Changes in soil fungal communities following logging and salvage logging disturbances decrease lodgepole pine (*Pinus contorta* var. *latifolia*) seedling performance

by

Jackson L. Beck

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

Master of Science

in

Forest Biology and Management

Department of Renewable Resources

University of Alberta

ABSTRACT

Disturbances are frequent events across the Canadian boreal forests and can affect both below and above ground ecosystem processes at various temporal and spatial scales. We have limited understanding of how changes in the below ground fungal communities affect above ground plant communities. Such understanding has become increasingly important in-light-of observed changes in frequency and severity of disturbances due to climate change. Thus, we investigated how soil inoculum collected from the four common disturbances (fire, mountain pine beetle outbreak, logging, and salvage logging) in lodgepole pine stands in Alberta affect pine seedling performance in the greenhouse. We asked, (1) whether fungal communities of lodgepole pine seedling roots change when seedlings were grown in pots inoculated with soil from one of the disturbed (fire, mountain pine beetle outbreak, logging, or salvage-logging) and paired control sites and (2) whether changes in fungal community composition have cascading impacts on seedling performance (below and above ground biomass, height, and survival). We found that the root fungal communities of logged and salvage-logged treatments differed from their paired controls while fire and beetle outbreak treatments did not. We also found significant variations on the root fungal communities among disturbance treatments. The most prominent difference was between burned and salvaged-logged sites. In addition, we found that these changes to the root associated fungal community resulted in decreased seedling performance both when comparing logging and salvage-logging treatments to their paired control sites and when comparing among all disturbance treatments (fire to salvage logging treatments). Our findings indicate that soil fungi may mediate negative impacts of anthropogenic disturbance (logging and salvage logging)on seedling growth. Additionally, these impacts may not be analogous to the soil fungi mediated response of seedlings following natural disturbances (wildfire and beetle outbreaks). Furthermore, seedlings inoculated with soil from salvage logged sites had reduced

performance when compared to fire disturbed sites. Land managers should consider that salvage-logging may have negative indirect impacts on seedlings when planning salvage harvests. Additional work is needed to investigate the soil fungi mediated long-term impacts of salvage logging on the regeneration of seedlings.

PREFACE

This document addresses an ecological study as outlined in an NSERC-Strategic Project Grant proposal. The objective of the ecological study outlined in the proposal was to elucidate the impact of individual and compound disturbances on soil fungal communities and highlight the functional role of soil fungi in seedling regeneration. The lead author of this proposal was Nadir Erbilgin (University of Alberta; U of A). The proposal was awarded to principle investigators, Nadir Erbilgin (U of A), Justine D. Karst (U of A), James F. Cahill Jr. (U of A), and Suzanne W. Simard (University of British Columbia). The findings of this ecological study are intended for publication as well as integration with other studies outlined in the proposal.

The results presented in this thesis are the findings of a greenhouse study where we investigate four common disturbances in lodgepole pine stands (fire, MPB outbreak, logging, and salvage logging) to determine the soil fungi mediated impacts of these disturbances on seedling performance.

Jonathan A. Cale and Jean Rodriguez-Ramos collected field soils used as fungal inoculum. Jonathan A. Cale assisted in the set-up of the greenhouse experiment as well as provided statistics and editing assistance. Jean Rodriguez-Ramos provided molecular biology assistance. Jackson L. Beck collected and processed all data sets, performed all statistics, and generated all the figures and tables. Nadir Erbilgin, Justine D. Karst and James F. Cahill provided statistics and editing assistance. Nadir Erbilgin was the academic primary supervisor to Jackson L. Beck.

To Laura

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Nadir Erbilgin for his help and encouragement as well as his sound advice though the highs and lows throughout my graduate experience. I would also like to thank my Committee members, Drs. JC Cahill and Justine Karst, for their guidance and support during my time at the University of Alberta.

Thank you to Dr. Charles Nock for taking the time to read and comment on my thesis. I would also like to thank our collaborator at the University of British Columbia Dr. Suzanne W. Simard.

Special thanks to postdoctoral research fellow Dr. Jon Cale for fielding innumerable questions as well as his advice and encouragement throughout my time at the University of Alberta. Thank you to the entire Erbilgin lab both current and former members for their conversation, advice and help throughout my graduate experience. I would also like to thank the research assistants; Ashley Kocsis, Ashley Hynes, Sanat Kanekar, Laura Brunner for their help in the greenhouse and processing seedling samples.

Thank you to Steven Williams and Adam Tomaszewski at the Department of Biological Sciences Greenhouse, University of Alberta, for their help and advice. Thank you to the MBSU staff for their assistance in the processing and sequencing of my fungal samples.

Thank you to the following industry partners for providing support for my project: Alberta Agriculture and Forestry, Canadian Forest Products, Ltd., Mikro-Tek Inc., ANC Timber, Ltd., Millar Western Forest Products, Ltd., Sundre Forest Products Inc., West Fraser, Weyerhaeuser, fRI Research and the Alberta Tree Improvement & Seed Centre.

Thank you to Drs. Nilesh Timilsina (University of Wisconsin-Stevens point; UWSP) and Holly Petrillo (UWSP) as well as Paul Cigan (Wisconsin DNR) for inspiring me to pursue graduate studies while an undergraduate student at UWSP.

Thank you to my family (Mom, Dad, Sarah Kate, Nate, and Katie), my in-laws (Tom, Lynn, and Allison) and my friends for their support throughout my time at the University of Alberta.

Last but certainly not least, thank you to my wife Laura for your patience, grace and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES CONTINUED

LIST OF FIGURES

LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
ESV	Exact sequence variant
ITS	Internal transcribed spacer
MBSU	Molecular biological sciences facility
MPB	Mountain pine beetle
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
QIIME2	Quantitative insights into microbial ecology 2
Tukey HSD	Tukey honest significant difference

Introduction

Natural and anthropogenic disturbances can alter the composition, function and structure of an ecosystem (White & Pickett 1985). Understanding the impact of forest disturbances has become increasingly important in-light-of potential changes to forest disturbance regimes (especially frequency and severity) in the future (Flannigan et al. 2005; Wotton et al. 2010; Price et al. 2013; Seidl et al. 2017). Furthermore, to address challenges from altered disturbance regimes associated with climate change, studies have suggested management strategies like salvage-logging that result in compound disturbances (Boucher et al. 2018). Since organisms are usually best suited to natural disturbance regimes that they have evolved with (Bergeron et al. 1999; Lindenmayer & Noss 2006), researchers have expressed concerns that multiple disturbances in short time (compound disturbance) may result in novel species assemblages, which have the potential to reduce the resilience of an ecosystem (Paine et al. 1998; Beshta et al. 2004; Lindenmayer & Noss 2006; Peterson & Leach 2008a; Buma 2015).

Lodgepole pine (*Pinus contorta*) is one of the most widely distributed pine species in western North America and, as a result, lodgepole pine forests are impacted by a wide variety of forest disturbances (Anderson 2003). Disturbances such as wildfire, bark beetle outbreaks, clear-cut logging, and salvage-logging are some of the most common disturbance types in lodgepole pine ecosystems throughout its natural range (Romme & Knight 1981; Peet 2000; Anderson 2003). Wildfire plays an important role in the life history of lodgepole pine as both an agent of mortality and regeneration (Lothan et al. 1985; Lamont 1991; Agee 1996; Keeley 2012; Tabacaru et al. 2016). Mountain pine beetle (*Dendroctonus ponderosae* Hopkins; hereafter MPB) also disturbs lodgepole pine forests, and while MPB outbreaks have occurred throughout most of the range of lodgepole pine, recent MPB range expansion into naïve lodgepole pine

stands in western Alberta are unprecedented (Cudmore et al. 2010). Given the economic value of lodgepole pine throughout its range and in western Alberta, clear-cut logging is a frequent anthropogenic disturbance and common silvicultural technique employed in lodgepole pine forests (Smithers 1961; Alexander et al. 1985; Cahalan 1985; Murphy et al. 1999; FAO 2016). Unlike the aforementioned disturbances, salvage-logging can sometimes represent a compound disturbance as it removes beetle-killed trees following MPB outbreak. Salvage-logging is commonly used to capture the timber value in MPB killed stands, protect watersheds, reduce fuel loads and, as a silvicultural technique, to create seedbeds more suitable to pine regeneration (Mitchell 2005; McIntosh & Macdonald 2013).

Studies have largely focused on how these disturbances affect ecosystem function or above ground plant communities (Thom & Seidl 2016; Thorn et al. 2018), though increasingly attention is being given to soil microbial communities (Bruns 1995; Goldman et al 2015; Karst et al. 2015; Pec et al. 2017). Fungi are a particularly important component of the soil microbial community as changes to the soil fungal community following a disturbance can be a critical mechanism of altered forest function. Soil fungi alter host plant nutrient and water acquisition (Miller et al. 1998; Despain et al. 2001; Smith and Read 2008) and/or contribute to ecological functions like carbon and nutrient cycling (Dighton 2003; Shah et al. 2016). Furthermore, disturbances such as wildfire, MPB outbreak, clear-cut logging and salvage-logging can impact the soil fungal community. Earlier work has demonstrated that MPB outbreak (Treu et al. 2014; Karst et al. 2015; Pec et al. 2017), wildfire (Cairney & Bastias 2007; Buscardo et al. 2011, Reazin et al. 2016; Taudière et al. 2017), clear-cut logging (Jones et al. 2003; Lazaruk et al. 2005; Kohout et al. 2018), and salvage-logging (Jennings et al. 2012; Kutorga et al. 2012; Ford et al. 2018) can all potentially alter the soil fungal communities of conifer forest stands.

Not only do soil fungal communities change with disturbance but, the biotic and abiotic filters that drive differences in soil fungal communities may change with the type of disturbance (Bruns 1995; Barker et al. 2013; Šturcová et al. 2014; Goldman et al. 2015; Ashley et al. 2016). Environmental conditions important to soil fungi such as soil pH, moisture, nutrients, phenolics and host plant composition can differ with disturbance, potentially resulting in differences in soil fungal community composition (Durall et al. 2005; Kutorga et al. 2012; Goldman et al. 2015; Kennedy et al. 2015; Ashley et al. 2016; Pec et al. 2017; Taudière et al. 2017). For example, wildfire and MPB outbreaks may filter the soil fungal community by changing the aboveground vegetation, however the removal of aboveground vegetation associated with wildfire may impose a different biotic filter on the soil fungal community when compared to the filter associated with changes in host plant composition following MPB outbreak (Buscardo et al. 2011; Treu et al. 2014; Taudière et al. 2017; Pec et al. 2017). Such changes may be of particular importance to lodgepole pine forests as not only are these disturbances common, but soil fungi are important components of these pine forests (Simard & Durall 2004; Smith et al. 2009; Karst et al. 2014; 2015) Furthermore, disturbance-induced shifts in the soil fungal community may be important as earlier work has demonstrated that such shifts to the soil fungal community can have cascading impacts on the next generation of trees (Karst et al. 2015). Lastly, since soil fungi may respond differently to diverse disturbance types, changes in soil fungi may result in different cascading impacts on the next generation of seedlings such as decreased seedling performance following one disturbance, relative to another.

To better understand the roles of forest disturbances and specifically the role of soil fungi in mediating the response of seedlings following a forest disturbance, we investigate if variation in seedling root fungal communities associated with different forest disturbances impact

lodgepole pine seedling performance. Specifically, we first investigate how seedling root-associated fungal communities differ when grown in pots inoculated with soil from different disturbed sites in the greenhouse. While soil inoculum from disturbed sites will contain organisms such as fungi, bacteria, nematodes, insects, and mites, given the importance of soil fungi to the success of seedling regeneration, we will investigate soil fungi. We hypothesize that seedlings will respond to disturbance altered fungal communities and changes to the soil fungal community will result in decreased seedling performance. Second, we investigate if seedlings perform differently when grown in pots inoculated with soil from disturbed stands. In addition to elucidating the soil fungi mediated impact of different disturbances on seedling performance, our findings may also have important management implications given the importance of the disturbances investigated in this study to lodgepole pine.

Materials and Methods

1 Experimental design and soil inoculation in the greenhouse

We investigate soil fungi mediated impacts of different disturbances on lodgepole pine seedling performance by conducting a greenhouse bioassay in which seedlings were grown in pots inoculated with small amounts of soil from one of the four types of disturbed pine forests paired with control sites. 'Disturbed' refers to seedlings grown in pots inoculated with soil from forests that have been recently disturbed by either fire, MPB outbreak, logging, or salvage-logging; 'paired control' refers to lodgepole pine seedlings grown in pots inoculated with soil from forests with no recent disturbance history; and 'non-inoculated control' refers to lodgepole pine seedlings grown in pots without field soil inoculum. Inoculating pots with field soil is standard

practice to investigate root-associated fungi in greenhouse experiments (Nunez et al. 2009; Pickles et al. 2015; Karst et al 2015).

1.1 Soil collection and greenhouse experiment set-up

1.1.1 Site selection and soil sampling

In the summer of 2016, soils in 14 lodgepole pine stands in west-central Alberta (54°17' N, -118°13'W; elevation 935 m) disturbed in the past six years by either clear-cut logging (n=4), wildfire (n=3), MPB (n=3), as well as MPB followed by salvage-logging (n=4) were sampled (Supplemental Fig. 1). All sites were mature stands dominated by lodgepole pine (>75% basal area) prior to disturbance. Those sites disturbed by MPB had a minimum of 50% pine basal area mortality. In addition, we identified a paired control lodgepole pine stand for each of the 14 disturbed sites using Alberta Vegetation Inventory data. All paired control stands had similar pre-disturbance characteristics such as height, stand composition, and percent crown closure when compared to their paired disturbed site (Supplemental Table 1). All control sites were located within 10 km of their paired disturbed sites. In total, 28 sites were sampled: 14 disturbed and 14 paired-control, spread over an 42,096 km^2 region of west central Alberta.

In each site we located 25 points using a 5 x 5-point grid, all points on the grid were spaced 7.5 m apart. At each point, a soil core (23 cm long with a 1.9 cm diameter) was collected using an Oakfield Apparatus Soil Probe (Oakfield Apparatus, Fond du Lac, WI, USA). Soil samples were transported on ice to the University of Alberta where they were stored at 4° C. The soil cores collected at each site were sifted with a 4 mm sieve to remove large particles such as gravel, rocks, etc. (Karst et al. 2015), and pooled within site, resulting in one soil source per site. Pooling was done to ensure that soils used in inoculation were representative of the fungal

community present at each site not that of a single microsite. Pooling samples within sites ensures consistent and representative inoculation for the sites sampled (Karst et al. 2015; Cahill et al. 2016) and this approach is consistent with any potential management implementation that could emerge. Lost is the ability to address questions of within-site variability (Reinhart & Rinella 2016; Cahill et al. 2017, and here we focus on larger scale impacts. Further, soil pooling increases statistical power (Cahill et al. 2017), something critical in the context of a study with potential for adoption as a forest industry practice.

1.1.2 Seed stock preparation

An earlier study suggested that genetic variation within lodgepole pine populations alters ectomycorrhizal colonization and growth of seedlings (Karst et al. 2008). We accounted for the genetic variation of lodgepole pine occurring in the study region by creating representative seedstock. Specifically, seed stock from seed zones corresponding with each of the 28 sites was pooled to create seedstock representative of the genetic variation across all the sites sampled (Supplemental Table 2). Furthermore, by using seed stock with genetic variation representative of the sites sampled, we increase the applicability of our work for land managers. Seed stock was provided by Smokey Lake Tree Nursery (Smokey Lake, Alberta). Pooled seed stock was stratified 30 days prior to sowing. Seeds were first surface sterilized with 5% (v/v) bleach for 15 minutes, rinsed in distilled water, then soaked in distilled water for 24 hours. Seeds were dried and placed in cold storage (4° C) for 28 days.

1.1.3 Potting soil preparation

We sterilized a bulk soil mix (75% sand, 25% topsoil) prior to planting, by autoclaving for 1 hour at 125° C and then autoclaving again 24 hours later under the same conditions. Each pot

was then filled with 350 ml of sterilized bulk soil to which 15 ml (4% total soil volume) of field soil collected from one of the 28 field sites was added to serve as soil inoculum. Field soil not only contained fungi but also contained other organisms such as bacteria, nematodes, insects, mites, etc. In this experiment we specifically investigate soil fungi given both the responsiveness of soil fungi to disturbance (Jones et al. 2003; Pec et al. 2017; Taudière et al. 2017; Ford et al. 2018; Kohout et al. 2018) and the importance of soil fungi to pine (Miller et al. 1998; Despain et al. 2001; Smith and Read 2008; Karst et al. 2014). However, it is possible that other soil organisms may also influence the performance of lodgepole pine seedlings in this experiment. In total, 420 field soil treated pots were planted, with 15 pots per site for each of the 28 sites. In addition, 27 non-inoculated pots contained 365 ml of autoclaved soil without field soil inoculum to control for greenhouse contaminants.

1.1.4 Greenhouse growth conditions

In total, we grew seedlings for 44 weeks: two 22 week-growth periods and a single eight-week dormancy period. During the two growth-periods, seedlings were grown in a greenhouse at 22-25° C under a natural light: dark regime supplemented with light when natural levels dropped below 12 hours aday^{-1}. We sowed five stratified seeds at a depth of 5 mm in each pot. After four weeks of growth, we thinned seedlings to one seedling per pot leaving only the most vigorous seedling (i.e. tallest and largest in size) in each pot (Karst et al. 2015; Kanekar et al. 2018). After this first 22 week growth period, we moved seedlings to a growth chamber to simulate winter conditions and induce dormancy. Dormancy was induced by gradually decreasing, holding and increasing the temperature of the growth chamber as in Kanekar et al 2018 (see supplemental information). Following dormancy, seedlings were returned to the greenhouse where they were grown as before for another 22 weeks. During the entirety of growth, seedlings were rotated

weekly to ensure that seedlings were exposed to equal light and temperature conditions in the greenhouse and growth chamber. Beginning five weeks post germination, seedlings were fertilized with 300 ppm phosphorus using a 10-52-10 (N:P:K) fertilizer mix and 18 ppm iron chelate every four weeks. Fertilizer was applied to avoid phosphorus and iron deficiencies that could be identified by the reddening and yellowing of seedling needles, respectively (Kanekar et al. 2018). Water acidified with phosphoric acid was administered to pots every two weeks to correct for basic tap water and maintain a soil pH in the pots below 6.5. At the end of the second growth period seedlings, 273 seedlings were harvested. Biomass and height was measured for all seedlings while 139 seedlings were used for molecular analysis (i.e., identification of root-associated fungi) and 134 seedlings were used for foliar nutrient analysis.

1.2 Molecular identification of root-associated fungi

We sampled the root systems of 139 harvested seedlings for molecular analysis (fire=16, logging=18, MPB=14, salvage=18, paired-controls=68, non-inoculated control=5). The roots of seedlings used for molecular analysis were carefully removed from potting soil and cut at the root collar. After sampling, root systems were stored on dry ice until they could be transported to -20° C freezer. Roots were then lyophilized using a Labconco freeze drier (Kansas City, MO, USA) for 72 hours at -45° C and weighed for mass. From the lyophilized roots, fine roots from each sample were selected and then cut into 1.5-2 cm fragments. Fine root fragments were evenly placed on a 2.5 cm^2 grid where we randomly sampled cells on the grid until 100 mg of root tissue has been sampled (Cowan et al. 2016; Pec et al. 2017). This sampled root tissue was then ground using TissueLyser II (Qiagen Inc., Mississauga, ON, Canada). We then twice extracted DNA from 100 mg of ground root samples using MP Biomedicals Fast spin kit for soil (MP Biomedicals, Solon, OH, USA) following manufacturer's protocols. Duplicate extractions

were then pooled for each sample (Deslippe et al. 2016). Extracted DNA was then quantified using ND-1000 Nanodrop (Thermo Fisher Scientific, MA, USA).

The ribosomal internal transcribed spacer region (ITS1) of nuclear DNA was amplified by two step polymerase chain reaction (PCR) using extracted genomic DNA as a template for the reactions. ITS1 region was selected given as it is commonly used in studies investigating soil fungal diversity and that this barcode yields similar results to ITS2 region barcodes (Blaalid et al. 2013; Smith & Peay 2014; Thompson et al. 2017). Specifically, an Illumina Nextera forward adapter and linker sequence added to the 5' end of ITS1-f primer was used as the forward primer given its ability to discriminate against plant DNA (Gardes & Bruns 1993) as well as Illumina Nextera reverse adapter and linker sequence to the 5' end of the ITS2 primer as the reverse primer were used to amplify ITS1 (Blaalid et al. 2013; Smith & Peay 2014; Thompson et al. 2017). The first PCR reaction consisted of 12.5 µl of Platinum™ SuperFi™ Green PCR Master Mix (Invitrogen, Carlsbad, California, USA), 1.25 µl of forward primer, 1.25 µl of reverse primer and 1 µl of DNA template. Thermocycler conditions for the first PCR reaction consisted of an initial 1 min denaturing cycle at 94° C followed by 35 cycles of 94° C for 30 sec, 52° C for 30 sec, 68° C for 30 sec and a single extension cycle of 68° C for 7 min (Blaalid et al. 2013; Thompson et al. 2017). PCR products were checked to ensure samples amplified successfully using 2% agarose gel electrophoresis with SYBR™ Safe DNA Gel Stain (Invitrogen) 100 V, 0.5 hr. and viewed using Gene Genius Bio imaging system (Syngene, Frederick, MD, USA). PCR products were then cleaned to remove primers and primer dimers using Mag-Bind TotalPure NGS Kit (Omega Bio-tek, Norcross, GA, USA) and checked using UV light and gel electrophoresis as above.

The second PCR reaction was conducted to barcode products from the first PCR reaction using primers from Nextera XT Index Kit v2 (Illumina, San Diego, California, USA). This reaction consisted of 17.5 µl of Platinum™ SuperFi™ Green PCR Master Mix, 2.5 µl of Illumina Nextera forward index primer, 2.5 µl of Illumina Nextera reverse index primer and 2.5 µl of DNA template. For both PCR runs, samples containing DES water (Invitrogen) instead of DNA template were run as negative controls. Thermocycler conditions for the second PCR reaction consisted of an initial 3 min denaturing cycle at 95° C followed by 8 cycles of 95° C for 30 sec, 55° C for 30 sec, 72° C for 30 sec and a single extension cycle of 72° C for 5 min (16S metagenomic sequencing library preparation). As before, PCR products were then cleaned to remove primers and primer dimers using Mag-Bind TotalPure NGS Kit (Omega Bio-tek). Cleaned second PCR products were quantified using Qubit 2.0 (Invitrogen, Carlsbad, CA, USA) and an Aligent 2100 bioanalyzer (Santa Clara, CA, USA) and 5 µl from each sample was pooled. The pooled library was submitted to the Molecular Biological Sciences Facility (MBSU) at the University of Alberta for sequencing. The amplicon library was sequence on an Illumina MiSeq sequencing platform using 2 x 300 bp paired-end reads with v3 chemistry (Illumina Inc., San Diego, CA, USA).

Bioinformatic analysis of Illumina paired-end reads was conducted using the "Quantitative insights into microbial ecology 2" (QIIME2 version 2018.6; https://qiime2.org/; Caporaso et al. 2010) pipeline using custom python scripts (Mckinney 2010). Raw sequence reads were demultiplexed with the Illumina Sequencing platform at the University of Alberta MBSU. Demultiplexed reads were checked for non-biological adapter and primer complements using FastQC (Andrews 2010). Primer complements and adapters were trimmed from reads using cutadapt plugin within the QIIME2 pipeline (Martin 2011; McDonald et al. 2012).

After trimming, sequences were filtered, dereplicated, sample inferences were made, chimeras were identified, and paired end reads were merged using default settings in the DADA2 workflow within QIIME2 (Callahan et al. 2016). First, low quality regions at the ends of the sequences were identified (average quality score dropped< 35) and trimmed within DADA2 to improve the combination rate when paired end reads were merged. Specifically, forward reads were trimmed to 286 reads and reverse reads were trimmed to 279 reads. Sequences were then filtered and exact sequence variants (ESVs) were resolved using the DADA2 inference algorithm (Callahan et al. 2016). After ESVs were resolved, paired-end reads were then merged, and chimeras removed using the DADA2 pipeline which implements a more sensitive method to remove chimeras for ESVs (Callahan et al. 2016).

ESV taxonomy was assigned using a Naïve-Bayes classier within the QIIME 2 feature-classifier plugin (Pedregosa et al. 2012, Bokulich et al. 2018). The Naïve-Bayes classifier was trained using dynamic reference sequences from the UNITE database (Abarenkov et al. 2010). Classified ESV feature table was then filtered to remove ESVs not identified to Kingdom Fungi as these ESVs could not be accurately assigned taxonomy (<70% confidence) using the UNITE database dynamic classifier.

1.3 Seedling performance measurements

We measured seedling response to different soil fungal inoculum treatments by measuring survival, height, biomass and foliar nutrient content.

We recorded seedling survival as the number of seedlings that survived dormancy and the two growth periods. Survival counts began after all pots in the experiment were thinned to one seedling per pot (four weeks post germination) and continued until seedlings were harvested.

When seedlings were harvested, we determined seedling height by measuring from the root collar to the tip of the apical meristem, and seedling biomass by measuring the dry and fresh weights of the above and below ground tissues. After seedling height measurements were taken, we carefully removed seedling roots from the soil, cut the seedlings at the root collar and weighed the above and below ground tissues separately. Seedlings used for nutrient analysis and the above ground biomass of seedlings used for molecular analysis were dried in an oven at 40° C for 96 hours until a constant weight was achieved (Massad et al. 2012). Whereas, the belowground biomass of seedlings used for molecular analysis was lyophilized for 72 hours and weighed.

The nutrient content of seedling foliar tissue was measured for a total of 134 seedlings (fire=13, logging=19, MPB=12, salvage=19, paired-control=66, non-inoculated control=5). Briefly, we ground dried foliar tissue using a TissueLyser II and submitted ground samples to the Natural Resources Analytics laboratory at the University of Alberta for total nitrogen and Ca, Cu, Fe, K, Mg, Mn, Zn, Na, S, P analysis. Foliar tissue total nitrogen content was analyzed by total nitrogen dry combustion method using a Thermo FLASH 2000 Organic Elemental Analyzer (Thermo Fisher Scientific Inc., Bremen, Germany). Foliar Ca, Cu, Fe, K, Mg, Mn, Zn, Na, S, P content was measured by coupled plasma-optical emission spectroscopy (ICP-OES) using Thermo iCAP6300 Duo inductively coupled plasma-optical emission spectrometer (Thermo Fisher Corp., Cambridge, UK; Skoog et al. 2007).

2 Data analysis

2.1 Overview

To investigate our two research objectives, we first tested if seedling root-associated fungal composition changed depending on disturbed vs paired control soil treatments and whether seedling root-associated fungal composition differs when comparing fire, MPB, logging, or salvage-logging disturbed soil treatments. Second, we analyze if seedling performance differs depending on disturbed vs paired control soil treatments and whether performance differs when comparing fire, MPB, logging, or salvage-logging disturbed soil treatments.

2.2 Root associated fungal community composition

Species accumulation curves for all samples were calculated within QIIME 2. These curves were used to identify a rarefication level, which represents a tradeoff between maximizing the number of samples and maximizing the taxon analyzed (Lekberg et al. 2018). We rarified samples to 343 sequences per sample based on distribution of samples with low numbers of sequences, as well as the curves generated in QIIME2 (Supplemental Fig. 2). At this level of rarefaction, a total of 14 samples of 134 samples were eliminated, including all five non-inoculated control seedlings. Samples were rarefied without replacement using the 'rrarefy' function in the Vegan package in R v3.5.1 (R Development Core Team, 2018).

All statistical analyses were conducted in R v3.5.1 (R Development Core Team, 2018). Bray-Curtis distance matrices were generated to identify differences in root fungal community composition first between individual disturbance treatments and their respective paired control treatments (control fire vs. fire, control MPB vs. MPB, etc.), then, among individual paired control inocula treatments (control fire vs. control logging vs. control MPB vs. control salvage)

and among individual disturbed inocula treatments (fire vs. logging vs. MPB vs. salvage) using PERMANOVA. PERMANOVA with 10,000 permutations and all other parameters set to default were performed with the Adonis function within the Vegan package in R. Pairwise comparisons of individual disturbed inoculum treatments and individual paired-control inoculum treatments were performed using pairwise PERMANOVAs with 10,000 permutations, Holm adjusted for multiple comparisons and all other parameters et to default with the pairwise.perm.manova function within the RVAideMemoire package version in R (Herve 2018).

We tested if fungal diversity metrics differed first between individual disturbance treatments and their respective paired control treatments (control fire vs. fire, control MPB vs. MPB, etc.), then, among individual paired control inocula treatments (control fire vs. control logging vs. control MPB vs. control salvage) and among individual disturbed inocula treatments (fire vs. logging vs. MPB vs. salvage). Fungal diversity metrics were tested with linear mixed models using the lmer function in the R lmerTest package (Kuznetsova et al. 2018) and the specific fungal diversity metrics tested were: log transformed richness as well as square root transformed Shannon's diversity and Simpsons diversity indices. Both diversity metrics were calculated in R with the Vegan package (Oksanen et al. 2018). Site and a greenhouse block was added to the model as random effects to account for variation due to site pooled soil inoculum and blocking within the greenhouse. Differences were considered to be significant at $\alpha = 0.1$.

2.3 Seedling performance

We tested whether seedling performance metrics differed first between individual disturbance treatments and their respective paired control treatments (control fire vs. fire, control MPB vs. MPB, etc.), then, among individual paired control inocula treatments (control fire vs. control logging vs. control MPB vs. control salvage) and among individual disturbed inocula treatments

(fire vs. logging vs. MPB vs. salvage) with linear mixed models using the lmer function in the R lmerTest package. The specific seedling performance metrics tested were: square root transformed root biomass, square root transformed shoot biomass, total biomass, and seedling height. Site and a greenhouse block was added to the model as random effects to account for variation due to site pooled soil inoculum and blocking within the greenhouse. Pairwise comparisons between individual disturbed inoculum treatments were performed using a general linear hypothesis test with Tukey Honest Significant Difference (HSD) adjustment for multiple comparisons using the glht function in the r multcomp package (Hothorn et al. 2008).

To test if seedling biomass values or height differed between non-inoculated control and field soil inoculated treatments, we used a linear mixed model with a greenhouse block as a random effect using the lmer function in the R lmerTest package. As above, the greenhouse block was added to the model to account for variation in greenhouse conditions.

To determine differences in seedling survival between non-inoculated control and field soil inoculated seedlings, we used a generalized linear mixed model with binomial distribution and a greenhouse block as random effect with the glmer function in the R lmerTest package.

Foliar nutrient content for non-inoculated control and field soil inoculum treatments was compared using Permutational Multivariate Analysis of Variance (PERMANOVA). PERMANOVA tests with 10,000 permutations and all other parameters set to default were performed on Bray-Curtis distance matrices with the Adonis function within the Vegan package in R.

Finally, we tested for correlations between root associated fungal diversity and seedling total biomass and height using a Kendall's non-parametric correlation analysis. Analyses were

conducted using the cor.test function in R. Specific fungal diversity metrics used for correlation analysis were: richness, Simpson's diversity and Shannon's diversity metrics.

Results

1 Root fungal community composition

1.1 General fungal community characterization

In total 4,961,408 sequences were obtained from a total 134 root samples. Denoising in the DADA2 work flow reduced the total number of sequences to 2,525,763 and 68 ESVs were identified. Following rarefication, a total of 41,160 sequences were analyzed (Supplemental Table 3). In addition, roots from all five non-inoculated control seedlings had fewer than 173 sequences and did not meet the minimum rarefaction sampling depth, which may indicate low fungal colonization. *Wilcoxina mikole* was the most ubiquitous fungal ESV, appearing in 85% of the samples and in all the soil inoculation treatments (Supplemental Table 3, Fig. 1). *Wilcoxina mikole* was the most abundant taxa on roots of seedlings inoculated with field soil from paired control treatments as well as fire, logging and MPB outbreak disturbed treatments (Fig. 1). While *Wilcoxina mikole* was present on the roots of seedlings from the salvage logging treatment, the most abundant fungal ESV was Sebacinales 1.

1.2 Comparison of root fungal community between disturbed and paired controls

Both the root-associated fungal communities of logging (F_1= 2.73, P=0.027) and salvage-logging (F_1= 8.13, P<0.001) treatments differed when compared to their respective paired control treatments (Fig. 2). In contrast, we did not detect a difference in the fungal community composition of fire (F_1= 1.08, P=0.198) and MPB (F_1= 1.14, P=0.295) treatments when

compared to their respective paired control treatments (Fig. 2), suggesting that logging causes unique filters in the soil fungal community.

1.3 Comparison of root fungal community among different disturbances

Root-associated fungal communities varied among control inocula treatments (fire-control, logging-control, MPB-control, salvage-control) (F_3= 2.8574, P=0.004). Specifically, root-associated fungal communities from the fire-control treatments were significantly different than those from MPB-control and logging-control treatments (Table 1, Fig. 3). However, no other differences in fungal community composition were observed among control treatments.

Root-associated fungal community differed among different disturbed inocula types (F_3= 3.33, P<0.001). Specifically, the root fungal composition of seedlings inoculated with soil from fire-disturbed treatments differed when compared to the root fungal composition of seedlings inoculated with soil from logging-disturbed treatments (Table 1, Fig. 3) and salvage-logging disturbed treatments (Table 1, Fig. 3). However, we did not observe a difference in the root fungal composition of MPB outbreak disturbed soil treatments when compared to fire, logging or salvage logging disturbed soil treatments (Table 1, Fig. 3). Lastly, we observed no difference in the root fungal composition of logging disturbed soil treatments when compared to salvage logging disturbed soil treatments (Table 1, Fig. 3).

1.4 Comparison of fungal diversity between disturbed and paired controls as well as among different disturbance types

Root fungal ESV richness, Shannon's diversity index or Simpson's diversity index did not differ among control inoculum treatments (Table 2) or among disturbed inoculum treatments (Table 3). However, when we compared individual disturbance treatments to their respective paired

controls, the root fungal diversity was greater on seedlings from salvage logged treatments when compared to seedlings from the paired control treatments (Table 4). Furthermore, the fungal root diversity of seedlings inoculated with soil from fire, MPB and logging disturbed sites did not differ from their respective paired control sites (Table 4). Finally, we did not detect a correlation between fungal ESV richness, Shannon's diversity index or Simpson's diversity index and seedling biomass or height (Table 5).

2 Seedling performance

2.1 Seedling response to soil inoculation treatments

Soil inoculation overall improved seedling performance. The survival of non-inoculated control seedlings (51 ±9.8%) was significantly lower than the survival of seedlings that received field soil inoculations (85±1.8%) (*z-value*= 4.133, P<0.001). Furthermore, the surviving non-inoculated control seedlings had 32% less mass when compared to inoculated seedlings ($F_{1,267}$= 7.24, P=0.008) and did not grow as tall as inoculated seedlings ($F_{1,268}$= 3.81, P=0.05).

2.2 Comparison of seedling performance between disturbed and paired control sites

Seedlings from the logging disturbed treatment had reduced total and shoot biomass compared to seedlings from their paired control treatment (Table 4). Furthermore, seedlings from the salvage logging disturbed treatment had reduced height compared to seedlings from their paired control treatment. Lastly, seedling height and biomass did not differ between fire and MPB disturbed treatments compared to their paired control treatments.

2.3 Comparison of seedling performance among different disturbances

Though fungal community composition differed, we observed no difference in seedling height and biomass among the control inocula treatments (Table 2). However, seedling height and biomass differed based on disturbed-soil inocula treatments (Table 3). Seedlings inoculated with fire-disturbed soil grew taller (16%) and had more biomass (34%) when compared to seedlings that were inoculated with soil disturbed by salvage-logging. However, the height and biomass of seedlings inoculated with soil from MPB or logging disturbed sites did not differ when compared to seedlings inoculated with soil from fire or salvage-logging disturbed sites (Table 3). Lastly, no difference was observed among disturbed inoculation treatments for seedling nutrient content (F_3= 0.37, P=0.923) (Supplemental Table 4).

Discussion

In the current study, we investigated the impact of four common disturbances to lodgepole pine forests of western Canada (i.e., fire, MPB outbreak, logging, and salvage-logging) (Romme & Knight 1981; Peet 2000; Anderson 2003) on soil fungi and seedling performance in the greenhouse. Two main findings emerged from our study, both critical to understanding the cascading impacts of the disturbances investigated as well as the management of these forest stands.

First, inoculation with field soil improved seedling performance relative to those that received no inoculum, demonstrating that field soil inoculations, and thus our approach, was successful and validated. We specifically investigated two main questions: 1) does seedling root fungal community composition change depending on disturbance type? and 2) what are the impacts of different disturbed treatments on seedling performance? For the first question, we

found that not all disturbances similarly affected the root fungal communities relative to their paired control sites. Specifically, the root fungal communities of logged and salvage-logged treatments differed from their paired controls while fire and MPB outbreak treatments did not. We also found significant variations on the root fungal communities among disturbance types. The most prominent difference was between burned and salvaged-logged sites. For the second question, we found that these changes to the root associated fungal community resulted in cascading impacts on seedling performance both when comparing different disturbance treatments to their paired control sites and when comparing among disturbance types.

1 Root fungal community composition

1.1 Individual disturbance treatments vs paired controls

Disturbance treatments differed when comparing the root fungal communities of individual disturbance treatments and their respective paired control treatments. In particular, the root fungal community composition differed between logged or salvage-logged treatments and their paired control treatments (Fig. 2). These findings are consistent with studies that have observed changes in fungal community composition following clear-cut logging (Jones et al. 2003; Lazaruk et al. 2005; Kohout et al. 2018) and salvage logging (Jennings et al. 2012; Kutorga et al. 2012; Ford et al. 2018). Past work comparing fungal community composition of the soil following fire, partial and clear-cut logging found that clear-cut logging resulted in the greatest reduction in ectomycorrhizal diversity (Lazaruk et al. 2005), while others found no difference in fungal communities following the same disturbances (Barker et al. 2013), suggesting that the impacts of clear-cut logging may vary depending on the forest system it is implemented. We did not detect a difference in the fungal community composition of fire and MPB disturbed treatments when compared to their paired controls (Fig. 2). In contrast, Karst et al. (2015)

reported differences in seedling fungal composition when comparing MPB outbreak disturbed vs. control soil inoculations in the greenhouse. In the earlier study, sites experiencing a minimum of 80% mortality due to MPB were sampled while we sampled sites with a minimum of 50% MPB mortality. Thus, this discrepancy in the impact of MPB outbreak on soil fungal community composition relative to undisturbed sites may be due to differences in the criterion used to select MPB disturbed sites.

While logging differed from its paired control, among disturbances, the fungal communities associated with the salvaged stands were the most distinct from its paired control stands, demonstrating that multiple disturbances in quick succession may have greater impacts on the fungal community of forest soil when compared to singular disturbance (Fig. 2) (Kutorga et al. 2012; Ford et al. 2018). These findings support earlier work that has expressed concerns that management practices, such as salvage logging, that cause multiple disturbances in quick succession (compound disturbances) may result in different species assemblages which have the potential to reduce seedling regeneration and/or the resilience of an ecosystem (Paine et al. 1998; Beshta et al. 2004; Peterson & Leach 2008a; Buma 2015).

1.2 Comparison among disturbance treatments

To determine baseline differences among disturbances, we first compared the fungal community and performance of seedlings among the controls of the different disturbance treatments. Our findings indicate that although there were differences in the fungal community composition of different control treatments (Fig. 3, Table 1), these differences did not influence seedling performance as seedling performance was similar among all paired control treatments (Table 2).

When different disturbances were compared, root associated fungal community composition differed between burned and salvage-logged treatments (Table 1). Considering that seedling in the fire-control and salvage-logged control treatments had similar fungal community composition (Table 1) and showed similar performance (Table 2), differences between salvage logging and fire treatments (Table 3) are likely due to different impacts of these disturbances. These findings are consistent with previous studies found that the way in which salvage logging alters the composition of soil fungi is different when compared to natural disturbances (i.e., fire) (Kutorga et al. 2012; Ford et al. 2018). Furthermore, salvage logging is unique in that it is the only compound disturbance investigated in this study. These findings agree with previous studies that have suggested that salvage logging, due in part to the fact that it is a compound disturbance, may result in novel species assemblages when compare to natural disturbances such as fire (Lindenmayer et al. 2012; Lindenmayer et al. 2017; Thorn et al. 2018).

2 Seedling performance

2.1 Individual disturbance treatments vs paired controls

As we predicted, changes to the root associated fungal community can have important impacts on seedling performance (Fig. 2, Table 4). We found that disturbances that had different fungal community composition when compared to their paired controls (Fig. 2) also had significant influence on seedling performance (Table 4). Since both logging and salvage logging are anthropogenic disturbances and given the cascading impacts of these disturbances on root fungal community composition (Fig. 2) and seedling performance (Table 4), our findings suggest that seedlings and their associated fungal communities are best adapted to natural disturbance regimes they have evolved with, supporting earlier studies (Bergeron et al. 1999; Lindenmayer & Noss 2006, Lindenmayer et al. 2012). Furthermore, since MPB is a novel disturbance in the

forests studied, our findings also suggest that seedlings and their associated fungal communities may be better adapted to a novel natural disturbance when compared to anthropogenic disturbance. This may be due in part to unique impacts on forest biotic and abiotic conditions associated with logging such as compaction during harvesting and changes to soil organic matter, which in the past have been demonstrated to alter soil fungal community composition (Hartman et al. 2012).

2.2 Comparison among disturbance treatments

When comparing different disturbance treatments, we found that seedlings from fire disturbed treatments had different fungal community composition (Fig. 3, Table 1) and greater biomass and height when compared to seedlings from salvage-logging treatments (Table 3). Considering that the fire-control and salvage-logged control seedlings had similar fungal community composition (Fig. 3, Table 1) and performance (Table 2), differences between salvage logging and fire treatments are likely due to different impacts of these disturbances. Once again, these findings indicate that fungal community composition may have important impacts on seedling performance .

These results indicate that of the disturbances investigated fire and salvage logging may represent opposite ends of a spectrum that other singular MPB outbreak and logging disturbances fall within. While the impact of salvage logging on seedling regeneration and performance has been debated (Donato et al. 2006; Newton et al. 2006; Royo et al. 2016; Lindenmayer et al. 2017, Thorn et al. 2018). These findings stress that salvage logging may result in greater changes to soil fungal community when compared to other disturbances investigated in this study (Fig.3, Table 1) and these changes may result in cascading negative impacts on seedling performance (Table 3). Furthermore, these findings indicate that disturbance context is important; fungal

communities can respond differently with different disturbances (Lazaruk et al. 2005) and soil fungi can mediate the response of the next generation of seedlings (Karst et al. 2015).

2.3 Seedling performance and root associated fungal community diversity

Root-associated fungal diversity did not explain differences in seedling biomass and height (Table 3, Table 5). Previous work has found that seedling performance does not necessarily improve when grown in association with multiple fungi and that combinations of different root associated fungi can result in both positive and negative impacts on seedling performance (Kennedy et al. 2007; Kanekar et al 2018). Furthermore, past work in post fire stands investigating the root associated fungal communities of pine found no correlation between sapling performance and fungal diversity (Buscardo et al. 2011). In the case of our greenhouse study, the fungal taxa growing in association with seedling roots, rather than their diversity, seemed to have a greater influence on seedling performance.

3 Study limitations

The use of field soil as inoculum for pots may introduce some potential limitations for the interpretation of our results. Specifically, while we investigate the role of soil fungi on seedling performance in this experiment, it is possible that other soil organisms such as nematodes, bacteria, mites, etc. present in the field soil inoculum may have also contributed to observed differences in the performance of lodgepole pine seedlings. However, given the importance of soil fungi to pine (Miller et al. 1998; Despain et al. 2001; Smith and Read 2008; Karst et al. 2014) as well as that changes in seedling performance in this experiment (Table 4, Table 3) typically were accompanied differences in seedling root-associated fungal communities (Fig. 2, Table 3) our results indicate that differences in seedling performance were due in large to

changing fungal communities. However, we caution against attributing all observed differences in seedling performance solely to soil fungi.

Furthermore, conditions in greenhouse may also introduce some potential limitations. For example, we grew seedlings for two growing seasons to investigate their responses to disturbed soil inoculum from 14 sites. While greenhouse bioassays may not necessarily translate to conditions in the field, these bioassays allow comparisons of disturbances under controlled conditions enabling us to investigate soil fungi as a potential mechanism involved in the indirect impacts of forest disturbance (Perry et al. 1982). Likewise, while our study provides evidence of the soil mediated impacts of anthropogenic disturbance, long term field studies with more sites may help to further elucidate our results regarding the soil fungi-mediated impacts on seedling regeneration following anthropogenic disturbance.

4 Management implications

Our work demonstrates that the way in which soil fungal communities are filtered can differ depending on the disturbance and these differences can have cascading impacts on the next generation of lodgepole pine seedlings. This is of concern to land managers because we found that logging (clear-cut logging and salvage logging), as mediated by soil fungi, may decrease seedling performance (Table 3, Table 4). These findings show that the impacts of logging disturbance in lodgepole pine stands may not be analogous to other natural disturbance. As mentioned earlier, more work is needed to better understand the long-term impacts of logging on seedling performance as mediated by soil fungi so that land managers can appropriately address potential regeneration challenges when prescribing management.

In the past, salvage logging operations were implemented to address short term economic losses and meet a variety of management objectives in post-MPB attacked stands (Mitchell 2005; BC Ministry of Forests and Range 2006; ASRD 2007; McIntosh and Macdonald 2013; Leduc et al. 2015). As a result, some authors have suggested that salvage logging may be an important management strategy in the future that can be implemented to address timber shortages associated with climate change altered disturbance regimes (Boucher et al. 2018). However, little is known about the impacts of salvage logging on the soil fungal community (Ford et al. 2018). We found that of all the disturbance types, salvage logging resulted in the greatest changes to the soil fungal community when compared to its paired control and decreased seedling performance when compared to fire disturbed seedlings. In light of our findings, land managers should consider the potential negative impacts on seedling performance that can be induced by salvage logging in lodgepole pine forests. Given the importance logging and especially the projected importance of salvage logging as a management strategy in the future, more work investigating the impacts and restoration of salvage logged sites is needed. Specifically, work investigating the use of soil inoculations to re-establish beneficial fungal communities at disturbed sites as well as long term field studies are needed to improve our understanding of the cascading impacts of salvage logging on the soil fungal community.

Figure 1. Percent mean relative abundance of *Pinus contorta* root associated fungal exact sequence variants (ESVs) occurring in at least 1% relative abundance within at least one of the different soil inoculum treatments. Soil inoculum treatments indicate that seedlings were grown in pots inoculated with soil from one of disturbed (fire, logging, MPB or Salvage logging) or control sites. Controls were sites that had similar pre-disturbance stand composition to a paired disturbed site. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

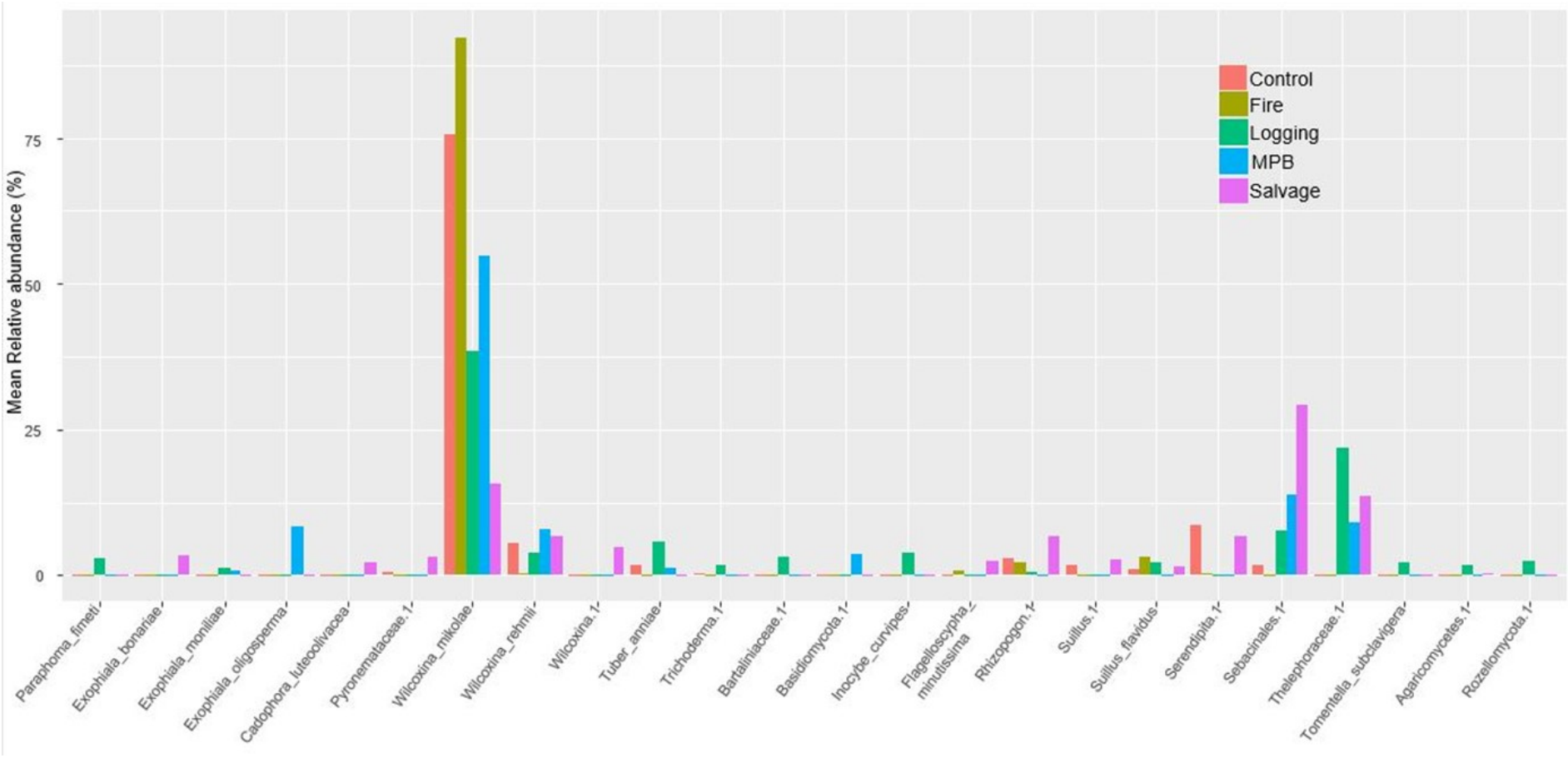


Figure 2. Principal coordinates analysis (PCoA) plots of *Pinus contorta* root associated fungal communities. Points on the plot indicate the root fungal community for individual seedlings. Dashed ellipse indicates disturbed soil inoculum treatment (either: fire, logging, MPB or salvage) and solid ellipse indicates paired control soil inoculum treatment. All ellipses indicate 95% confidence intervals. Paired controls were sites that had similar pre-disturbance stand composition to a disturbed site. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

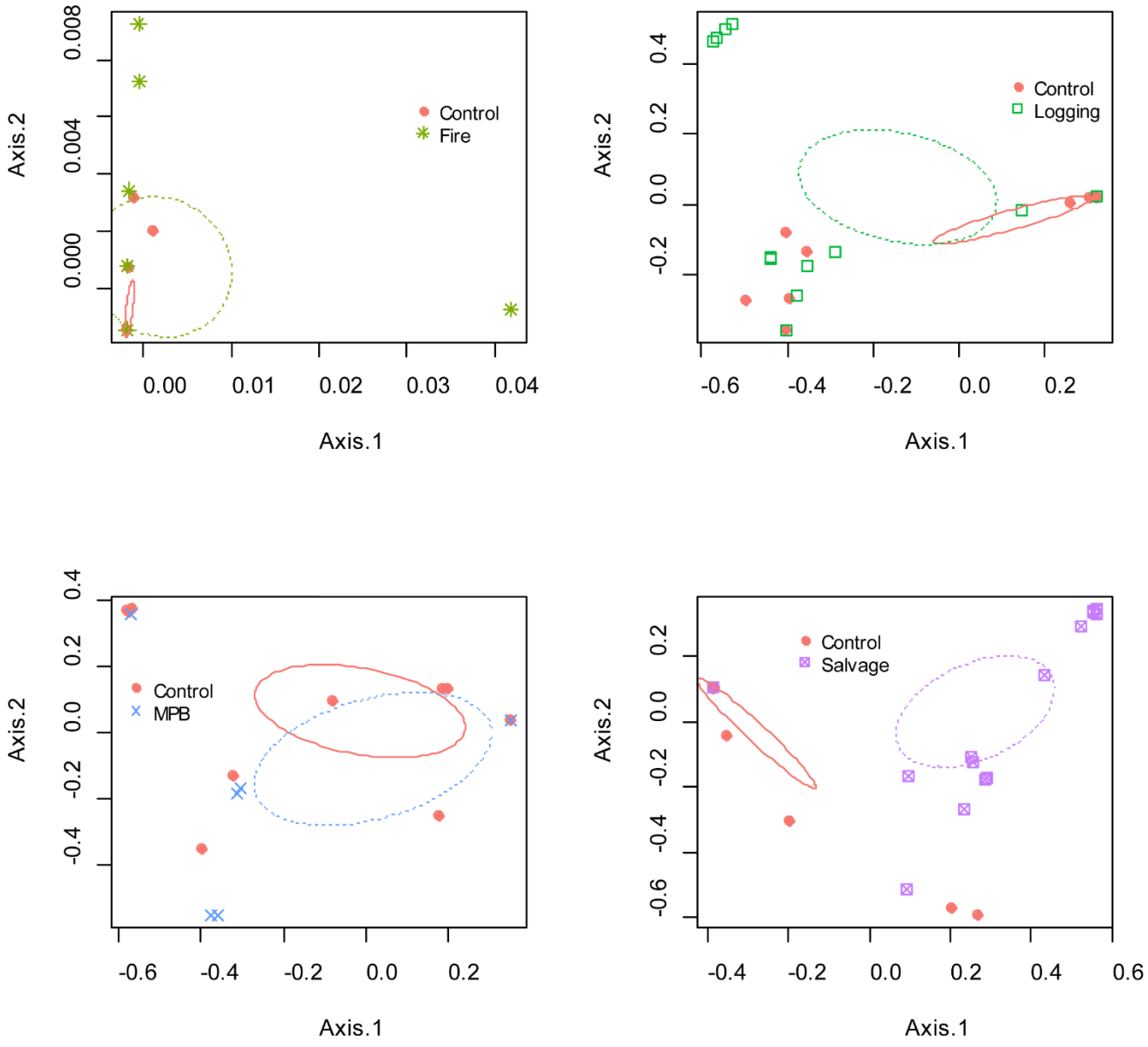


Figure 3. Principal coordinates analysis (PCoA) plot of *Pinus contorta* root associated fungal communities. Points on the plot indicate the root fungal community of individual seedlings. Colors and ellipses indicate different soil inoculum treatments. Dashed ellipses indicate disturbed soil inoculum treatment and solid ellipses indicate paired control soil inoculum treatment. All ellipses indicate 95% confidence intervals. Soil inoculum treatments indicate that seedlings were grown in pots inoculated with soil from one of disturbed (fire, logging, MPB or Salvage logging) or control sites. Controls were sites that had similar pre-disturbance stand composition to a paired disturbed site. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

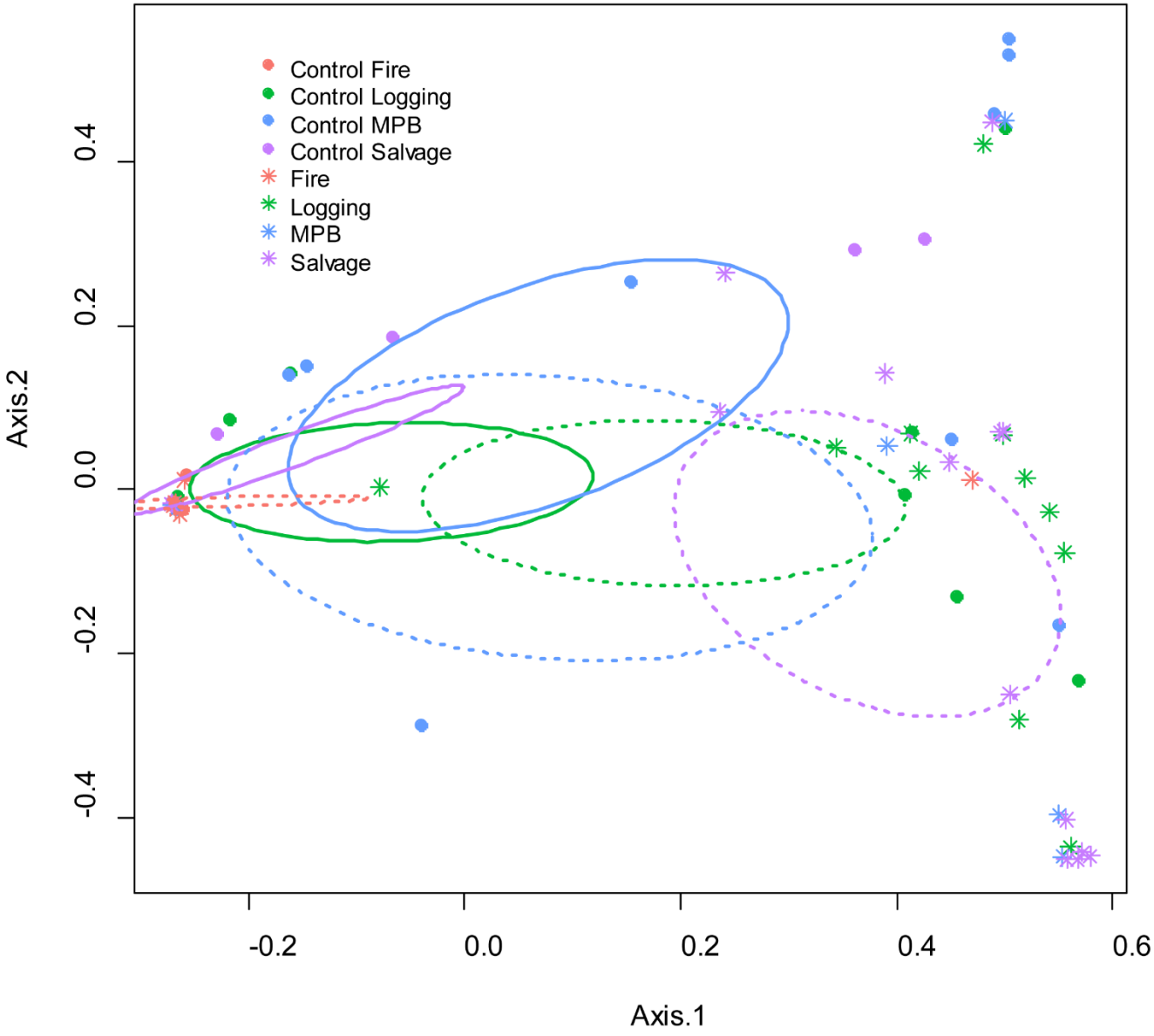


Table 1. Holm adjusted P-values from Pairwise PERMANOVA analysis between control soil inocula treatments (fire-control, logging-control, MPB-control, salvage-control) (left) and between disturbed soil inocula treatments (fire, logging, MPB, salvage) (right) on the root associated soil fungal communities of *Pinus contorta*. Soil inoculum treatments indicate that seedlings were grown in pots inoculated with soil from one of disturbed or control sites. Controls were sites that had similar pre-disturbance stand composition to a paired disturbed site. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

			MPB	Logging	Fire	
Logging Control	**0.087**		0.320	0.322	**<0.001**	Salvage
MPB Control	**0.002**	0.328		0.716	0.157	MPB
Salvage Control	0.142	0.329	0.255		**0.013**	Logging
	Fire Control	Logging Control	MPB Control			

Red and blue shading below indicates either a different or similar relationship when comparing the result of a given control treatment combination to the same combination for the disturbed treatments. Red shading highlights a different relationship when comparing a control treatment pairwise comparison result to the same pairwise comparison for a given disturbed treatment combination (i.e. control-salvage and control-fire treatments do not differ but salvage and fire treatments are significantly different = red shading) while blue shading indicates similar relationship (i.e. control-MPB and control-salvage are not significantly different and MPB and salvage are not significantly different = blue shading).

Table 2. Results of linear mixed model testing the effect of soil inoculum from fire-control, logging-control, MPB-control and salvage-control stands on *Pinus contorta* performance and root fungal diversity. Controls were sites that had similar pre-disturbance stand composition to a paired disturbed site. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

Seedling performance	Df (num, den)	*F*	*P*
Total dry biomass (g)	3, 8.965	1.1005	0.398
Root dry biomass (g)	3, 6.593	1.3788	0.330
Shoot dry biomass (g)	3, 10.934	1.0428	0.412
Height (cm)	3, 10.1	1.4714	0.280
Root fungal diversity			
Richness	3, 10.727	1.7063	0.225
Shannon	3, 12.472	2.5325	0.114
Simpson	3, 12.272	2.346	0.123

Table 3. Results of linear mixed model testing the effect of soil inoculum from stands disturbed by fire, logging, mountain pine beetle (MPB, *Dendroctonus ponderosae*) or salvage logging on the performance and root fungal diversity of *Pinus contorta* seedlings. Below raw data means, and standard error values are graphed for individual soil inoculum treatments. Letters indicate significant differences based on the results of pairwise Tukey HSD tests.

Seedling performance	Mean (± SE)				Df (num, den)	*F*	*P*
	Salvage	MPB	Logging	Fire			
Total dry biomass (g)	2.31 (0.17) [b]	3.12 (0.25) [ab]	2.76 (0.16) [ab]	3.53 (0.24) [a]	3, 10.32	3.9174	**0.042**
Root dry biomass (g)	1.19 (0.09) [b]	1.67 (0.17) [ab]	1.45 (0.11) [ab]	1.81 (0.13) [a]	3, 10.38	3.6207	**0.051**
Shoot dry biomass (g)	1.12 (0.09) [b]	1.5 (0.11) [ab]	1.31 (0.07) [ab]	1.72 (0.12) [a]	3, 10.258	3.3145	**0.064**
Height (cm)	6.6 (0.3) [b]	7.5 (0.3) [ab]	7.3 (0.2) [ab]	7.9 (0.3) [a]	3, 7.41	3.0369	**0.098**
Root fungal diversity							
Richness					3,8.875	2.068	0.176
Shannon					3,9.3492	2.077	0.112
Simpson					3,10.524	2.545	0.171

Table 4. Results of linear mixed model testing the effect of soil inoculum from disturbed sites to their respective paired control sites on *Pinus contorta* root fungal diversity (Shannon and Simpson) and performance (biomass, height and survival). Controls were sites that had similar pre-disturbance stand composition to a paired disturbed site. Below raw data means, and standard error values are graphed for individual soil inoculum treatments. Letters indicate significance between disturbed treatments and paired controls. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

Fire vs paired control	Df (num, den)	*F*	*P*	Mean (± SE) *Disturbed*	Mean (± SE) *Control*
Total dry biomass (g)	(1, 4.028)	1.223	0.330	3.5(0.24)	2.8(0.21)
Root dry biomass (g)	(1, 4.031)	1.829	0.247	1.8(0.13)	1.4(0.11)
Shoot dry biomass (g)	(1, 4.030)	0.743	0.437	1.5(0.10)	1.7(0.12)
Height (cm)	(1, 4.051)	0.954	0.383	7.4(0.32)	8.0(0.32)
Shannon fungal diversity	(1, 3.152)	0.9643	0.395	0.14(0.11)	0.03(0.02)
Simpson fungal diversity	(1, 3.144)	0.9273	0.403	0.06(0.05)	0.01(0.01)
Logging vs paired control					
Total dry biomass (g)	(1, 6.027)	3.780	**0.096**	**2.8(0.16)^{b}**	**3.4(0.21)^{a}**
Root dry biomass (g)	(1, 6.105)	2.540	0.161	1.4(0.13)	1.7(0.11)
Shoot dry biomass (g)	(1, 6.074)	4.012	**0.091**	**1.3(0.06)^{b}**	**1.7(0.11)^{a}**
Height (cm)	(1, 6.336)	3.084	0.127	7.3(0.25)^{b}	8.0(0.30)^{a}
Shannon fungal diversity	(1, 6.105)	0.514	0.500	0.41(0.11)	0.26(0.10)
Simpson fungal diversity	(1, 6.096)	0.685	0.439	0.23(0.06)	0.13(0.05)
MPB vs paired control					
Total dry biomass (g)	(1, 1.197)	0.236	0.701	3.1(0.25)	2.6(0.21)
Root dry biomass (g)	(1, 1.428)	1.661	0.368	1.6(0.17)	1.3(0.13)
Shoot dry biomass (g)	(1, 3.854)	1.135	0.349	1.5(0.11)	1.2(0.09)
Height (cm)	(1, 3.422)	0.229	0.661	6.9(0.23)	7.4(0.33)
Shannon fungal diversity	(1, 2.304)	5.207	0.133	0.17(0.09)	0.48(0.11)
Simpson fungal diversity	(1, 2.485)	5.648	0.116	0.09(0.05)	0.28(0.07)
Salvage vs paired control					
Total dry biomass (g)	(1, 6.0412)	1.1826	0.318	2.3(0.17)	2.7(0.18)
Root dry biomass (g)	(1, 6.094)	0.5812	0.474	1.2(0.09)	1.3(0.09)
Shoot dry biomass (g)	(1, 5.942)	2.3527	0.176	1.1(0.09)	1.4(0.09)
Height (cm)	(1, 6.3747)	6.009	**0.047**	**6.6(0.29)^{b}**	**7.6(0.30)^{a}**
Shannon fungal diversity	(1, 4.3462)	5.576	**0.072**	**0.47(0.12)^{b}**	**0.11(0.06)^{a}**
Simpson fungal diversity	(1, 5.4103)	4.5618	**0.082**	**0.25(0.07)^{b}**	**0.07(0.04)^{a}**

Table 5. Results of Kendall's correlation analysis testing the correlations between *Pinus contorta* root associated fungal richness, Shannon diversity, Simpson diversity and *P. contorta* seedling height (cm) and biomass (g).

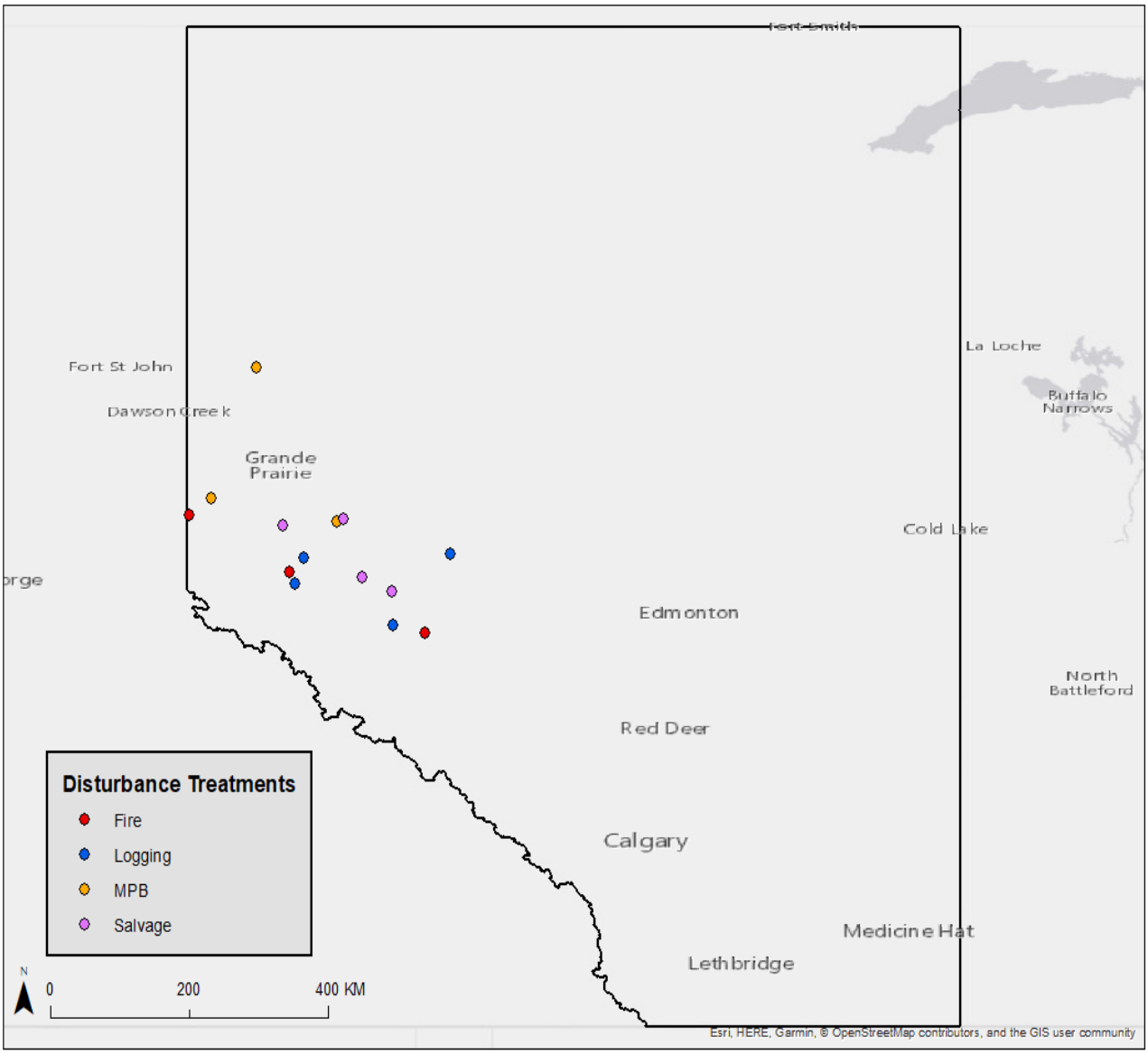
Seedling total dry biomass (g)	Tau	Z-value	P
Fungal richness	0.04429	0.65066	0.515
Simpson fungal diversity	0.07951	1.2015	0.230
Shannon fungal diversity	0.07632	1.1533	0.249
Seedling height (cm)			
Fungal richness	0.06164	0.89784	0.369
Simpson fungal diversity	0.03344	0.50107	0.616
Shannon fungal diversity	0.03666	0.54925	0.583

Supplemental information

Supplemental Table 1. Table of Alberta Vegetation Inventory data used to assign paired controls to individual disturbed study sites. The data below represents the pre-disturbance stand conditions of disturbed stands as well as the conditions of the paired control stands. Composition indicates the crown composition (%) of lodgepole pine (*Pinus contorta*) as well as the composition (%) of other species such as black spruce (*Picea mariana*), white spruce (*Picea glauca*), and trembling aspen (*Populus tremuloides).* MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

Site	Disturbance category	Crown closure (%)	Height (m)	lodgepole pine composition (%)	composition (%) of other species	composition (%) of other species
G16-5c	Control	6-30	17	100		
G16-5	MPB	6-30	19	100		
G16-6c	Control	31-50	16	90	10- black spruce	
G16-6	Fire	51-70	14	90	10- black spruce	
G16-1c	Control	51-70	22	80	10- white spruce	10- trembling aspen
G16-1	Salvage	51-70	25	70	20- white spruce	10- trembling aspen
G15-8c	Control	71-100	19	70	30- trembling aspen	
G15-8	MPB	51-70	21	70	30- trembling aspen	
G15-1c	Control	51-70	16	100		
G15-1	MPB	31-50	21	100		
G15-2c	Control	51-70	21	90	10- trembling aspen	
G15-2	Salvage	51-70	21	80	20- trembling aspen	
W14-1c	Control	51-70	26	100		
W14-1	Logging	51-70	26	100		
G15-6c	Control	71-100	22	100		
G15-6	Logging	71-100	22	100		
E8-7c	Control	6-30	16	70	20- black spruce	10- white spruce
E8-7	Fire	6-30	16	70	20- black spruce	10- white spruce
E8-3c	Control	51-70	17	100		
E8-3	Logging	51-70	15	100		
E14-4c	Control	51-70	23	100		
E14-4	Salvage	51-70	23	100		
E14-2c	Control	51-70	20	100		
E14-2	Salvage	51-70	22	100		
E14-6c	Control	51-70	22	100		
E14-6	Logging	51-70	21	100		
E14-8c	Control	51-70	21	90	10-trembling aspen	
E14-8	Fire	51-70	22	90	10-trembling aspen	

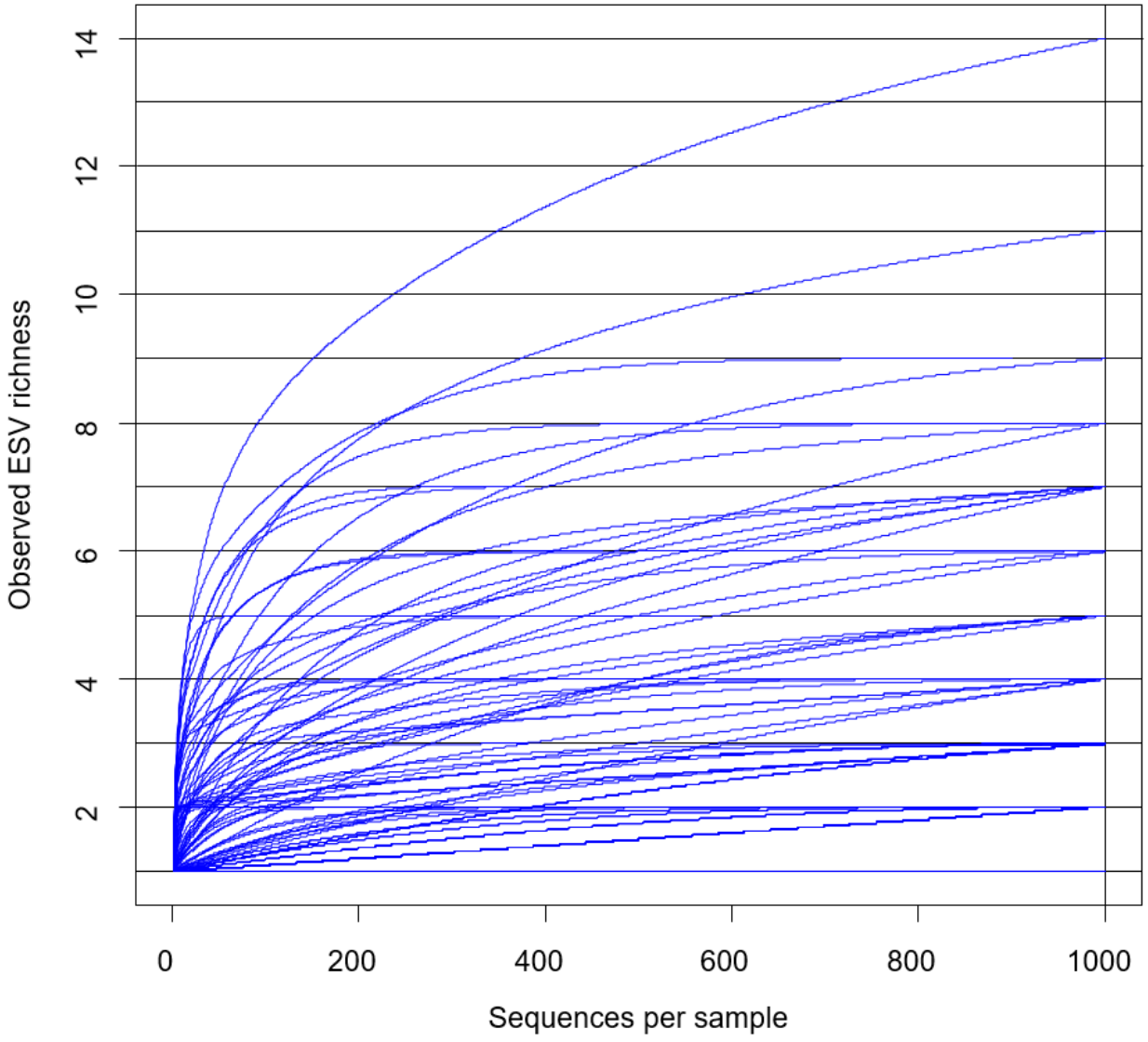
Supplemental Figure 1. Map of the 14 disturbed sites (represented by colored points) in west-central Alberta disturbed by either clear-cut logging (n=4, blue), wildfire (n=3, red), MPB (n=3, yellow) and salvage logging (n=4, purple). MPB: Mountain pine beetle (*Dendroctonus ponderosae*).



Supplemental Table 2. List of seed lots used to create pooled seed stock that corresponded with each of the 28 sites we sampled in west central Alberta, Canada. Seed stock was provided by Smokey Lake Tree Nursery (Smokey Lake, Alberta, Canada).

Seed lots:
TIC 1-55-23-5-2008 PL
TIC 36-57-26-5-2010
NES2 50-23-5-1979 PL
TIC 4-50-20-5-2012 PL
TIC 26-59-4-6-2008 PL
NES1 58-5-6-1983 PL
NWB1 64-8-6-1981 PL
NWB8 64-25-5-1979 PL
NWB8 62-3-6-1981 PL
NES3 63-22-5-1990 PL
TIC 36-59-4-6-2008 PL
NWB2 86-7-6-1982 PL
WEG 14-64-6-6-2012 PL
WEG 36-63-5-6-2010 PL
TIC 26-67-12-6-2008 PL
NWB1 65-9-6-1984 PL
TIC 14-59-20-5-2015 PL

Supplemental Figure 2. Species accumulation curves of observed fungal exact sequence variant (ESV) richness as a function of the number of sequences per seedling root sample. Each curve represents an individual seedling root sample.



Supplemental Table 3. List of all fungal Exact Sequence variants (ESV) and their presence (indicated by "+") on the roots of *Pinus contorta* seedlings grown in pots inoculated with soil disturbed by fire, logging, MPB (*Dendroctonus ponderosae*), salvage logging or control sites (controls were sites that had similar pre-disturbance stand composition to a paired disturbed site).

Fungal ESV	Fire Control	Logging Control	MPB Control	Salvage Control	Fire	Logging	MPB	Salvage
Fungi 1		+	+		+	+	+	+
Ascomycota 1					+	+		+
Plenodomus biglobosus						+		+
Paraphoma fimeti		+				+		
Chaetothyriales 1		+				+		
Cladophialophora 1							+	
Cladophialophora chaetospira			+	+	+	+	+	+
Cladophialophora 1			+					
Exophiala bonariae						+		+
Exophiala equina		+	+		+	+		+
Exophiala moniliae	+					+	+	
Exophiala oligosperma	+	+					+	
Exophiala psychrophila		+						
Exophiala salmonis		+				+		
Cadophora luteo.olivacea						+		+
Cadophora orchidicola		+			+	+		+
Cadophora orientoamericana	+							
Arthrobotrys superba			+					
Dactylella rhopalota		+						
Pyronemataceae 1			+					+
Wilcoxina mikolae	+	+	+	+	+	+	+	+
Wilcoxina rehmii		+	+	+	+	+	+	+
Wilcoxina 1								+
Tuber anniae 1		+				+	+	
Metarhizium carneum 1			+					
Trichoderma 1			+			+		
Trichoderma oblongisporum			+					
Bartaliniaceae 1					+	+		+
Basidiomycota 1						+	+	
Cortinarius 1					+			
Inocybe curvipes						+		
Flagelloscypha minutissima	+	+			+			+
Piloderma 1			+					
Rhizopogon 1		+	+	+	+	+		+
Suillus 1	+	+	+			+	+	+
Suillus flavidus		+	+	+	+	+		+

Suillus pungens		+						
Suillus quiescens		+						
Sebacinales 1		+	+					
Serendipita 1	+	+	+	+	+		+	+
Sebacinales 2	+	+	+		+	+	+	+
Thelephoraceae 1		+				+	+	+
Tomentella subclavigera						+		
Agaricomycetes 1						+		+
Powellomyces 1								+
Rozellomycota 1		+	+			+		+
Syncephalis 1						+		
Fungi 2								+

Supplemental Table 4. Raw data means, and standard error values of foliar nutrients from *Pinus contorta* seedlings. Control-fire, control-logging, control-MPB and Control-salvage were sites that had similar pre-disturbance stand composition to a paired disturbed site. Non-inoculated controls were seedlings that were grown in pots that were not inoculated with field soil. MPB: Mountain pine beetle (*Dendroctonus ponderosae*).

Nutrients	Mean (± SE)								
	Control Fire	Control Logging	Control MPB	Control Salvage	Fire	Logging	MPB	Salvage	Control Non-inoc.
N (%)	1.373 (0.87)	1.368 (0.07)	1.629 (0.105)	1.456 (0.07)	1.446 (0.07)	1.442 (0.07)	1.533 (0.08)	1.579 (0.1)	1.4 (0.09)
Ca (%)	0.588 (0.026)	0.589 (0.019)	0.707 (0.038)	0.601 (0.022)	0.663 (0.027)	0.643 (0.031)	0.643 (0.023)	0.618 (0.022)	0.784 (0.141)
Cu (µg/g)	5.2 (0.071)	3.526 (0.455)	3.929 (0.691)	3.611 (0.512)	3.846 (0.406)	3.158 (0.353)	3.583 (0.484)	3.842 (0.361)	2.8 (0.8)
Fe (µg/g)	233.3 (28.4)	1.88.2 (32.0)	157.1 (25.7)	197.2 (21.9)	176.9 (32.1)	281.2 (34.8)	281.3 (75.5)	259.2 (57.2)	160 (51.6)
K (%)	0.514 (0.034)	0.448 (0.024)	0.559 (0.04)	0.507 (0.028)	0.568 (0.04)	0.519 (0.034)	0.523 (0.032)	0.561 (0.031)	0.552 (0.082)
Mg (%)	0.212 (0.011)	0.18 (0.008)	0.195 (0.011)	0.2 (0.008)	0.203 (0.006)	0.211 (0.008)	0.208 (0.01)	0.206 (0.008)	0.206 (0.013)
Mn (µg/g)	473.3 (57.5)	568.4 (44.3)	4.66.4 (50.3)	540 (43.4)	330.8 (31.0)	486.8 (48.8)	486.7 (55.0)	534.2 (33.0)	478 (69.4)
Zn (µg/g)	41.3 (2.4)	33.7 (2.1)	40.7 (4.5)	40 (2.7)	37.7 (2.8)	41.1 (2.8)	36.7 (3.1)	38.4 (2.2)	18 (2)
Na (µg/g)	75.1 (9.4)	62.2 (7.9)	56.7 (5.5)	68.3 (7.0)	79.8 (11.1)	74.2 (10.5)	63.6 (7.9)	76.2 (8.4)	71.2 (14)
S (%)	0.146 (0.007)	0.152 (0.009)	0.178 (0.009)	0.16 (0.007)	0.162 (0.008)	0.169 (0.008)	0.173 (0.008)	0.174 (0.008)	0.192 (0.02)
P (%)	0.235 (0.014)	0.198 (0.009)	0.236 (0.018)	0.222 (0.013)	0.245 (0.016)	0.216 (0.01)	0.2 (0.011)	0.22 (0.014)	0.152 (0.014)

Supplemental methods

Seedling dormancy protocol adapted from Kanekar et al. 2018. Seedlings were gradually acclimated to cold storage for two weeks; first stored at 15° C during the day and 10° C at night for the first week and then 6° C for the second week. After seedlings were acclimated to the cold, they were stored at 4° C for five weeks and then the temperature in the cold storage room was gradually increased 3° C every day for a week until the temperature in the growth chamber reached 23° C. While dormancy was being simulated in the growth chamber, seedlings were watered weekly and kept under a 12h:12h (light: dark) light regime. No fertilization application took place during dormancy.

Literature cited

Abarenkov, K., Nilsson, R. H., Larsson, K. H., Alexander, I. J., Eberhardt, U., Erland, S., … Kõljalg, U. (2010). The UNITE database for molecular identification of fungi - recent updates and future perspectives. *New Phytologist*, *186*(2), 281–285. doi:10.1111/j.1469-8137.2009.03160.x

Agee, J. K. (1996). Fire ecology of Pacific Northwest forest. *Island Press*. doi:10.1071/WF9940195

Alexander, R. R., Lotan, J. E., Larson, M. J., & Volland, L. A. (1985). Silvicultural systems for the major forest types of the United States. *Forest Ecology and Management*, *11*(4), 63–67. doi:10.1016/0378-1127(85)90107-0

Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

ASRD. (2007). Alberta Sustainable Resource Development. Mountain Pine Beetle Management Strategy, Edmonton, AB. *Alberta Development*.

Astrup, R., Coates, K. D., & Hall, E. (2008). Recruitment limitation in forests: lessons from an unprecedented mountain pine beetle epidemic. *Forest Ecology and Management*, *256*(10), 1743–1750. doi:10.1016/J.FORECO.2008.07.025

Barker, J., Jones, M. D., & Simard, S. W. (2013). Ectomycorrhizal fungal community assembly on regenerating Douglas-fir after wildfire and clearcut harvesting. *Oecologia*, (172), 1179–1189. doi:10.1007/s00442-012-2562-y

Bässler, C., Müller, J., Svoboda, M., Lepšová, A., Hahn, C., Holzer, H., & Pouska, V. (2012). Diversity of wood-decaying fungi under different disturbance regimes-a case study from

spruce mountain forests. *Biodiversity and Conservation*, *21*(1), 33–49. doi:10.1007/s10531-011-0159-0

Bergeron, Y., Harvey, B., Leduc, A., & Gauthier, S. (1999). Forest management guidelines based on natural disturbance dynamics: stand- and forest-level considerations. *The Forestry Chronicle*, *75*(1), 49–54. doi:10.5558/tfc75049-1

Beschta, R. L., Rhodes, J. J., Kauffman, J. B., Gresswell, R. E., Minshall, G. W., Karr, J. R., … Frissell, C. A. (2004). Postfire management on forested public lands of the Western United States. *Conservation Biology*, *18*(4), 957–967. doi:10.1111/j.1523-1739.2004.00495.x

Blaalid, R., Kumar, S., Nilsson, R. H., Abarenkov, K., Kirk, P. M., & Kauserud, H. (2013). ITS1 versus ITS2 as DNA metabarcodes for fungi. *Molecular Ecology Resources*, *13*(2), 218–224. doi:10.1111/1755-0998.12065

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., … Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*(1), 90. doi:10.1186/s40168-018-0470-z

Boucher, D., Boulanger, Y., Aubin, I., Bernier, P. Y., 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*(5), 1245–1259. doi:10.1002/eap.1724

Bradbury, S. M., Danielson, R. M., & Visser, S. (1998). Ectomycorrhizas of regenerating stands of lodgepole pine. *Canadian Journal of Botany*, *76*(2), 218–227. doi:10.1139/cjb-76-2-218

British Columbia Ministry of Forests and Range. (2006). Mountain Pine Beetle Action Plan 2006–2011, British Columbia Ministry of Forests and Range, Victoria, BC, Canada.

https://www2.gov. bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/ forest-health/mountain-pine-beetle/mountain_pine_beetle_action_ plan_2006.pdf

Bruns, T. (1995). Thoughts on processes that maintain local species diversity of ectomycorrhizal fungi. *Plant and Soil*, *170*, 63–73.

Buma, B. (2015). Disturbance interactions: characterization, prediction, and the potential for cascading effects. *Ecosphere*, *6*(4), 1–15. doi:10.1890/ES15-00058.1

Buscardo, E., Freitas, H., Pereira, J. S., & de Angelis, P. (2011). Common environmental factors explain both ectomycorrhizal species diversity and pine regeneration variability in a post-fire Mediterranean forest. *Mycorrhiza*, *21*(6), 549–558. doi:10.1007/s00572-011-0363-5

Cahill JF, JA Cale, J Karst, T Bao, G Pec**,** N Erbilgin. (2017). No silver bullet: Different soil handling techniques are useful for different research questions, exhibit differential type I and II error rates, and are sensitive to sampling intensity. [A response to K.O. Reinhart and M. J. Rinella. 2016. A common soil handling technique can generate incorrect estimates of soil biota effects on plants. New Phytologist 210:786-789.]. *New Phytologist.* 216: 11-14

Cairney, J. W. G., & Bastias, B. A. (2007). Influences of fire on forest soil fungal communitites. *Canadian Journal of Forest Research*, *37*, 207–215.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. doi:10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). Correspondence QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature*

Publishing Group, *7*(5), 335–336. doi:10.1038/nmeth0510-335

Cowan, A. D., Smith, J. E., & Fitzgerald, S. A. (2016). Recovering lost ground: effects of soil burn intensity on nutrients and ectomycorrhiza communities of ponderosa pine seedlings. *Forest Ecology and Management*, *378*, 160–172. doi:10.1016/j.foreco.2016.07.030

Craig, A. J., Woods, S., & Hoeksema, J. D. (2016). Influences of host plant identity and disturbance on spatial structure and community composition of ectomycorrhizal fungi in a northern Mississippi uplands ecosystem. *Fungal Ecology*, *24*(Nov), 7–14. doi:10.1016/j.funeco.2016.08.007

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*(5), 1036–1043. doi:10.1111/j.1365-2664.2010.01848.x

Deslippe, J. R., Hartmann, M., Grayston, S. J., Simard, S. W., & Mohn, W. W. (2016). Stable isotope probing implicates a species of *Cortinarius* in carbon transfer through ectomycorrhizal fungal mycelial networks in Arctic tundra. *New Phytologist*, *210*(2), 383–390. doi:10.1111/nph.13797

Despain, D. G. (2001). Dispersal ecology of lodgepole pine (*Pinus contorta* Dougl.) in its native environment as related to Swedish forestry. *Forest Ecology and Management*, *141*(1–2), 59–68. doi:10.1016/S0378-1127(00)00489-8

Dighton, J. (2016). Fungi in Ecosystem Processes. *CRC Press*.

Donato, D. C., Fontaine, J. B., Campbell, J. L., Robinson, W. D., Kauffman, J. B., & Law, B. E. (2006). Post-wildfire logging hinders regeneration and increases fire risk. *Science*, *311*(5759),

352. doi:10.1126/science.1122855

Durall, D. M., Jones, M. D., & Lewis, K. J. (2005). Effects of forest management on fungal communities, In: Dighton, J.,White, J.F., Oudemans, P. (Eds.), The Fungal Community: Its Organization and Role in the Ecosystem, 3 rd ed. *Taylor & Francis*.

Erbilgin, N. (2018). Phytochemicals as mediators for host range expansion of a native invasive forest insect herbivore. *New Phytologist*. doi:10.1111/nph.15467

Erbilgin, N., Ma, C., Whitehouse, C., Shan, B., Najar, A., & Evenden, M. (2014). Chemical similarity between historical and novel host plants promotes range and host expansion of the mountain pine beetle in a naive host ecosystem. *New Phytologist*, *201*(940–950). doi:10.1111/nph.12573

Fahey, T. J., & Knight, D. H. (1986). Lodgepole pine ecosystems. *BioScience*, *36*(9), 610–617.

FAO. (2016). Sustainable Forest Management. *FAO Focus Areas*, 2–5. doi:10.1201/9781420032857.ch2

Flannigan, M. D., Kochtubajda, B., & Logan, K. A. (2005). Forest fires and climate change in the 21st Century. *Mitigation and Adaptation Strategies for Global Change*, *11*(4), 847–859. doi:10.1007/978-3-540-73936-4_23

Ford, S. A., Kleinman, J. S., & Hart, J. L. (2018). Effects of wind disturbance and salvage harvesting on macrofungal communities in a *Pinus* woodland. *Forest Ecology and Management*, *407*(January), 31–46. doi:10.1016/j.foreco.2017.10.010

Goldmann, K., Schöning, I., Buscot, F., & Wubet, T. (2015). Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems.

Frontiers in Microbiology, *6*(NOV), 1–11. doi:10.3389/fmicb.2015.01300

Hartmann, M., Howes, C. G., Vaninsberghe, D., Yu, H., Bachar, D., Christen, R., … Mohn, W. W. (2012). Significant and persistent impact of timber harvesting on soil microbial communities in Northern coniferous forests. *ISME Journal*, *6*(12), 2199–2218. doi:10.1038/ismej.2012.84

Herve, M. (2018). Testing and Plotting Procedures for Biostatistics Package ' RVAideMemoire .'

Hothorn, T., Bretz, F. & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal,* 50(3), 346-363.

Jennings, T. N., Smith, J. E., Cromack, K. jr., Sulzman, E. W., McKay, D., Caldwell, B. A., & Beldin, S. I. (2012). Impact of postfire logging on soil bacterial and fungal communities' and soil biogeochemistry in a mixed-conifer forest in central Oregon. *Plant and Soil*, (350), 393–411.

Jones, M. D., Durall, D. M., & Cairney, J. W. G. (2003). Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. *New Phytologist*, *1*(157), 399–422.

Kanekar, S. S., Cale, J. A., & Erbilgin, N. (2018). Ectomycorrhizal fungal species differentially affect the induced defensive chemistry of lodgepole pine. *Oecologia*, *188*(2), 395–404. doi:10.1007/s00442-018-4231-2

Karst, J., Erbilgin, N., Pec, G. J., Cigan, P. W., Najar, A., Simard, S. W., & Cahill, J. F. (2015). Ectomycorrhizal fungi mediate indirect effects of a bark beetle outbreak on secondary chemistry and establishment of pine seedlings. *New Phytologist*, *208*(3), 904–914. doi:10.1111/nph.13492

Karst, J., Randall, M. J., & Gehring, C. A. (2014). Consequences for ectomycorrhizal fungi of the

selective loss or gain of pine across landscapes. *Botany*, *92*(12), 855–865. doi:10.1139/cjb-2014-0063

Karst, J., Jones, M. D., & Turkington, R. (2009). Ectomycorrhizal colonization and intraspecific variation in growth responses of lodgepole pine. *Plant Ecology*, *200*(2), 161–165. doi:10.1007/s11258-008-9443-9

Keeley, J. E. (2012). Ecology and evolution of pine life histories. *Annals of Forest Science*, *69*(4), 445–453. doi:10.1007/s13595-012-0201-8

Kennedy, P. G., Hortal, S., Bergemann, S. E., & Bruns, T. D. (2007). Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. *Journal of Ecology*, *95*(6), 1338–1345. doi:10.1111/j.1365-2745.2007.01306.x

Kennedy, N. M., Robertson, S. J., Green, D. S., Scholefield, S. R., Arocena, J. M., Tackaberry, L. E., … Egger, K. N. (2015). Site properties have a stronger influence than fire severity on ectomycorrhizal fungi and associated N-cycling bacteria in regenerating post-beetle-killed lodgepole pine forests. *Folia Microbiologica*, *60*(5), 399–410. doi:10.1007/s12223-014-0374-7

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. *ISME Journal*, *12*(3), 692–703. doi:10.1038/s41396-017-0027-3

Kutorga, E., Adamonyte, G., Iršenaite, R., Juzenas, S., Kasparavičius, J., Markovskaja, S., … Treigiene, A. (2012). Wildfire and post-fire management effects on early fungal succession in *Pinus mugo* plantations, located in Curonian Spit (Lithuania). *Geoderma*, *191*, 70–79. doi:10.1016/j.geoderma.2012.02.007

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2018). lmerTest Package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13). doi:10.18637/jss.v082.i13

Lamont, B. B., Le Maitre, D. C., Cowling, R. M., & Enright, N. J. (1991). Canopy seed storage in woody plants. *The Botanical Review*, *57*(4), 277–317. doi:10.1007/BF02858770

Lazaruk, L. W., Kernaghan, G., Macdonald, S. E., & Khasa, D. (2005). Effects of partial cutting on the ectomycorrhizae of *Picea glauca* forests in northwestern Alberta. *Canadian Journal of Forest Research*, *35*(6), 1442–1454. doi:10.1139/x05-062

Leduc, A., Bernier, P. Y., Mansuy, N., Raulier, F., Gauthier, S., & Bergeron, Y. (2015). Using salvage logging and tolerance to risk to reduce the impact of forest fires on timber supply calculations. *Canadian Journal of Forest Research*, *45*(4), 480–486. doi:10.1139/cjfr-2014-0434

Lekberg, Y., Vasar, M., Bullington, L. S., Sepp, S.-K., Antunes, P. M., Bunn, R., … Öpik, M. (2018). More bang for the buck? Can arbuscular mycorrhizal fungal communities be characterized adequately alongside other fungi using general fungal primers? *New Phytologist*, (2017). doi:10.1111/nph.15035

Lewis Murphy, T. E., Adams, D. L., & Ferguson, D. E. (1999). Response of advance lodgepole pine regeneration to overstory removal in eastern Idaho. *Forest Ecology and Management*, *120*(1–3), 235–244. doi:10.1016/S0378-1127(98)00544-1

Lindenmayer, D. B., & Noss, R. F. (2006). Salvage logging, ecosystem processes, and biodiversity conservation. *Conservation Biology*, *20*(4), 949–958. doi:10.1111/j.1523-1739.2006.00497.x

Lindenmayer, D. B., Burton, P. J., & Franklin, J. F. (2012). *Salvage logging and its ecological consequences*. Island Press.

Lindenmayer, D., Thorn, S., & Banks, S. (2017). Please do not disturb ecosystems further. Nature Ecology and Evolution, 1, 1–8.

Lotan, J., Brown, J. and Neuenschwander, L. (1985). Role of fire in lodgepole pine forests, pp. 133-152 in D. Baumgartner et al. (eds) Lodgepole pine the species and its management Symposium Proceedings. Washington State University, Pullman.

Man, G. (2010). Major forest insect and disease conditions in the United States: 2009 update. *USDA Forest Service*, (July), 54. Retrieved from http://www.fs.fed.us/foresthealth/publications/ConditionsReport_2013.pdf

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10.

Massad, T. J., Dyer, L. A., & Vega C., G. (2012). Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. *PLoS ONE*, *7*(10). doi:10.1371/journal.pone.0047554

McDonald, D., Clemente, J. C., Kuczynski, J., Rideout, J. R., Stombaugh, J., Wendel, D., … Caporaso, J. G. (2012). The Biological Observation Matrix (BIOM) format or: How I learned to stop worrying and love the ome-ome. *GigaScience*, *464*(1), 1–6. doi:10.1186/2047-217X-1-7

McIntosh, A. C. S., & Macdonald, S. E. (2013). Potential for lodgepole pine regeneration after mountain pine beetle attack in newly invaded Alberta stands. *Forest Ecology and Management*, *295*, 11–19. doi:10.1016/j.foreco.2012.12.050

McKinney, W. (2010). Data Structures for Statistical Computing in Python. *Proceedings of the 9th Python in Science Conference*, *1697900*(Scipy), 51–56. Retrieved from

http://conference.scipy.org/proceedings/scipy2010/mckinney.html

Meddens, A., Hicke, J., & Ferguson, C. (2012). Spatiotemporal patterns of observed bark beetle-caused tree mortality in British Columbia and the western United States. *Ecological Applications*, *22*(7), 1876–1891. doi:doi: 10.1890/11-1785.1

Miller, S. L., McClean, T. M., Stanton, N. L., & Williams, S. E. (1998). Mycorrhization, physiognomy, and first-year survivability of conifer seedlings following natural fire in Grand Teton National Park. *Canadian Journal of Forest Research*, *28*(1), 115–122. doi:10.1139/x97-195

Mitchell, J. (2005). Review and synthesis of regeneration methods in beetle-killed stands following mountain pine beetle (*Dendroctonus ponderosae*) attack: A Literature Review. *Mountain Pine Beetle Initiative*, 1–25.

Newton, M., Fitzgerald, S., Rose, R. R., Adams, P. W., Tesch, S. D., Sessions, J., … Skinner, C. (2006). Comment on "post-wildfire logging hinders regeneration and increases fire risk." *Science*, *313*(5787), 615a–615a. doi:10.1126/science.1126478

Nuñez, M. A., Horton, T. R., & Simberloff, D. (2009). Lack of belowground mutualisms hinders Pinaceae invasions. *Ecology*, *90*(9), 2352–2359. doi:10.1890/08-2139.1

Oksanen, A. J., Blanchet, F. G., Kindt, R., Legen-, P., Minchin, P. R., Hara, R. B. O., … Wagner, H. (2018). Package ' vegan ' version 2.5-2. doi:ISBN 0-387-95457-0

Paine, R. T., Tegner, M. J., & Johnson, E. a. (1998). Ecological surprises. *Ecosystems*, *1*(July), 535–545. doi:10.1007/s100219900049

Pec, G. J., Karst, J., Taylor, D. L., Cigan, P. W., Erbilgin, N., Cooke, J. E. K., … Cahill, J. F.

(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*(2), 864–873. doi:10.1111/nph.14195

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., … Duchesnay, É. (2012). Scikit-learn: Machine Learning in Python. *Journal of Machine Learning Research*, *12*, 2825–2830. doi:10.1007/s13398-014-0173-7.2

Perry, D. A., Meyer, M. M., Egeland, D., Rose, S. L., & Pilz, D. (1982). Seedling growth and mycorrhizal formation in clearcut and adjacent, undisturbed soils in Montana: a greenhouse bioassay. *Forest Ecology and Management*, *4*, 261–273.

Perry, D. A., Amaranthus, M. P., Borchers, J. G., Borchers, S. L., & Brainerd, R. E. (1989). Bootstrapping in ecosystems. *BioScience*, *39*(4), 230–237. doi:10.2307/1311159

Peterson, C. J., & Leach, A. D. (2008). Limited salvagelogging effects on forest regeneration after moderate-severity windthrow. *Ecological Applications*, *18*(2), 407–420.

Pickles, B. J., Gorzelak, M. A., Green, D. S., Egger, K. N., & Massicotte, H. B. (2015). Host and habitat filtering in seedling root-associated fungal communities: taxonomic and functional diversity are altered in 'novel' soils. *Mycorrhiza*, *25*(7), 517–531. doi:10.1007/s00572-015-0630-y

Price, D. T., Alfaro, R. I., Brown, K. J., Flannigan, M. D., Fleming, R. A., Hogg, E. H., … Venier, L. A. (2013). Anticipating the consequences of climate change for Canada's boreal forest ecosystems. *Environmental Reviews*, *21*(4), 322–365. doi:10.1139/er-2013-0042

Reazin, C., Morris, S., Smith, J. E., Cowan, A. D., & Jumpponen, A. (2016). Fires of differing intensities rapidly select distinct soil fungal communities in a Northwest US ponderosa pine

forest ecosystem. *Forest Ecology and Management*, *377*, 118–127. doi:10.1016/j.foreco.2016.07.002

Reinhart, K. O., Rinella, M. J. (2016). A common soil handling technique can generate incorrect estimates of soil biota effects on plants. *New Phytologist, 210*, 786–789.

Royo, A. A., Peterson, C. J., Stanovick, J. S., & Carson, W. P. (2016). Evaluating the ecological impacts of salvage logging: can natural and anthropogenic disturbances promote coexistence? *Ecology*, *97*(6), 1566–1582. doi:10.1890/15-1093.1

Safranyik, L., Carroll, A. L., Régnière, J., Langor, D. W., Riel, W. G., Shore, T. L., … Taylor, S. W. (2010). Potential for range expansion of mountain pine beetle into the boreal forest of North America. *Canadian Entomologist*, *142*(5), 415–442. doi:10.4039/n08-CPA01

Seidl, R., Thom, D., Kautz, M., Martin-benito, D., & et.al. (2017). Forest disturbances under climate change. *Nature Climate Change*, *7*, 395–402. doi:10.1038/nclimate3303.Forest

Shah, F., Nicolás, C., Bentzer, J., Ellström, M., Smits, M., Rineau, F., … Tunlid, A. (2016). Ectomycorrhizal fungi decompose soil organic matter using oxidative mechanisms adapted from saprotrophic ancestors. *New Phytologist*, *209*(4), 1705–1719. doi:10.1111/nph.13722

Shore, T. L., Safranyik, L., Hawkes, B. C., & Taylor, S. W. (2006). Effects of the mountain pine beetle on lodgepole pine stand structure and dynamics. the mountain pine beetle: A synthesis of biology, management, and impacts on lodgepole pine. Retrieved from http://www.cfs.nrcan.gc.ca/pubwarehouse/pdfs/26116.pdf#page=106

Skoog, D.A., Holler, F.J. and Crouch, S.R., 2017. Principles of instrumental analysis. *Cengage learning, 10, 254-280*

Simard, S. W., & Durall, D. M. (2004). Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany*, *82*(8), 1140–1165. doi:10.1139/b04-116

Smith, D. P., & Peay, K. G. (2014). Sequence Depth, not PCR replication, improves ecological inference from next generation DNA sequencing. *PLoS ONE*, *9*(2), e90234. doi:10.1371/journal.pone.0090234

Smith, M. E., Douhan, G. w., Fremier, A. K., & Rizzo, D. M. (2009). Are true multihost fungi the exception or the rule? Dominant ectomycorrhizal fungi on *Pinus sabiniana* differ from those on co- occurring Quercus species. *New Phytologist*,*182*: 287–290.

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. *Academic Press*. doi:10.1016/B978-0-12-370526-6.50010-6

Smithers L.A. (1961). Lodgepole pine in Alberta. *Canada Department of Forestry*, pp. 1-153.

Štursová, M., Šnajdr, J., Cajthaml, T., Bárta, J., Šantrůčková, H., & Baldrian, P. (2014). When the forest dies: the response of forest soil fungi to a bark beetle-induced tree dieback. *ISME Journal*, *8*(9), 1920–1931. doi:10.1038/ismej.2014.37

Tabacaru, C. A., Park, J., & Erbilgin, N. (2016). Prescribed fire does not promote outbreaks of a primary bark beetle at low-density populations. *Journal of Applied Ecology*, *53*(1), 222–232. doi:10.1111/1365-2664.12546

Taudière, A., Richard, F., & Carcaillet, C. (2017). Review on fire effects on ectomycorrhizal symbiosis, an unachieved work for a scalding topic. *Forest Ecology and Management*, *391*, 446–457. doi:10.1016/j.foreco.2017.02.043

Thom, D., & Seidl, R. (2016). Natural disturbance impacts on ecosystem services and biodiversity

in temperate and boreal forests. *Biological Reviews of the Cambridge Philosophical Society*, *91*(3), 760–781. doi:10.1111/brv.12193

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., …, Jansson, J. K., Gilbert, J. A., Knight, R., & The Earth Microbiome Project Consortium. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature, 551:457-463.* doi:10.1038/nature24621.

Thorn, S., Bässler, C., Brandl, R., Burton, P. J., Cahall, R., Campbell, J. L., … Müller, J. (2018). Impacts of salvage logging on biodiversity: A meta-analysis. *Journal of Applied Ecology*, *55*(1), 279–289. doi:10.1111/1365-2664.12945

Treu, R., Karst, J., Randall, M., Pec, G. J., Cigan, P. W., Simard, S. W., … Cahill, J. F. (2014). Decline of ectomycorrhizal fungi following a mountain pine beetle epidemic. *Ecology*, *95*(4), 1096–1103. doi:10.1890/13-1233.1

White, P. S., & Pickett, S. T. A. (1985). The Ecology of Natural Disturbance and Patch Dynamics. *Elsevier Inc.* Academic Press, INC. doi:10.1016/B978-0-08-050495-7.50006-5

Wotton, B. M., Flannigan, M. D., & Marshall, G. A. (2017). Potential climate change impacts on fire intensity and key wildfire suppression thresholds in Canada. *Environmental Research Letters*, *12*(095003). Retrieved from http://iopscience.iop.org/article/10.1088/1748-9326/aa7e6e/pdf

Wotton, B. M., Nock, C. A., & Flannigan, M. D. (2010). Forest fire occurrence and climate change in Canada. *International Journal of Wildland Fire*, *19*(3), 253–271. doi:10.1071/WF09002.