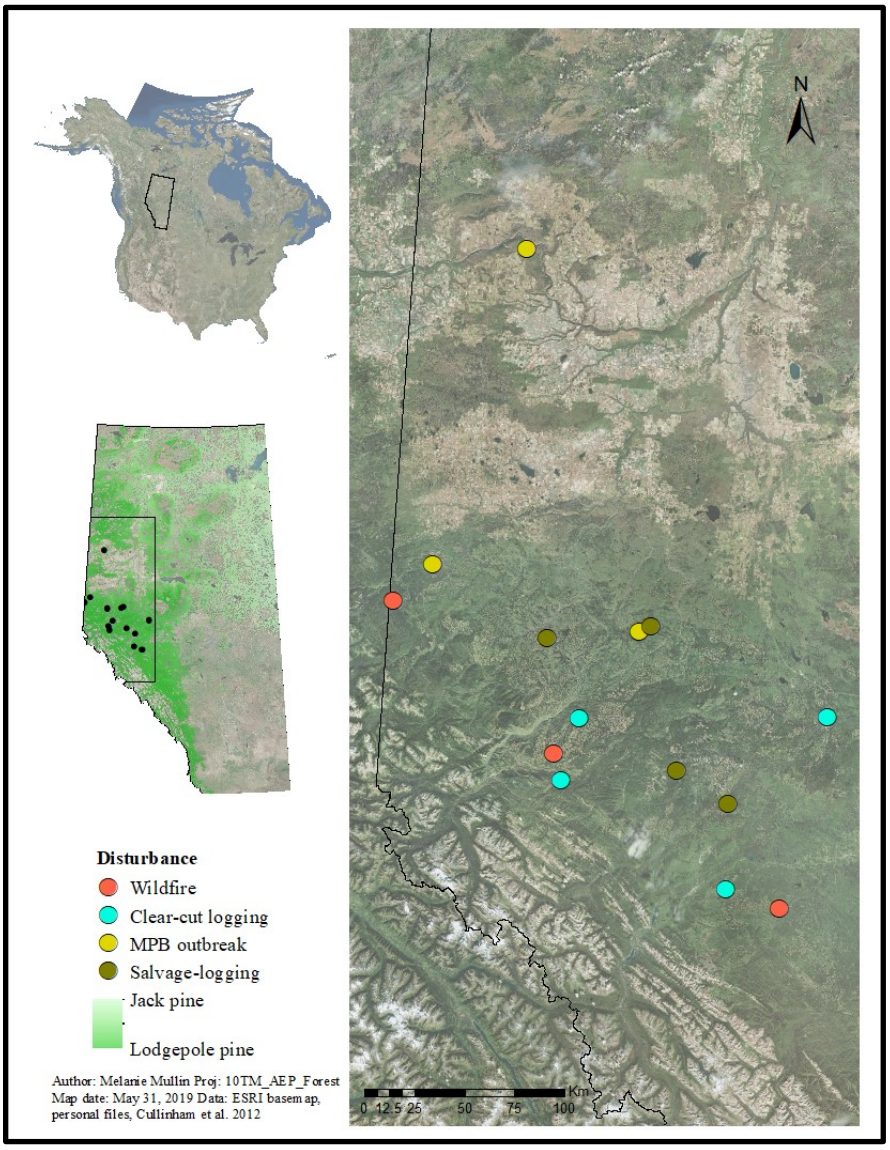
Zhao S, Erbilgin N. 2019. Larger resin ducts are linked to the survival of lodgepole pine trees during mountain pine beetle outbreak. *Frontiers in Plant Science*, **10**: 1459.

Zhou J, Ning D. 2017. Stochastic community assembly: does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews* **8**(4): e00002-17.

Zscheischler J, Westra S, van den Hurk BJJM, Seneviratne SI, Ward PJ, Pitman A, AghaKouchak A, Bresch DN, Leonard M, Wahl T, et al. **2018**. Future climate risk from compound events. *Nature Climate Change* **8**: 469–477.

Appendix

Appendix 2.1 Map of the sampled disturbed sites in west-central Alberta, Canada. For each disturbed site, a control site located within 10 km was also sampled. Wildfire (n = 3, red), clear-cut logging (n = 4, light blue), MPB outbreak (n = 3, yellow), salvage-logging (n = 4, green). MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

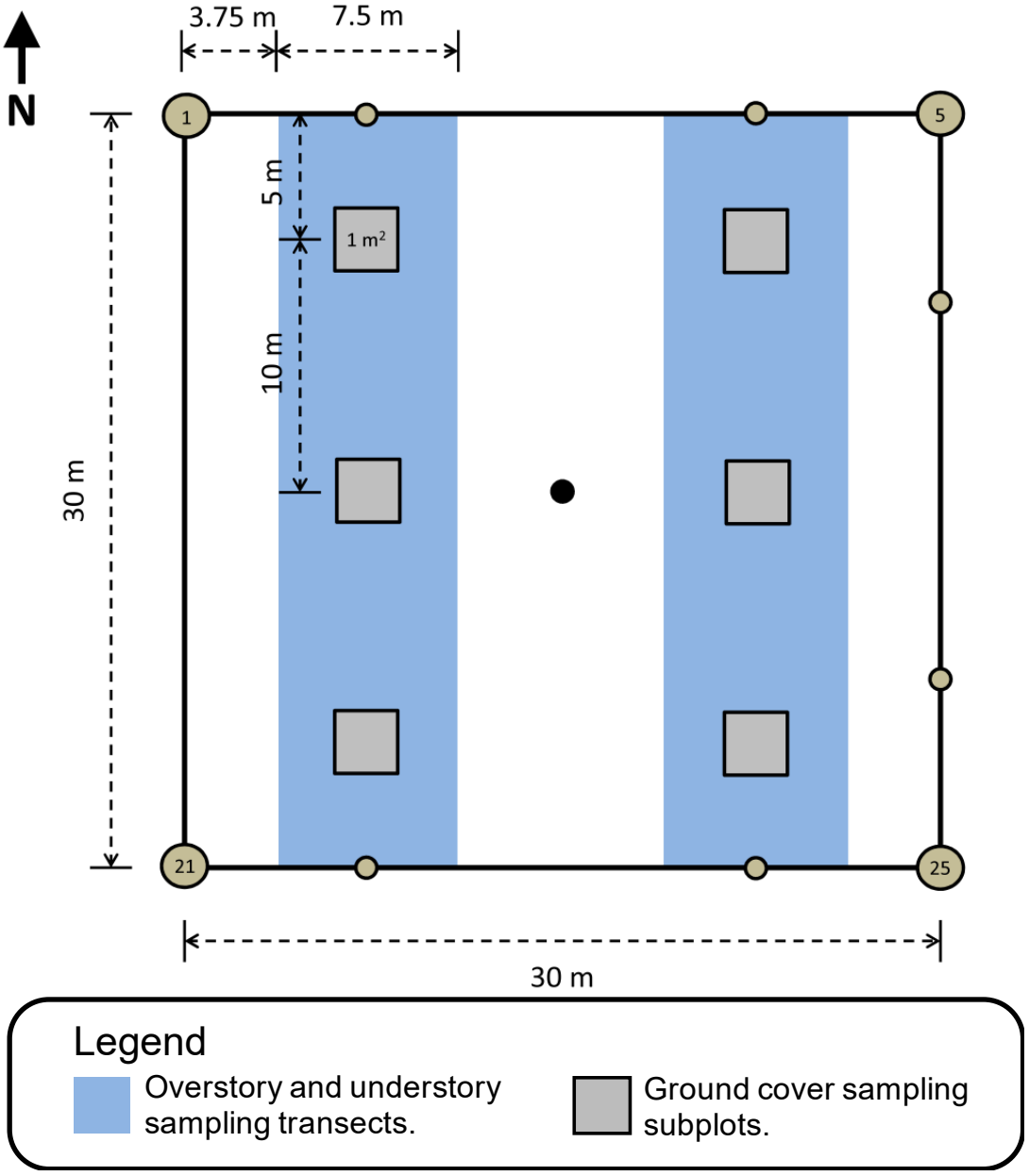


Appendix 2.2 Data from Alberta Vegetation Inventory used to select matched control sites for each disturbed site. The data below represent the state of control sites followed by their disturbed pair, prior to the disturbance event. Composition indicates the canopy composition (%) of lodgepole pine (*Pinus contorta*) as well as the composition (%) of other species (black spruce (*Picea mariana*), white spruce (*Picea glauca*), and trembling aspen (*Populus tremuloides*)). Clear-cut logging (CCL); Salvage-logging (SL); MPB: Mountain pine beetle (*Dendroctonus ponderosae*) outbreak.

Site	Disturbance	Year	Canopy closure (%)	Height (m)	Lodgepole pine composition (%)	Composition (%) of other species	Composition (%) of other species
G16-5c	Control		6-30	17	100		
G16-5	MPB	2010	6-30	19	100		
G16-6c	Control		31-50	16	90	10- *P. mariana*	
G16-6	Wildfire	2014	51-70	14	90	10- *P. mariana*	
G16-1c	Control		51-70	22	80	10- *P. glauca*	10- *P. tremuloides*
G16-1	SL	2013	51-70	25	70	20- *P. glauca*	10- *P. tremuloides*
G15-8c	Control		71-100	19	70	30- *P. tremuloides*	
G15-8	MPB	2010	51-70	21	70	30- *P. tremuloides*	
G15-1c	Control		51-70	16	100		
G15-1	MPB	2010	31-50	21	100		
G15-2c	Control		51-70	21	90	10- *P. tremuloides*	
G15-2	SL	2011	51-70	21	80	20- *P. tremuloides*	
W14-1c	Control		51-70	26	100		
W14-1	CCL	2011	51-70	26	100		
G15-6c	Control		71-100	22	100		
G15-6	CCL	2011	71-100	22	100		
E8-7c	Control		6-30	16	70	20- *P. mariana*	10- *P. glauca*
E8-7	Wildfire	2014	6-30	16	70	20- *P. mariana*	10- *P. glauca*
E8-3c	Control		51-70	17	100		
E8-3	CCL	2013	51-70	15	100		

E14-4c	Control		51-70	23	100	
E14-4	SL	2010	51-70	23	100	
E14-2c	Control		51-70	20	100	
E14-2	SL	2015	51-70	22	100	
E14-6c	Control		51-70	22	100	
E14-6	CCL	2010	51-70	21	100	
E14-8c	Control		51-70	21	90	10- *P. tremuloides*
E14-8	Wildfire	2009	51-70	22	90	10- *P. tremuloides*

Appendix 2.3 Sampling design of experimental sites consisted of a 900 m^2 plot with a 25-point grid sampling.



Appendix 2.4 Forest site characteristics in control and disturbed sites. Data represents mean ± SE for each treatment. Mean forest over and understory values calculated from two 7.5 m × 30 m transect, and groundcover values from three 1 m × 1 m subplots along each 7.5 m × 30 m transect. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

	Control	MPB outbreak	Clear-cut logging	Salvage-logging	Wildfire
Forest overstory					
Live lodgepole pine basal area (m^2 ha^{-1})	285.4 ± 24.28	146.8 ± 65.41	0.1 ± 0.06	0.3 ± 0.22	8.7 ± 8.75
Forest					
Non-conifer individuals	18.7 ± 6.55	0.0 ± 0.00	13.3 ± 8.10	10.5 ± 6.70	0.3 ± 0.33
Conifer individuals	3.0 ± 2.35	1.0 ± 1.00	0.8 ± 0.48	1.3 ± 1.25	0.0 ± 0.00
Ground cover					
Shrubs	11.4 ± 2.23	22.3 ± 8.91	18.6 ± 4.23	13.4 ± 7.27	9.6 ± 8.58
Grass	1.4 ± 0.62	50.7 ± 26.82	20.8 ± 8.06	21.2 ± 9.87	0.1 ± 0.05
Forbs	19.2 ± 4.86	11.9 ± 5.75	10.8 ± 6.44	21.7 ± 7.27	22.6 ± 10.51
Moss	46.1 ± 8.01	38.7 ± 19.28	5.0 ± 1.95	0.5 ± 0.23	16.8 ± 5.27
Woody	11.7 ± 2.01	8.5 ± 3.88	17.8 ± 5.25	13.8 ± 5.00	20.8 ± 10.34
Leaf litter	22.5 ± 5.44	4.1 ± 1.63	11.5 ± 4.14	17.3 ± 11.63	4.4 ± 4.44
Exposed soil	0.0 ± 0.01	0.1 ± 0.06	13.3 ± 3.76	2.7 ± 1.75	35.0 ± 18.28

Appendix 2.5 Depth of the soil organic layer, ergosterol concentration, and KCl extractable nutrient concentration in control and disturbed forests. Depth and ergosterol concentration data represent mean ± SE for each site; nutrient and pH represent the value of five pooled and homogenized soil samples per site. Clear-cut logging (CCL); Salvage-logging (SL); Mountain pine beetle (*Dendroctonus ponderosae*) outbreak (MPB).

Site ID	Disturbance	Depth (cm)	Ergosterol concentration ($\mu g\ g^{-1}$)	PO_4 (mg kg^{-1})	NH_4 (mg kg^{-1})	NO_3 (mg kg^{-1})	pH
E14-2C	Control	4.3 ± 1.19	2.4 ±0.25	20.12	5.76	0.96	4.70
E14-4C	Control	4.3 ± 0.20	5.4 ± 0.86	21.44	2.88	0.67	4.92
E14-6C	Control	5.4 ± 0.34	7.4± 3.14	14.36	7.87	0.76	5.39
E14-8C	Control	3.2 ± 0.17	2.2 ± 0.31	18.87	7.88	0.69	5.07
E8-3C	Control	4.7 ± 0.18	3.4 ± 0.32	20.01	2.48	0.61	4.64
E8-7C	Control	4.4 ± 0.19	3.2 ± 0.96	17.92	2.22	0.66	4.23
G15-1C	Control	8.3 ± 0.39	9.7 ± 1.84	25.84	4.04	1.18	4.20
G15-2C	Control	6.7 ± 0.37	8.6 ± 4.61	10.44	4.57	1.67	4.78
G15-6C	Control	4.4 ± 0.23	5.8 ± 2.96	23.55	5.57	0.84	4.55
G15-8C	Control	8.0 ± 0.40	10.7 ± 2.47	18.16	10.1	1.94	5.59
G16-1C	Control	4.0 ± 0.24	8.3 ± 1.66	8.17	2.36	0.74	4.60
G16-5C	Control	3.5 ± 0.18	4.1 ± 1.06	19.79	2.34	0.86	5.19
G16-6C	Control	4.3 ± 0.35	5.3 ± 1.07	9.29	13.96	8.62	5.33
W14-1C	Control	3.7 ± 0.22	2.7 ± 1.14	7.51	3.33	1.34	4.96
E14-8	Wildfire	2.2 ± 0.17	2.0 ± 0.25	17.72	5.5	1.97	5.24
E8-7	Wildfire	3.0 ± 0.32	1.2 ± 0.20	33.76	6.07	0.81	4.61
G16-6	Wildfire	3.1 ± 0.27	1.0 ± 0.20	22.89	8.72	1.03	4.84
E14-6	CCL	3.4 ± 0.25	4.8 ± 1.15	12.35	13.65	13.51	5.33
E8-3	CCL	2.7 ± 0.19	2.3 ± 0.38	18.8	2.25	0.69	4.74
G15-6	CCL	0.7 ± 0.20	0.7 ± 0.20	26	4.42	0.72	4.60
W14-1	CCL	1.2 ± 0.20	3.4 ± 1.42	29.37	2.15	2.05	5.34
G15-1	MPB	7.5 ± 0.79	11.7 ± 3.29	57.03	1.94	1.45	4.07
G15-8	MPB	5.8 ± 0.36	9.5 ± 1.82	9	6.61	1.70	5.73
G16-5	MPB	4.1 ± 0.19	3.6 ± 0.54	33.33	2.77	0.78	4.77
E14-2	SL	3.3 ± 0.38	1.5 ± 0.41	6.76	7.43	3.47	4.82
E14-4	SL	2.6 ± 0.27	3.1 ± 0.77	14.65	4.85	0.81	4.75
G15-2	SL	5.7 ± 0.49	3.5 ± 0.73	7.79	3.03	1.42	4.71
G16-1	SL	3.1 ± 0.39	4.6 ± 0.71	7.69	7.92	0.93	4.99

Appendix 2.6 Summary of the fitted linear mixed effects model on the soil fungal biomass in control and disturbed forests. MPB: Mountain pine beetle, σ^2: level-one variance, $\tau_{00\ ID}$: level-two variance, ICC: intraclass Correlation Coefficient, and $N_{\ ID}$: number of groups.

	Ergosterol concentration	
Predictors	*Estimates*	*P-value*
(Intercept)	0.67	<0.001
Wildfire	-0.56	<0.001
Clear-cut logging	-0.36	0.005
MPB outbreak	0.17	0.241
Salvage-logging	-0.23	0.076
Random effects		
σ^2	0.06	
$\tau_{00\ ID}$	0.04	
ICC	0.40	
$N_{\ ID}$	28	
Observations	112	
Marginal R^2 / Conditional R^2	0.338 / 0.605	

Appendix 2.7 Results from post-hoc test with Tukey's adjustments for multiple comparisons on differences in soil fungal biomass in control and disturbed forests, following linear mixed effects model test. Values represent *P*-values. Bold text represents results deemed significant. Mountain pine beetle (MPB).

Disturbances	Control	Wildfire	Clear-cut logging	MPB outbreak
Wildfire	**0.001**	-	-	-
Clear-cut logging	**0.038**	0.72	-	-
MPB outbreak	0.723	**0.001**	**0.019**	-
Salvage -logging	0.309	0.309	0.724	0.131

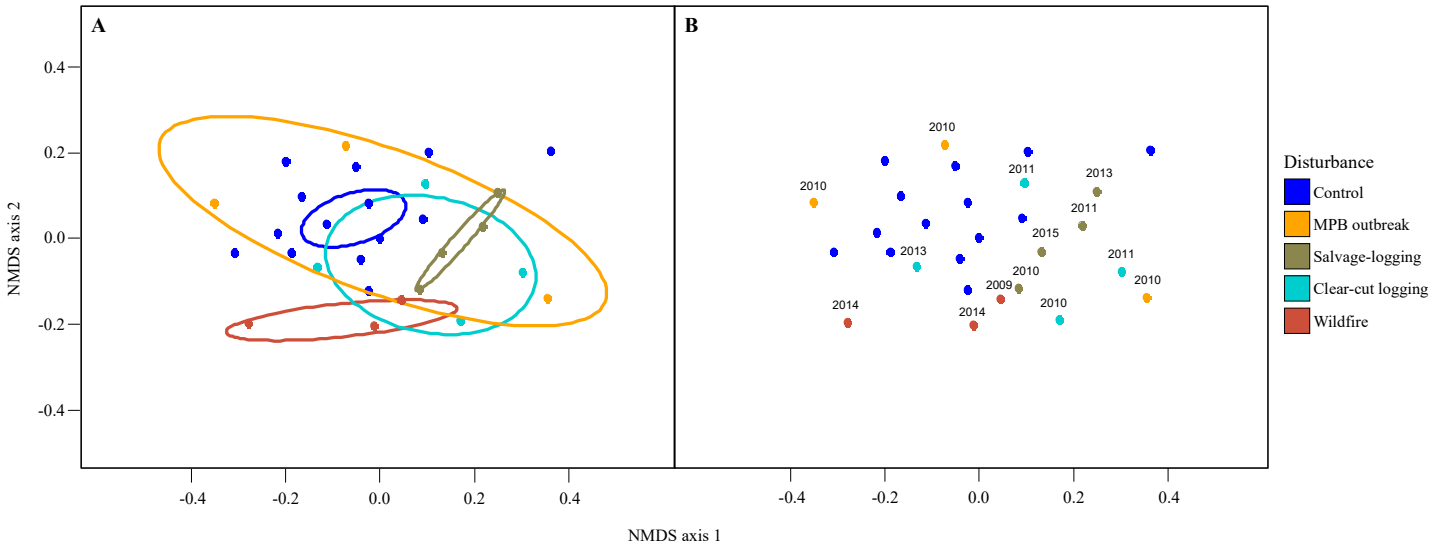
Appendix 2.8 Results from perMANOVA tests on the community composition of fungal guilds with disturbance as predictor variable. Degrees of freedom (*df*), sum of squares (*Sum Sq*), mean squares (*MeanSq*). Bold text represents results deemed significant.

Statistical values	*df*	*Sum Sq*	*MeanSq*	*F-value*	R^2	*P-value*
Whole community (all guilds)						
Disturbance	4	0.902	0.22549	1.431	0.19925	**0.01**
Residuals	23	3.625	0.15760		0.80075	
Total	27	4.527			1.00	
Ectomycorrhizal fungi						
Disturbance	4	1.873	0.468	2.291	0.251	**< 0.001**
pH	3	1.336	0.445	2.179	0.179	**< 0.001**
Dist:pH	34	0.997	0.249	1.220	0.133	
Residual	16	3.269	0.204		0.437	
Total	27	7.474			1.000	
Saprotrophic fungi						
Disturbance	4	1.011	0.253	1.288	0.183	0.08
Residual	23	4.513	0.196		0.817	
Total	27	5.524			1.000	
Pathogenic fungi						
Disturbance	4	1.647	0.412	1.354	0.19159	**0.04**
Residual	23	6.995	0.304		0.809	
Total	27	8.642			1.000	
Arbuscular mycorrhizal fungi						
Disturbance	4	0.211	0.053	1.178	0.170	0.32
Residual	23	1.029	0.045		0.830	
Total	27	1.240			1.000	

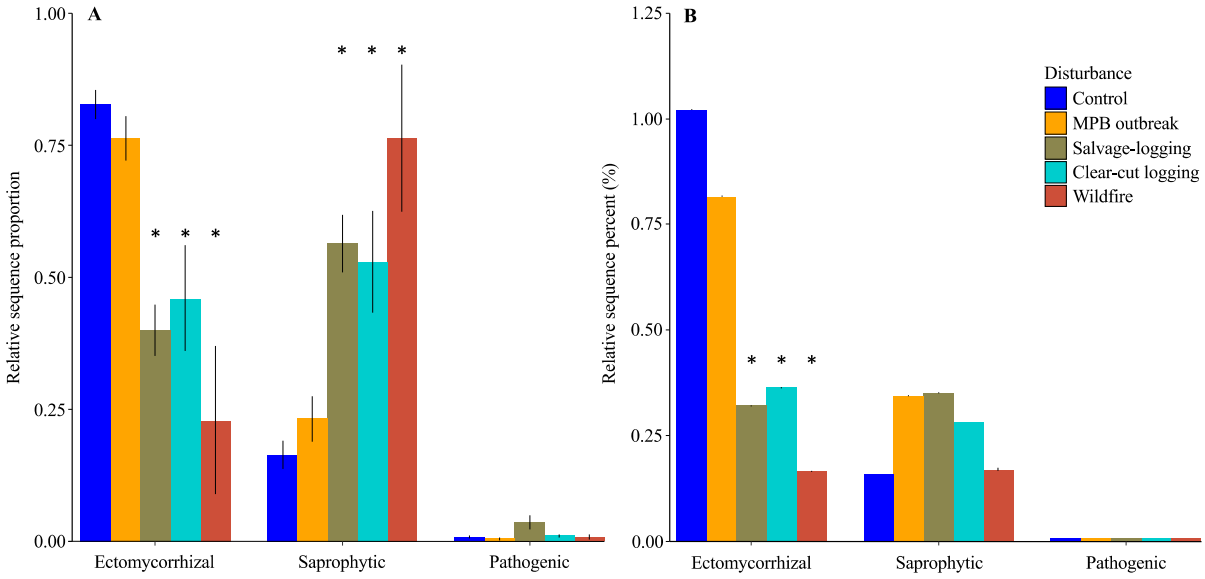
Appendix 2.9 Results from post-hoc test with Holm adjustments for multiple comparisons on community composition of guilds, following perMANOVA tests. Values represent *P-values*. Bold text represents results deemed significant. Mountain pine beetle (MPB).

Disturbance	Control	Wildfire	Clear-cut logging	MPB outbreak
		Whole community		
Wildfire	**0.03**	-	-	-
Clear-cut logging	0.22	1.00	-	-
MPB outbreak	1.00	1.00	1.00	-
Salvage -logging	**0.03**	0.40	1.00	1.00
		Ectomycorrhizal fungi		
Wildfire	**0.03**	-	-	-
Clear-cut logging	**0.03**	1.00	-	-
MPB outbreak	1.00	1.00	1.00	-
Salvage -logging	**0.02**	0.20	1.00	1.00
		Saprotrophic fungi		
Wildfire	0.66	-	-	-
Clear-cut logging	1.00	1.00	-	-
MPB outbreak	1.00	1.00	1.00	-
Salvage -logging	0.29	0.29	1.00	1.00
		Pathogenic fungi		
Wildfire	**0.05**	-	-	-
Clear-cut logging	1.00	1.00	-	-
MPB outbreak	1.00	0.80	1.00	-
Salvage -logging	0.81	0.32	1.00	1.00
		Arbuscular mycorrhizal fungi		
Wildfire	1.00	-	-	-
Clear-cut logging	0.22	1.00	-	-
MPB outbreak	1.00	1.00	1.00	-
Salvage -logging	1.00	1.00	1.00	1.00

Appendix 2.10 Non-metric multidimensional scaling plots of the effect of forest disturbances whole community composition (all fungal guilds) with and without year of disturbance (A, B, respectively). Dots represent individual sites and ellipses 95% confidence intervals. MPB: mountain pine beetle (*Dendroctonus ponderosae*).



Appendix 2.11 Relative sequence proportion of guilds characterized using ITS1, only using sequence reads from the same guilds (A), and percentage of sequences in each guild based on overall ITS1 sequence count (B). Data are mean ± SE (n = 14 for control, n = 4 for salvage-logging and clear-cut logging, and n = 3 for wildfire and MPB outbreak). Asterisks indicate significant differences from controls at $\alpha = 0.1$ from Kruskal-Wallis test of ranks followed by Tukey's HSD. In figure A, $\chi^2_4 = 19.75$, $P < 0.001$ in ectomycorrhizal, $\chi^2_4 = 19.93$, $P < 0.001$ for saprotrophic, and $\chi^2_4 = 7.90$, $P = 0.095$ for pathogenic fungi. In figure B, $\chi^2_4 = 19.75$, $P < 0.001$ in ectomycorrhizal, $\chi^2_4 = 19.08$, $P < 0.001$ for saprotrophic, and $\chi^2_4 = 11.61$, $P = 0.021$ for pathogenic fungi $\chi^2_4 = 6.25$, $P = 0.182$. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

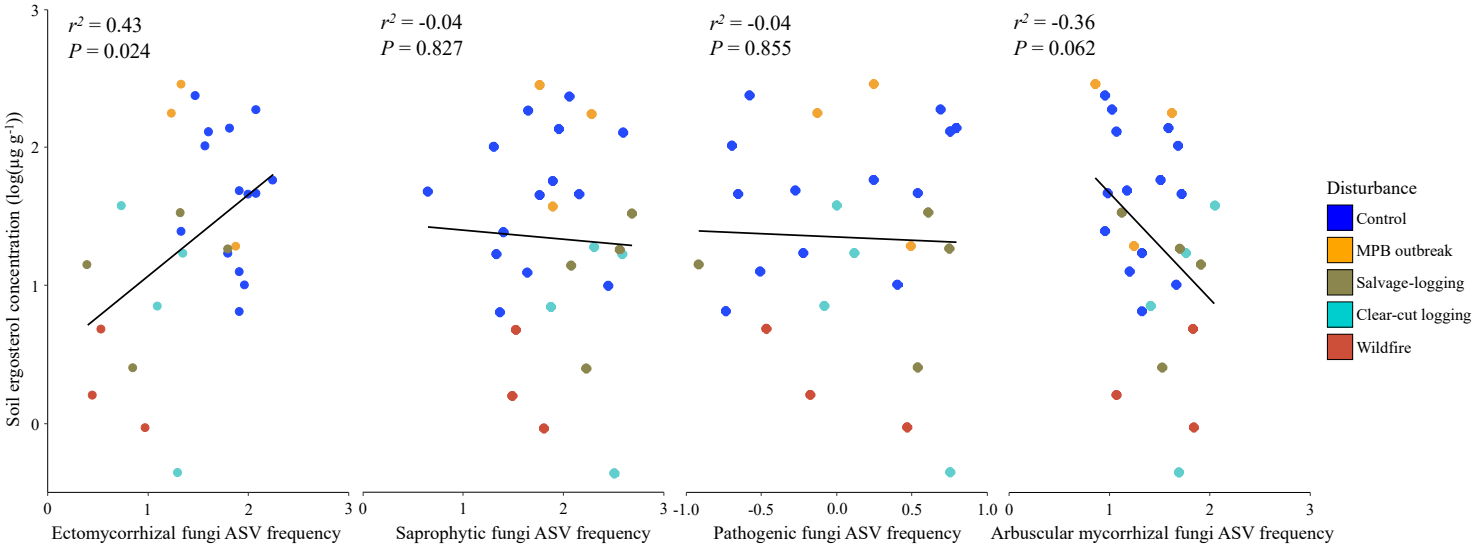


Appendix 2.12 Results from statistical tests on the proportion or frequency of guilds and effective number of species data, followed by post-hoc test with Tukey's adjustment for multiple comparisons. Proportion and frequency of guilds were tested with Kruskal-Wallis test of ranks and effective number of species for each of the guilds was tested with one-way ANOVA. Values represent *P*-values. Bold text represents results deemed significant. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

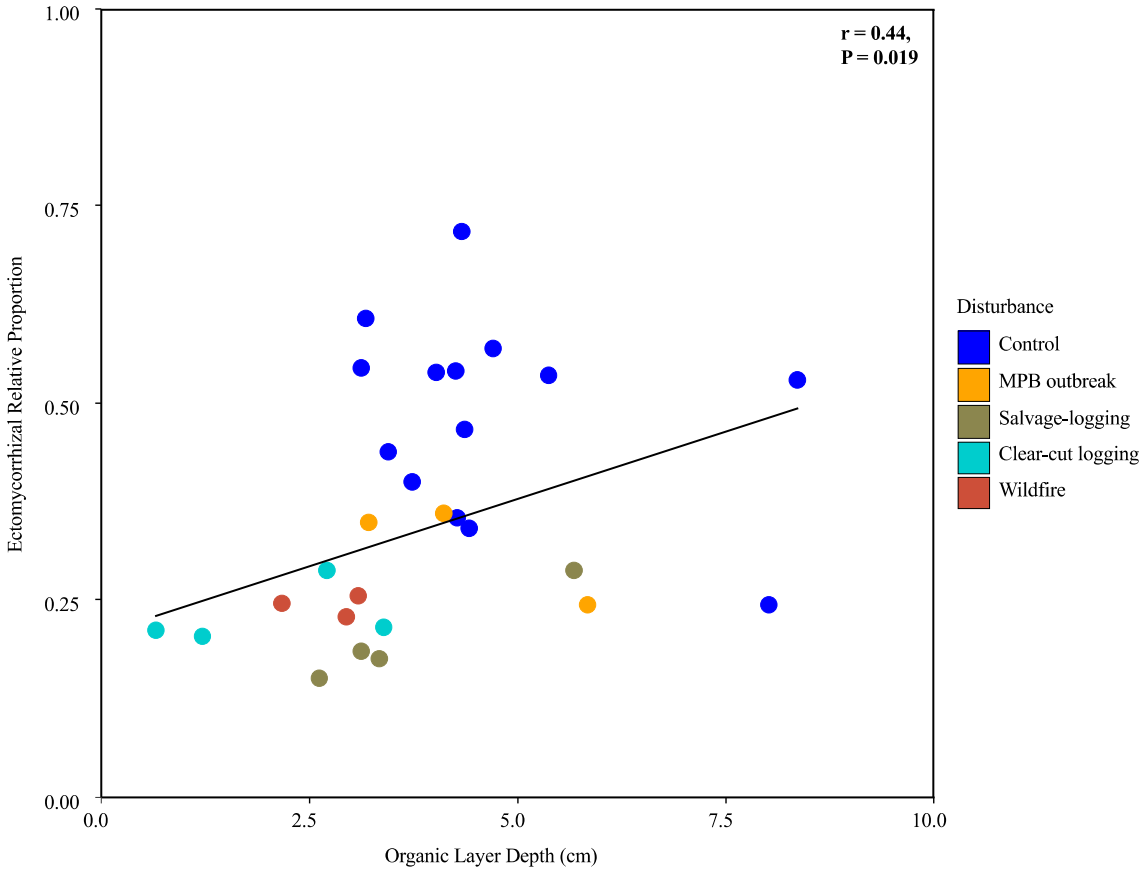
Disturbance	Control	Wildfire	Clear-cut logging	MPB outbreak
Ectomycorrhizal fungi proportion (χ^2_4=18.63, $P < 0.001$)				
Wildfire	**0.02**	-	-	-
Clear-cut logging	**0.07**	1.00	-	-
MPB outbreak	0.33	0.60	0.60	-
Salvage -logging	**0.01**	1.00	1.00	0.60
Saprotrophic fungi proportion (χ^2_4=17.45, $P = 0.002$)				
Wildfire	0.17	-	-	-
Clear-cut logging	0.01	1.00	-	-
MPB outbreak	0.47	1.00	1.00	-
Salvage -logging	0.02	1.00	1.00	0.69
Pathogenic fungi proportion (χ^2_4=4.78, $P = 0.310$)				
Wildfire	0.68	-	-	-
Clear-cut logging	1.00	1.00	-	-
MPB outbreak	1.00	1.00	1.00	-
Salvage -logging	1.00	1.00	1.00	1.00
Arbuscular mycorrhizal fungi proportion (χ^2_4=8.02, $P = 0.091$)				
Wildfire	**0.06**	-	-	-
Clear-cut logging	1.00	1.00	-	-
MPB outbreak	1.00	0.90	1.00	-
Salvage -logging	1.00	1.00	1.00	1.00
Ectomycorrhizal fungi diversity ($F_{4,23}$=6.245, $P = 0.001$)				
Wildfire	**< 0.01**	-	-	-

Clear-cut logging	**0.07**	0.46	-	-
MPB outbreak	0.44	0.23	0.97	-
Salvage -logging	0.23	0.24	0.99	1.00
Saprotrophic fungi diversity ($F_{4,23}$=3.584, P = 0.021)				
Wildfire	0.89	-	-	-
Clear-cut logging	0.74	0.48	-	-
MPB outbreak	1.00	0.86	0.97	-
Salvage -logging	**0.02**	**0.03**	0.45	0.22
Pathogenic fungi diversity ($F_{4,23}$= 0.463, P = 0.762)				
Wildfire	0.64	-	-	-
Clear-cut logging	1.00	0.73	-	-
MPB outbreak	0.49	1.00	0.61	-
Salvage -logging	0.65	0.22	0.85	0.16
Arbuscular mycorrhizal fungi diversity ($F_{4,23}$=1.98, P = 0.132)				
Wildfire	0.82	-	-	-
Clear-cut logging	0.53	1.00	-	-
MPB outbreak	0.51	0.26	0.12	-
Salvage -logging	0.91	1.00	0.98	0.31

Appendix 2.13 Response of soil fungal biomass to the frequency of ectomycorrhizal, saprotrophic, pathogenic, and arbuscular mycorrhizal fungal frequency. Results from Pearson's correlation of log-transformed frequencies and ergosterol concentration. ASV: Amplicon sequence variant; MPB: Mountain pine beetle (*Dendroctonus ponderosae*).



Appendix 2.14 Relationship between relative proportion of ectomycorrhizal fungi and the depth of the soil organic layer in control and disturbed sites. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).



Appendix 2.15 Frequency of genera in disturbances and their paired control sites used for Fig. 6. Data represent mean ± SE for each disturbance. Clear-cut logging (CCL); Salvage-logging (SL); MPB: Mountain pine beetle (*Dendroctonus ponderosae*) outbreak.

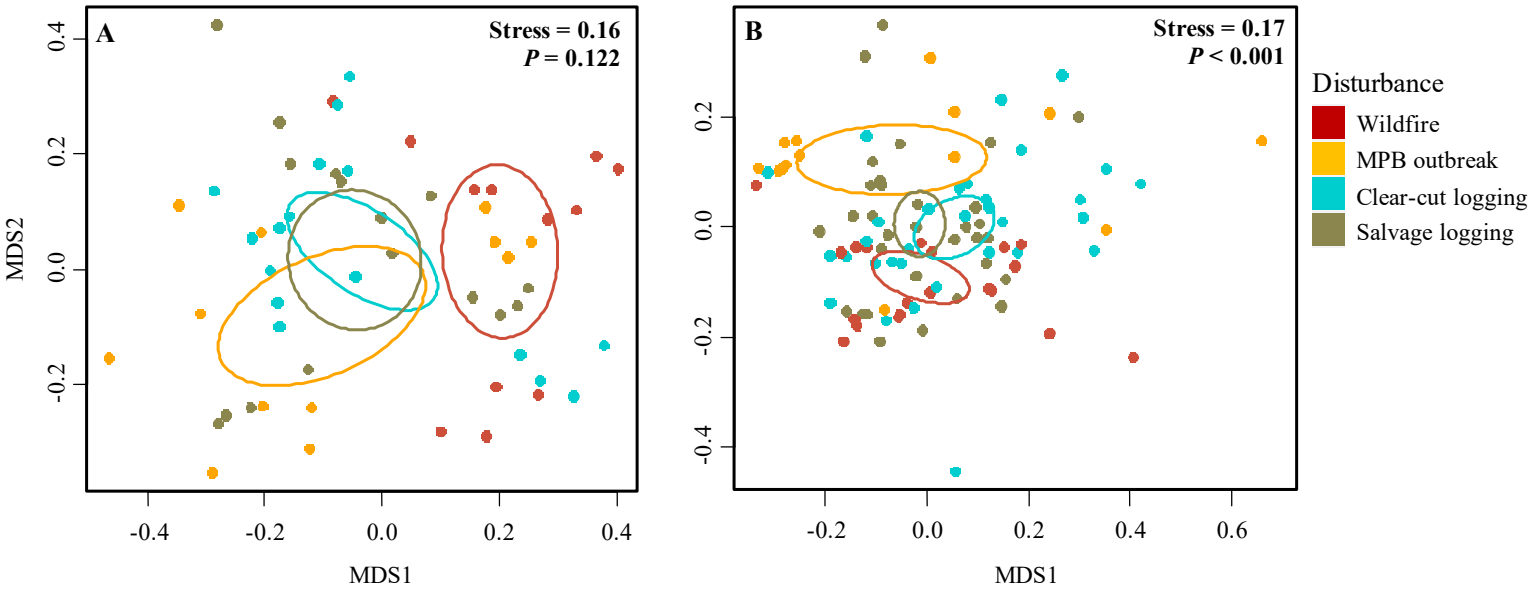
Taxa (genus)	Wildfire	Wildfire-Control	SL	SL-Control	CCL	CCL-Control	MPB	MPB-Control
Hygrophorus	0.1 ± 0.04	0.2 ± 0.09	0.0 ± 0.02	0.1 ± 0.04	0.1 ± 0.01	0.2 ± 0.06	0.1 ± 0.05	0.2 ± 0.12
Russula	0.2 ± 0.18	0.8 ± 0.51	0.8 ± 0.34	1.2 ± 0.33	0.4 ± 0.11	0.9 ± 0.12	1.0 ± 0.37	1.2 ± 0.22
Tomentella	0.2 ± 0.13	0.5 ± 0.43	0.5 ± 0.19	1.2 ± 0.35	0.5 ± 0.11	0.5 ± 0.17	0.7 ± 0.34	0.5 ± 0.09
Lactarius	0.0 ± 0.01	0.2 ± 0.09	0.1 ± 0.03	0.4 ± 0.04	0.0 ± 0.03	0.3 ± 0.21	0.1 ± 0.09	0.3 ± 0.19
Cortinarius	0.1 ± 0.01	0.6 ± 0.08	0.1 ± 0.04	0.5 ± 0.15	0.2 ± 0.07	0.5 ± 0.10	0.4 ± 0.17	0.8 ± 0.32
Piloderma	0.2 ± 0.04	1.7 ± 0.05	0.1 ± 0.04	1.4 ± 0.12	0.4 ± 0.15	1.4 ± 0.16	0.8 ± 0.40	1.2 ± 0.48
Tuber	0.0 ± 0.01	0.0 ± 0.01	0.1 ± 0.05	0.0 ± 0.02	0.1 ± 0.12	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.01
Tricholoma	0.0 ± 0.03	0.0 ± 0.01	0.1 ± 0.05	0.1 ± 0.04	0.0 ± 0.01	0.0 ± 0.00	0.3 ± 0.28	0.3 ± 0.10
Cladophialaphora	0.2 ± 0.07	0.2 ± 0.06	0.7 ± 0.10	0.2 ± 0.09	0.7 ± 0.17	0.2 ± 0.08	0.5 ± 0.17	0.9 ± 0.01
Mortierella	1.4 ± 0.27	1.1 ± 0.34	2.7 ± 0.46	1.5 ± 0.49	2.3 ± 0.75	1.7 ± 0.59	2.6 ± 0.70	2.2 ± 1.03
Clavaria	0.4 ± 0.14	0.3 ± 0.13	0.5 ± 0.22	0.2 ± 0.08	0.6 ± 0.12	0.4 ± 0.24	0.2 ± 0.07	0.1 ± 0.02
Xenopolyscytelum	0.1 ± 0.09	0.0 ± 0.00	0.4 ± 0.20	0.1 ± 0.06	0.4 ± 0.23	0.1 ± 0.08	0.3 ± 0.23	0.3 ± 0.23
Fayodia	0.3 ± 0.09	0.0 ± 0.03	0.3 ± 0.06	0.0 ± 0.01	0.1 ± 0.08	0.1 ± 0.02	0.0 ± 0.04	0.1 ± 0.03
Hyphodontiella	0.0 ± 0.00	0.0 ± 0.01	0.1 ± 0.08	0.0 ± 0.02	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.08
Gymnopus	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.01	0.1 ± 0.01	0.0 ± 0.01	0.1 ± 0.03	0.1 ± 0.04	0.1 ± 0.03
Archaeorhizomyces	0.0 ± 0.00	0.0 ± 0.01	0.1 ± 0.06	0.2 ± 0.13	0.0 ± 0.01	0.0 ± 0.02	0.0 ± 0.00	0.0 ± 0.03
Mycena	0.1 ± 0.06	0.3 ± 0.10	0.6 ± 0.20	0.4 ± 0.14	0.4 ± 0.05	0.4 ± 0.08	0.5 ± 0.23	0.4 ± 0.22
Galerina	0.1 ± 0.06	0.1 ± 0.03	0.1 ± 0.02	0.0 ± 0.00	0.1 ± 0.03	0.1 ± 0.03	0.0 ± 0.01	0.1 ± 0.04
Venturia	0.4 ± 0.00	0.1 ± 0.12	0.1 ± 0.06	0.1 ± 0.01	0.1 ± 0.05	0.1 ± 0.04	0.0 ± 0.01	0.1 ± 0.03
Chalara	0.2 ± 0.00	0.1 ± 0.10	0.2 ± 0.09	0.1 ± 0.06	0.1 ± 0.06	0.1 ± 0.08	0.0 ± 0.01	0.1 ± 0.01
Clarodieglomus	0.8 ± 0.44	0.3 ± 0.12	0.7 ± 0.27	0.4 ± 0.15	0.7 ± 0.24	0.5 ± 0.10	0.5 ± 0.32	0.3 ± 0.25

Glomus	1.2 ± 0.54	0.6 ± 0.31	1.2 ± 0.27	0.8 ± 0.17	1.6 ± 0.40	1.4 ± 0.28	0.5 ± 0.30	0.7 ± 0.44
Archaeospora	0.1 ± 0.04	0.0 ± 0.00	0.1 ± 0.04	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.00	0.0 ± 0.03	0.0 ± 0.00
Diversispora	0.1 ± 0.06	0.0 ± 0.01	0.1 ± 0.04	0.0 ± 0.01	0.3 ± 0.18	0.0 ± 0.01	0.0 ± 0.03	0.1 ± 0.14
Ambiospora	0.4 ± 0.18	0.2 ± 0.01	0.2 ± 0.10	0.1 ± 0.03	0.3 ± 0.18	0.3 ± 0.05	0.1 ± 0.07	0.1 ± 0.04

Appendix 3.1 Results from linear mixed effects model tests on the seedling metrics with disturbance and soil transfer volumes as predictor variable. Sum of squares (*Sum Sq*), mean squares (*MeanSq*), numerator and denominator degrees of freedom (*NumDF* and *DenDF*).

Statistical values	*Sum Sq*	*Mean Sq*	*NumDF*	*DenDF*	*F-value*	*P-value*
Seedling germination						
Disturbance	0.55318	0.18439	3	11.22	0.9446	0.45168
Soil transfer volume	0.03439	0.03439	3	35.00	0.1762	0.67722
Disturbance:Volume	2.45099	0.81700	9	35.00	4.1855	0.01241
Seedling establishment						
Disturbance	0.0061199	0.00203997	3	14.726	0.1852	0.9048
Soil transfer volume	0.0005266	0.00052660	3	35.000	0.0478	0.8282
Disturbance:Volume	0.0020242	0.00067472	9	35.000	0.0612	0.9798
Seedling biomass 1 yr old						
Disturbance	3.3464	1.11547	3	8.592	3.0689	0.0864352
Soil transfer volume	2.2682	0.75607	3	282.218	2.0801	0.1030263
Disturbance:Volume	12.1046	1.34495	9	282.321	3.7003	0.0002101
Seedling shoot length 1 yr old						
Disturbance	0.57468	0.19156	3	8.92	2.8625	0.0970942
Soil transfer density	0.49050	0.16350	3	281.62	2.4432	0.0643849
Disturbance:Volume	2.14265	0.23807	9	281.66	3.5575	0.0003336
Seedling biomass 2 yr old						
Disturbance	1.7869	0.59564	3	9.089	1.4704	0.286373
Soil transfer volume	1.2105	0.40349	3	180.681	0.9960	0.395981
Disturbance:Volume	10.3406	1.14895	9	180.636	2.8363	0.003806
Seedling shoot length 2 yr old						
Disturbance	0.27158	0.090528	3	9.03	1.3574	0.31645
Soil transfer volume	0.58749	0.195829	3	180.54	2.9363	0.03473
Disturbance:Volume	0.99129	0.110144	9	180.50	1.6515	0.10385

Appendix 3.2 Non-metric multidimensional scaling plot using Bray-Curtis distance of soil (A), and root (B) saprotrophic fungal communities. Points represent individual samples colored by disturbance type, and ellipses standard errors. Significant differences in communities at $\alpha = 0.05$ was tested using perMANOVA. MPB: mountain pine beetle (*Dendroctonus ponderosae*).



Appendix 3.3 Results from perMANOVA tests on the community composition of fungal guilds with disturbance as predictor variable. Degrees of freedom (*df*), sum of squares (*Sum Sq*), mean squares (*MeanSq*). Bold text represents results deemed significant.

Statistical values	*df*	*Sum Sq*	*MeanSq*	*F-value*	R^2	*P-value*
Total soil community						
Disturbance	3	4.401	1.46702	10.4242	0.12496	**< 0.001**
Soil transfer volume	3	0.637	0.21220	0.66269	0.01808	0.983
Site	10	16.813	1.68130	11.9468	0.47737	**< 0.001**
Residuals	95	13.370	0.14073		0.37959	
Total	111	35.220			1.00	
Total root community						
Disturbance	3	5.978	1.99280	11.8549	0.17001	**< 0.001**
Soil transfer volume	3	0.800	0.26679	0.76812	0.02275	0.823
Site	10	13.763	1.52918	9.0969	0.39136	**< 0.001**
Residuals	87	14.625	0.16810		0.41587	
Total	111	35.220			1.00	
Soil ectomycorrhizal fungi						
Disturbance	3	2.0350	0.67834	2.0065	0.08147	**< 0.001**
Soil transfer volume	3	1.2190	0.40632	1.2019	0.04880	0.105
Site	10	8.5398	0.85398	2.5260	0.34188	**< 0.001**
Residuals	95	13.1848	0.33807		0.52784	
Total	111	24.9786			1.00	
Root-associated ectomycorrhizal fungi						
Disturbance	3	6.544	2.18134	8.1960	0.15545	**< 0.001**
Soil transfer volume	3	1.407	0.46885	1.1404	0.03373	0.217
Site	10	11.259	1.25098	4.7003	0.26744	**< 0.001**
Residuals	86	22.889	0.26615		0.54370	
Total	101	42.098			1.00	

Appendix 3.4 Indicator ectomycorrhizal fungal amplicon sequence variants (ASVs) associated with roots of lodgepole pine (*Pinus contorta*) seedling growing in the different disturbed forests. MPB: mountain pine beetle (*Dendroctonus ponderosae*).

Wildfire	Indicator value
Tomentella badia	0.65
Sphaerosporella brunnea	0.52
Mallocybe unidentified	0.55
Tomentella unidentified	0.50
Clavulina cinerea	0.46
Clear-cut logging	
Wilcoxina rehmii	0.61
MPB outbreak	
Russula caerulea	0.71
Russula unidentified	0.70
Suillus glandulosipes	0.61
Tylospora unidentified	0.61
Russula densifolia	0.56
Lactarius unidentified	0.50
Inocybe leptophylla	0.50
Lactarius helvus	0.44
Tylospora unidentified.1	0.43
Tylospora fibrillosa	0.43
Cortinarius casimiri	0.35
Hygrophorus unidentified	0.35
Tricholoma equestre	0.35
Hygrophorus hypothejus	0.35

Appendix 4.1 Alpha diversity accumulation curve of fungal amplicon sequence variants (ASVs) in total soil and root samples.