Soil fungi after pine beetle outbreak: Diagnosis of fungal community composition and treatment of outplanted seedlings with tailored soil inoculum

by

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Abstract

Soil fungal communities play vital roles in boreal forests as key organisms that cycle nutrients, facilitate uptake of resources for mycorrhizal plants, and store carbon. Forest disturbances often result in shifts in soil fungal community composition, yet, it is unclear if these effects are consistent across soils widely varying in abiotic properties. I used the landscape-scale outbreaks of mountain pine beetle in lodgepole pine forests of western Alberta to elucidate whether soil physical and chemical properties underpin soil fungal community composition and function after disturbance. In addition to characterizing fungal communities across the landscape, I also tested whether pine seedling performance (survival and height) differs when grown with soil inoculum from different sources (beetle-killed, undisturbed, or no inoculum) after being outplanted into beetle-killed pine forests. I found two groups of soil fungi in beetle-killed forests: those inhabiting soils with greater ammonium and silt content ('Loamy Sites'), and those inhabiting soils with higher phosphate and sand content ('Sandy Sites'). Across all sites, saprotrophic fungi were more diverse than ectomycorrhizal fungi, yet Loamy Sites were more diverse in both functional groups compared to Sandy Sites. Indicator ectomycorrhizal fungi present at the two site groups corroborated previous research outlining nutrient preferences, yet indicators were not necessarily representative of disturbances in previous studies. Survival of seedlings did not differ by inoculum type. Pine seedling heights differed among inoculation treatments, yet only in those outplanted to Sandy Sites. These results indicate that the composition and function of soil fungal communities after beetle outbreaks diverges based on physical and chemical properties of soils. I also demonstrate that seedlings grown with tailored soil inoculum differ in performance when outplanted into beetle-killed forests, but site variation modifies seedling outcomes. Though the result of this insect outbreak appears uniform at the tree

canopy level, future research should exercise caution when predicting consequences of fungal community structure and function within these forests. A seemingly homogenous aboveground disturbance includes substantial variation belowground. Further work is necessary to investigate the long-term succession of soil fungal communities relative to soil abiotic properties, as well as the influence of inoculation treatments on performance of outplanted seedlings.

Preface

No data from this thesis has been published prior to thesis defense and publication.

Nullius in verba

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Introduction

Forest soils harbor diverse fungal communities that vary in composition and function, with possible consequences for above- and belowground ecosystem processes (Smith & Read, 2008). Soil fungi play vital roles in nutrient cycling, as both decomposers and plant mutualists (Smith & Read, 2008). Due to their importance in forest ecosystems, much research has focused on soil fungi, particularly in forestry (Smith & Read, 2008). Mycorrhizal fungi form mutualisms with plants, wherein fungal hyphae improve the uptake of water and nutrients through plant roots in exchange for carbon photosynthate from living host plants (Smith & Read, 2008) and decompose soil organic matter (Shah *et al*., 2016). Consequently, mycorrhizal fungi are essential for plant establishment and performance. Saprophytic fungi, on the other hand, decompose plant litter and woody debris and are fundamental in mobilizing soil organic nutrients into plant available inorganic nutrients such as ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) (Cairney & Meharg, 2002; Lindahl *et al.*, 2007). While soil fungi influence nutrient cycling in forests, fungi can, conversely, be sensitive to soil chemistry (Wallenda & Kottke, 1998; Egerton-Warburton & Jumpponen, 2005; Read & Perez-Moreno, 2003; Kivlin *et al.*, 2014; Treseder *et al.*, 2014; Suz *et al.*, 2017; Van der Linde *et al.*, 2018; Lilleskov *et al.*, 2019).

Forest disturbances often result in changes in soil chemistry, as well as removal of photoautotrophic hosts. Loss of host-derived carbon and changes in soil chemistry can directly and indirectly influence both mycorrhizal and saprotrophic fungal communities, with consequences for forest health and succession (Dickie *et al.*, 2013; Karst *et al.*, 2014; Sapsford *et al.*, 2017). It has been widely reported how mycorrhizal and saprotrophic fungal communities shift after disturbance, and separately, as communities occur in soils of varying nutrient content (Erland & Taylor, 2002; Toljander *et al.*, 2006; Sterkenberg *et al.*, 2015). However, shifts in

fungal community composition and function after large-scale disturbance such as insect outbreak, particularly in the context of soil physical and chemical properties, is less understood. Because soil fungi are integral for both nutrient cycling and tree health, the characterization of their composition and diversity both after disturbance, and across varying soil physical and chemical properties is essential for understanding forest health and succession on a regional scale. In this thesis, I examine fungal community composition and function among beetle-killed pine stands over a wide geographic range in Alberta.

In the boreal forest, where most N is bound in the organic form (Tamm, 1991), and thought to be the most limiting resource (Vitousek *et* al, 1997; Franklin & MacMahon, 2000), ectomycorrhizal (EcM) fungi are thought to be the dominate mycorrhizal symbiosis (Read, 2001), as host trees lack the catabolic enzymes necessary to mobilize N (Read & Perez-Moreno, 2003). Ectomycorrhizal (EcM) fungi, however, vary in their ability to acquire nutrients, both morphologically (Agerer, 2001) and enzymatically (Buée *et al.*, 2007; Talbot *et al.*, 2008; Baldrian, 2009; Bödeker *et al.*, 2009). While some EcM fungi invest more carbon into thicker or more highly-branched explorative hyphal morphotypes, others are more conservative, forming few or no emanating hyphae (Agerer, 2001). When plant available inorganic N (such as ammonium or nitrate) is introduced to forests, host trees generally invest less carbon to the mycobiont, inevitably leading to a shift in EcM fungal composition (Smith & Read, 2008; Karst *et al.*, 2014). This shift is often towards EcM fungi that are less carbon demanding (Karst *et al*., 2014) or can supplement their carbon quotas with mycelial mats (Landeweert *et al.*, 2001) and enzymatic activity that allows them to exploit more recalcitrant organic sources of N (Buée *et al.,* 2007 Talbot *et al.*, 2008; Baldrian, 2009; Bödeker *et al.*, 2009; Bödeker *et al.*, 2014).

Changes in the composition of fungal communities with deposition of inorganic N have been studied exhaustively (Kårén & Nylund, 1997; Wallenda & Kottke, 1998; Peter *et al*, 2001; Lilleskov *et al.*, 2002), and similar community changes can occur along natural gradients of N (Erland & Taylor, 2002; Egerton-Warburton & Jumpponen, 2002; Toljander *et al*. 2006, Sterkenberg *et al.*, 2015). Nutrient pulses with litter deposition after insect-caused disturbance have also been demonstrated for N and P (Kaňa *et al.,* 2013; Šturšová *et al.*, 2014; Cigan *et al.*, 2015). However, in contrast to inorganic N deposition, litter often serves as a carbon and nutrient source for saprotrophic fungi, which have been shown to increase (Šturšová *et al.*, 2014) or decrease (Pec *et al.*, 2017) in abundance in forest soils after large-scale beetle-induced mortality.

While the input and presence of N in forests has been well studied with regards to EcM and saprotrophic fungi, less attention has been drawn to EcM and saprotrophic preferences to soil P (Bolan, 1991; Wallenda & Kottke, 1998; Tedersoo *et al.*, 2014). Substantial research has been devoted to the influences of P on arbuscular mycorrhizal fungi (Johnson, 1993; Treseder & Allen, 2002; Smith & Read, 2008; Johnson, 2010; Johnson *et al.*, 2010; Talbot *et al.*, 2014), yet the effects of P on EcM fungi are limited to studies focusing on few species of fungi and hosts (Jones *et al.*, 1990; Pampolina *et al.*, 2002). Additionally, these studies were not conducted in the context of disturbances resulting in the large-scale elimination of EcM hosts and deposition of litter.

While the influence of N and P on forests and fungi in general have been explored, they do so rarely over large geographic ranges that likely vary in soil texture. Soils with greater proportions of silt and clay are known to have a higher cation exchange capacity (Lal, 2005), yielding greater retention of nutrient cations, such as ammonium as well as soil organic matter (Chandler, 1939; Borchers & Perry, 1992; Banfield *et al*, 2002), whereas sandier soils with

greater pore size may retain less ammonium (Borchers & Perry, 1992). While the importance of these properties of soil texture in the context of EcM and saprotrophic fungal distribution have been stressed (Fernandez & Kennedy, 2016), relatively few studies have reported associations between soil texture and soil fungal community composition (Raulund-Rasmussen & Vejre, 1995; Lekberg *et al.*, 2007). That soil fungal communities differ with input of nutrients, along nutrient gradients, and by soil texture, it is reasonable to test whether these soil properties underlie fungal community composition after a large-scale disturbance.

Forest disturbances can directly impact fungal community composition through removal of mature trees (Sapsford *et al.*, 2017) and indirectly by altering the amount of litter (Karst *et al.*, 2014). In consequence, EcM fungi depending on carbon photosynthates from host trees are widely reported to decrease in abundance and richness (Karst *et al.*, 2014), while saprotrophic fungi can vary in their response (Šťursová *et al.*, 2014; Treu *et al.*, 2014). Disturbances such as clearcut harvest (Bradbury *et al.*, 1998), wildfire (Stendell *et al.*, 1999; Dahlberg, 2002), and insect outbreaks (Del Vecchio *et al.*, 1993; Šťursová *et al.*, 2014; Saravesi *et al.*, 2015;), often result in shifts in soil fungal community composition. While both wildfire and insect outbreak reduce EcM abundance, likely due to host tree mortality, these disturbances may affect soil chemistry differently. Insect-induced mortality, for instance, differs from severe wildfire in that litter input and woody debris increase with increased tree mortality (Kaňa *et al.*, 2013; Saravesi *et al.*, 2014; Cigan *et al.*, 2015; Šťursová *et al.*, 2015), whereas severe wildfires eliminate organic soil layers (Certini, 2005), often sterilizing the top mineral layers (Visser *et al.*, 1995; Stendell *et al.*, 1999). The belowground succession (Dickie *et al.*, 2013) of forest disturbances by wildfire and clearcut harvest have been well studied (Karst *et al.*, 2014), however, the increasing scale and severity of insect-induced mortality of forests poses an ongoing threat with changing climate

(Sturrock *et al.*, 2011; Anderegg *et al*. 2015). As such, investigating soil fungal communities that are integral in nutrient cycling (Read & Perez-Moreno, 2003; Clemmensen *et al.*, 2013), seedling establishment and performance (Smith & Read, 2008) in the context of insect mortality is critical to understanding above- and belowground forest succession.

In western Canada, mountain pine beetle (*Dendroctonus ponderosae* Hopkins, Coleoptera: Curculionidae, Scolytinae) has expanded beyond its historical range (de la Giroday *et al.*, 2012), resulting in the landscape-scale mortality of lodgepole pine (*Pinus contorta* Douglas ex Loud. Var *latifolia* Egelm) into the boreal forest (Cullingham *et al.*, 2011). Previous work in western Alberta has found elevated soil nutrient concentrations (NH_4^+, NO_3^-, and PO_4^{3-}) and litter compared to undisturbed stands of *P. contorta* (Cigan *et al.*, 2015), as well as shifts in fungal community composition (Treu *et al.*, 2014; Pec *et al.*, 2017). Specifically, previous work has found a decrease in richness of EcM sporocarps and in the abundance of EcM fungal hyphae (Treu *et al.*, 2014), while DNA sequencing indicated a decrease in EcM and saprotrophic fungi in soils (Pec *et al.*, 2017). Additionally, Karst *et al.* (2015) reported that *P. contorta* seedlings in these forests also had lower richness of fungi colonizing their root tips compared to those in undisturbed forest stands, which was associated with lower pine seedling survival. In the same study, seedlings were grown in a greenhouse with soil inoculum sourced from beetle-killed and undisturbed stands. Aligned with their field results, they found that the decline of EcM richness on roots had negative consequences to seedling performance.

While previous studies in *P. contorta* forests demonstrated fungal community shifts (Pec *et al.*, 2017) between undisturbed and beetle-killed *P. contorta* forests, they did so only in a small region (625 km^2) that was just a fraction of the area affected by *D. ponderosae* (> 470,000 km^2 in North America; Raffa *et al.*, 2013). Given that soil physical and chemical properties vary

across a wide geographic range affected by beetle outbreaks, soil fungal community composition and function may differ accordingly. Additionally, if seedlings grown with inoculum sourced from undisturbed forests had greater survival than those inoculated with soil sourced from beetle-killed stands, it may be possible to outplant seedlings with tailored inoculum to improve seedling performance in beetle-killed *P. contorta* forests.

In this study, I characterized soil texture (percent sand, silt, and clay) and nutrients (NH_4^+, NO_3^-, and PO_4^-), and used DNA sequencing to characterize soil fungal community composition and function in beetle-killed *P. contorta* forests across a wide geographic range. Additionally, I assessed the survival and height of seedlings receiving soil inoculum from beetle-killed and undisturbed *P. contorta* forests without beetle outbreaks, and those receiving no inoculum, after being outplanted into beetle-killed forests. I used this information to answer the following research questions: Do soil fungi vary in community composition and function in beetle-killed *P. contorta* forests? Does variation in soil physical and chemical properties explain variation in soil fungal community composition and function? Does *P. contorta* seedling performance differ when grown with soil inoculum from different sources (beetle-killed, undisturbed, or no inoculum) after being outplanted into beetle-killed *P. contorta* forests? Taken together, I aim to characterize the belowground consequences of a novel disturbance and assess the efficacy of treating these impacts on a profoundly larger regional scale. This will expand the previous and on-going works on how disturbances affect soil fungal composition and function in western Canada.

Methods

Study region

The study region is within an approximate 125 km radius from Grande Prairie, AB (55° 10′ 15″ N, 118° 47′ 41″ W, 650 m above sea level) within the Foothills and Boreal natural regions of west central Alberta (Fig. 1) (Beckingham *et al.*, 1996). Soils are classified as poorly drained Orthic Gray Luvisols in the Foothills natural region, and well to moderately drained Eutric Brunisols in the Boreal natural region (Soil Classification Working Group, 1998). Forests are mainly comprised of *Pinus contorta* Douglas ex Loud. var. *latifolia* Engelm., with *Picea glauca* (Moench) Voss, *Picea mariana* Mill. Britton, Sterns, & Poggenb., *Abies balsamea* (L.) Mill., *Betula papyrifera* Marshall, *Populus tremuloides* Michx., and *Populus balsamifera* (L.) in the subcanopy.

The regional topography of the Foothills region is rolling, characterized by morainal deposits with lacustrine and organic deposits in valleys (Beckingham *et al.*, 1996), while the Boreal region is characterized by generally flat lowland regions, with some rolling hills. Mean annual temperatures of the Foothills and Boreal natural regions are 3°C and 1.5 °C, respectively; mean annual precipitations of these regions are 589 mm and 501 mm, respectively (Beckingham *et al.*, 1996).

Site selection was intended to capture a broad range of environmental variation across beetle-killed stands. Towards this goal, I selected 30 sites where mature (120 ± 0.4 SE years old) *Pinus contorta* var. *latifolia* comprised >70% of stand basal area ($m^2\ ha^{-1}$) and stands showed recent (since 2007) *Dendroctonous ponderosae* activity. *Dendroctonus ponderosae* attacks in this region (northern Rocky Mountains) were first reported in 2006 and continued through 2012.

This area was not previously considered part of the beetle's historical range (de la Giroday *et al.*, 2012). Details on stand structure, composition, and tree attack status of study regions are described in Treu *et al.* (2014), Cigan *et al.* (2015), Erbilgin *et al.* (2017), and Steinke (2018). At the time of these studies, *D. ponderosae* activity was confirmed in these sites by presence of pitch tubes, exit holes, and subcortical galleries (Safranyik, 2006). Although several sites were located on the edge of the Boreal natural region of Alberta, and subsequently in the lodgepole pine x jack pine (*Pinus banksiana* Lamb) hybrid zone, I inferred that they were not hybrids based on genetic ancestry maps (Cullingham *et al.*, 2012).

Canopy and understory vegetation surveys

Within each site (30 m x 30 m) in late July through early August 2016, two north-south transects (30 m x 7.5 m) were established at 7.5 m and 22.5 m on the east-west sides of the sites. Within these transects, I recorded the diameter at breast height (1.3 m; DBH), species identity, and health status (e.g. alive or dead, beetle-attacked or no sign of beetle-attack) of every tree taller than 1.4 m with a DBH of ≥ 5 cm. For each site, basal area of each tree species was scaled to $m^2\ ha^{-1}$ and expressed as a percentage of the total basal area ($m^2\ ha^{-1}$) of all trees. Mortality of *P. contorta* in each site was calculated as the basal area ($m^2\ ha^{-1}$) of attacked trees over the total basal area ($m^2\ ha^{-1}$) of *P. contorta* and expressed as a percentage. Understory vegetation was surveyed along the same two transects used to survey tree species. Along the center of each transect, three quadrats, each 1 m x 1 m, were established, starting five meters from the northern border of the site, ten meters apart (from the center of the previous quadrat), for a total of six vegetation quadrats per site. Understory vegetation was measured as presence/absence of species at each quadrat, averaged at each site, and expressed as a frequency.

Soil survey and analyses for nutrients and texture

To determine soil mineral nutrients and texture, I randomly collected three soil samples (approximately 20 cm deep and 15 cm in diameter) within each site. In total, 90 soil samples for nutrient and texture analysis (3 soil samples x 30 sites) were collected during the canopy and understory survey. Soil samples were kept on ice and transported within two days to the University of Alberta and stored at -20 °C until further processing.

In August 2016, each soil sample was thawed, individually homogenized and subsampled. Afterwards, subsamples (n = 3 per site) were analyzed for soil texture using a modified hydrometer protocol (Bouyoucos & Agron, 1962; American Society for Testing and Materials International, 1976; Kroetsch & Wang, 2008). Texture was calculated from the hydrometer readings and averaged by site. Remaining soil from the homogenized samples was analyzed by the University of Alberta Department of Renewable Resources Natural Research Analytics Laboratory for inorganic P and N. A modified Kelowna protocol (Soil and Crop Diagnostic Center, 1995) was used to extract and quantify phosphate (PO_4^{3-}). A 2M KCl protocol (Maynard *et al*., 1991) was used to extract nitrate (NO_3^-) and ammonium (NH_4^+), which were then quantified (Jones, 2001). Nutrients were quantified using colorimetry, and values were expressed as mg kg^{-1} of soil were averaged by site.

Biotic soil conditions of field sites: fungal communities

To sample soil fungal communities, I collected ten 20 cm deep x 2.5 cm diameter soil cores within each site, using 10% bleach to sterilize soil corers between each sample. In total, 299 soil cores for soil fungal community characterization (10 samples x 30 sites, one sample

lost) were collected during the canopy and understory survey. Soil samples were kept on ice and transported within two days to the University of Alberta and stored at -20 °C until further processing.

Soil cores were thawed, and individually homogenized with sterile instruments. Approximately 4 g of soil per core were placed in a pre-chilled FreeZone 2.5 L Freeze Dryer (Labcono Corporation, Kansas City, MO, USA) at -50 °C and 0.28 mBar, and lyophilized for 72 hours. Freeze-dried soils were twice-ground and homogenized to a fine powder using a Tissuelyser II (QIAGEN Inc., Mississauga, ON, Canada) for 3 minutes at a frequency of 1 s^{-1}. Genomic DNA was isolated from 250 mg of ground soil using DNeasy® PowerSoil® Kits (QIAGEN Inc., Mississauga, ON, Canada). Nuclease-free water samples (Life Technologies, Carlsbad, CA, USA) were extracted as negative controls.

A two-step polymerase chain reaction (PCR) was conducted to amplify the ITS1 (Internal Transcribed Spacer) region of nuclear rDNA extracted from soil samples. The first amplification of the ITS1 region was performed using primers ITS1F (Gardes & Bruns, 1993) for forward and ITS2 (White *et al.*, 1990) for reverse directions at 5µM concentrations. The first PCR was carried out in 25 µL reactions at 1 µL of each primer, 2.5 µL of DNA extract, 8 µL of nuclease-free water, and 12.5 µL of 2x Platinum Superfi Green PCR MasterMix (Invitrogen, Carlsbad, CA, USA). Each reaction for the first PCR was performed in a Model 6321 Eppendorf Mastercycler Pro S thermal cycler (Eppendorf Canada, Mississauga, ON, Canada) under the following conditions: initial denaturation at 95 °C for 3 minutes, followed by 35 cycles at 95 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds, with a final elongation for 10 minutes at 72 °C. To confirm presence of target amplicons, all reactions were analyzed by 2%

agarose gel electrophoresis using a 50 bp ladder (O'GeneRuler DNA Ladder, Thermo Scientific, USA) as a size standard.

Amplicons generated during the first PCR were diluted 1:0.85, and purified using AMPure XP beads (Beckman Coulter Genomics, Brea, CA, USA), following manufacturer protocols with the following modifications: 17 µL of AMPure beads and two 200 µL 80% ethanol washes were used to purify 20 µL of PCR amplicons, after which the product was eluted in 42.5 µL.

For the second PCR, Illumina Nextera Index barcode primers (Illumina Inc., San Diego, CA, USA) were used, N7xx for forward and N5xx for reverse directions, both at 1 mM concentrations. The second PCR was carried out in 25 µL reactions at 2.5 µL of each index primer, 2.5 µL purified amplicons from the first PCR, 5 µL of nuclease-free water, and 12.5 µL of 2x Platinum Superfi Green PCR MasterMix (Invitrogen, Carlsbad, CA, USA). Each reaction for the second PCR was performed in a Model 6321 Eppendorf Mastercycler Pro S thermal cycler (Eppendorf Canada, Mississauga, ON, Canada)under the following conditions: initial denaturation at 95 °C for 3 minutes, followed by 8 cycles at 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, with a final elongation for 5 minutes at 72 °C.

Amplicons of the second PCR were analyzed by gel electrophoresis as described for the first PCR. Amplicons were then diluted 1:0.75 and purified again using AMPure XP beads (Beckman Coulter Genomics, Brea, CA, USA), following manufacturer protocols with the following modifications: 15 µL of AMPure beads and two 200 µL 80% ethanol washes were used to purify 20 µL of PCR amplicons, after which the product was eluted in 20 µL. Following two-step PCR and purification, ITS1 amplicons were quantified fluorescently using a dsDNA HS

Assay kit on a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA) and pooled into equimolar concentrations. Amplicon library sequencing was performed by the University of Alberta Molecular Biological Sciences Unit, using 2 x 300 bp paired-end reads on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA).

Illumina MiSeq forward and reverse reads (2 x 300 bp) were demultiplexed by the University of Alberta Molecular Biological Sciences Unit, and trimmed, denoised, filtered, rarefied, and classified taxonomically to exact sequence variants (ESVs) with the Quantitative Insights Into Molecular Ecology 2 (QIIME2) pipeline (https://qiime2.org; Caporaso *et al.* 2010; Bolyen *et al.*, 2018). Forward (ITS1F) and reverse (ITS2) primers, reverse compliments, and Nextera adapters were trimmed from sequences using the *q2-cutadapt* plugin (Martin, 2011). Sequences were further trimmed to 239 bp for forward reads, and 220 for reverse reads to maintain a median quality score > 31 across the sequence library. This quality filtering, denoising, merging, and removal of chimeras was achieved using the *q2-dada2* plugin (Callahan *et al.*, 2016).

The taxonomic classifier was trained using the *q2-fit-classifier-naive-bayes* plugin (Pedregosa *et al.*, 2011) against the UNITE developer database and taxonomy was subsequently assigned to representative sequences against the trained classifier with the q*2-classify-sklearn* plugin (Pedregosa *et al.*, 2011). Sequence data was rarefied using the *rarefy* command in QIIME2. Sequences were rarefied to the minimum sequence depth of all soil samples (3648 reads), to account for uneven sequence depths across samples (Gihring *et al.*, 2012).

A total of 638 sequences classified as fungi in the family Malasseziaceae were found across all samples and 448 sequences belonging to this family were present in two negative

control samples. These contaminants were not included in downstream analysis, as they are known opportunistic human skin pathogens and not fungi of interest. Additionally, these taxa were extremely infrequent, and therefore unlikely to impact further statistical analyses on community composition (Gobet *et al.*, 2010). Sequences classified to this family, as well as any sequences unassigned to Kingdom Fungi, were filtered from all samples using the *q2-filter-table* command in QIIME2.

Performance of inoculated seedlings in the growth chamber

I tailored soil fungal communities of outplanted *P. contorta* seedlings by adding small amounts of soil sourced from *P. contorta* forests differing in health. In September 2016, soil was collected from six mature *P. contorta* forests: three of the initial 30 beetle-killed sites, and three with low levels of beetle attack (<10% basal area attacked by beetle), hereafter referred to as 'undisturbed' sites. Beetle-killed sites from which I collected soil inoculum were located at the following coordinates: 55° 37' 19.17" N, -117° 58' 14.06" W; 54° 44' 11.07" N, -118° 57' 11.93" W; 54° 39' 25.3" N, -118° 59' 43.16" W. Undisturbed sites from which I collected soil inoculum were located at the following coordinates: 54° 13' 0.12" N, -116° 2' 56.29" W; 54° 12' 34.16" N, -116° 6' 4.21" W; 54° 12' 32.76" N, -115° 58' 21.22" W.

Within each of these sites, I collected four soil samples, each at a depth of approximately 20 cm. I took these samples at a minimum of ten meters apart to capture within-stand variation of fungal community composition. In total, approximately 8000 cm^3 of soil was collected from each site. Soil inocula was transported on ice and frozen at -20 °C until used to inoculate seedlings, approximately 21 weeks later. At this time, soil inoculum was thawed and homogenized by stand type (beetle-killed versus undisturbed). Homogenizing soils by stand type was done to capture

the average fungal community composition representative of each stand type, capturing variation in fungal community composition at a regional scale.

Seedlings with tailored soil inoculum were grown in a growth chamber prior to outplanting. Fifty mL Ray Leach "Cone-tainer" pots (RCL3; Stuewe & Sons, Tangent, OR, USA) were soaked in 15% bleach and rinsed with deionized water, then filled with a common 70:30 sand:topsoil mix (Canar Rock Products, Edmonton, AB, Canada). Soil was sterilized by autoclaving at 121°C for 90 minutes at 103.4 kPa repeated 24 hours later. To tailor soil fungal communities of seedlings without presumably influencing soil abiotic properties, less than 5% of the total pot was comprised of inoculum soil (2.5 of 50 mL). Pots received soil inoculum from beetle-killed or undisturbed *P. contorta* forests, in addition to a set of control pots receiving no soil inoculum.

Pinus contorta seeds, sourced from seed zones to which the seedlings would be outplanted, were obtained from the Alberta Tree Improvement & Seed Center (Government of Alberta Agriculture and Forestry, Smokey Lake, AB, Canada). Seeds were surface sterilized with 5% bleach solution for 15 minutes, followed by rinsing seeds with deionized water and soaking seeds for 24 hours, followed by a 28 day incubation at 4 °C. Four seeds were sown per pot and culled to one seedling after five weeks in the growth chamber. Growth chamber conditions were maintained at 23 °C, with a 16 hour photoperiod.

Seedlings were grown in three treatments: seedlings receiving soil inoculum sourced from beetle-killed *P. contorta* forests, seedlings receiving soil inoculum from undisturbed sites, and seedlings grown with no inoculum. Each of these inoculation treatments was comprised of 860 pots, for a total of 2580 pots (860 pots x three inoculation treatments). Seedlings were grown

in trays of 200 pots each. To limit the possible variation in the growth chamber, trays of seedlings were rotated 180° every two days, and all trays were rotated around the growth chamber every other day. Randomly moving seedling pots within or between trays was impractical due to the risk of harming seedlings in early stages of germination and establishment. Seedlings were watered daily and fertilized (10-52-10 NPK, iron chelate 0.25 mL L^{-1} dH20, and phosphoric acid 1mL L^{-1} dH20) every four weeks, starting at the fifth week as they showed signs of nutrient stress. Seedling survival was assessed after four months in the growth chamber. Before outplanting, 25 seedlings (nine receiving inoculum from beetle-killed forests, nine receiving inoculum from undisturbed forests, and seven from the control treatment receiving no inoculum soil) were sampled to assess fungal colonization on root tips.

Performance of outplanted seedlings in beetle-killed forests

In June 2017, a total of 722 (347 inoculated with soil from beetle-killed forests, 260 inoculated with soil from undisturbed forests, and 115 receiving no inoculum) seedlings were outplanted into 15 of the beetle-killed sites surveyed in July 2016. Sites were selected based on a principal component analysis (see Results, Fig. 3 and *Statistical analysis*, below) representing variation in soil texture and nutrients (see Results), properties known to influence the composition of fungal communities (Gehring *et al*., 1998; Egerton-Warburton & Jumpponen, 2005; Finlay & Rosling, 2006; Toljander *et al.*, 2006; Treseder *et al.*, 2014). At each site, I established two 2 m x 2 m plots, comprised of four, 1 m x 1 m subplots (Fig. 2). Plots were established at a minimum distance of 2.5 m apart to capture within-site variation. I removed the duff layer from the forest floor within each plot, and trenched (20 cm wide x 15 cm deep) between each subplot to prevent root and fungal ingress. Additionally, all vegetation within 50

cm of the border of each plot was pruned to a height of approximately 5-10 cm to minimize competition with seedlings. Planted seedlings were grouped by inoculation treatment into the bare mineral soil of subplots, which were randomly assigned an inoculation treatment. Seedlings were planted a minimum of 10 cm from the subplot edges, and from each other, with soil plugs from their pots still intact to minimize root disturbance. In August 2018, height and survival of seedlings was assessed.

Statistical analysis

Abiotic conditions of field sites

All statistical analyses were carried out using the R v.3.5.1 (R Development Core Team, 2018) statistical software. To investigate the variation among the 30 sites based on soil abiotic properties, I used a principal components analysis (PCA) of variables: percent sand, silt, and clay; PO_4^{3-}, NO_3^-, and NH_4, as implemented by the *princomp* function in the *stats* package (R Development Core Team, 2018). Sites were then plotted in an ordination, with soil abiotic variables as vectors. These results were used to establish prospective sites for outplanting seedlings, based on site placement with regards to variable vectors.

Understory communities of field sites

In addition to soil nutrients and texture, sites were also characterized by understory vegetation. The *pamk* function in the *fpc* package (Hennig, 2018) was used to cluster sites based on similarity in understory vegetation community composition. Indicator species analysis of communities in these clusters was performed using the *multipatt* function in the *indicspecies* package (De Cáceres & Legendre, 2009).

Biotic soil conditions of field sites: fungal communities and guilds

Frequency of an ESV was calculated by converting sequence abundance (number of reads) in a sample into presence or absence, and then averaged by site. This method of estimating species abundance circumvents some biases introduced during PCR amplification and sequencing, especially biases in abundance between ESVs (Amend *et al.*, 2010; Benjamini & Speed, 2012; Lindahl *et al.*, 2013; Kebschull & Zabor, 2015). To examine the representation of sequence abundance of ESVs by these frequency values, I analyzed the correlation between the frequency and average sequences of each ESV.

To investigate the variation among the 30 sites based on soil fungal community composition, I created a Bray-Curtis distance matrix from frequency values of each ESV at each site. A non-metric multidimensional spacing (NMDS) ordination was then carried out with the *nmds* function in the *vegan* package (Oksanen *et al.*, 2018). Sites were represented as points in the ordination, with positions to one another corresponding to similarity of fungal community composition. Using indirect gradient analysis, abiotic soil variables and percent live, and percent dead trees were fitted on the NMDS ordination as vectors. Percent live, and percent dead trees were calculated by dividing the basal area (m^2 ha^{-1}) of live trees and dead trees by the total basal area of all trees, respectively.

The *pamk* function in the *fpc* package was used to cluster sites based on similarity and dissimilarity in fungal community composition. The resulting output indicated an optimal grouping of two clusters, which were represented by point shape on the NMDS ordination. To confirm significant differences between the two clusters of sites, I performed a permutational multivariate analysis of variance (perMANOVA) with the *adonis* function in the *vegan* package,

using 999 iterations. Soil abiotic variables of both clusters were then further described with summary statistics, and differences were discerned using two-tailed t-tests among clusters.

Community composition of soil fungi was described at the phylum level and order level and expressed as percentages of all fungal ESVs. These percentage values were calculated for the entire community dataset across all sites, as well as within each cluster. Frequency values of each ESV were used to calculate percent relative abundance of ESVs across all sites and within clusters, and a rank abundance curve was generated and used to identify the most abundant taxa. Indicator species analysis was performed among the clusters using the *multipatt* function in the *indicspecies* package.

Functional guilds were assigned to ESVs by taxonomic affiliation using the default parameters in the FUNGuild database (Nguyen *et al.*, 2016). Only assignments deemed as 'Highly Probable' or 'Probable' were included in analyses of functional guilds, and ESVs assigned to more than one guild were excluded. Additionally, only assignments to the guilds 'Ectomycorrhizal' and 'Plant Pathotroph', and 'Saprotroph' were used for analyses; ESVs assigned to 'Leaf Saprotroph', 'Litter Saprotroph', 'Plant Saprotroph', 'Soil Saprotroph', 'Undefined Saprotroph', and 'Wood Saprotroph' were pooled into 'Saprotroph'. Diversity of each guild (Ectomycorrhizal, Saprotrophic, Pathotrophic) at each site was calculated using the Shannon diversity index, and t-tests were used to discern differences in mean guild diversity among clusters.

Performance of outplanted seedlings in the growth chamber

To test whether inoculation treatment influenced seedling survival after 17 weeks in the growth chamber, I used a generalized linear binomial logistic regression model with the function *glm* in the package *nlme* (Pinheiro *et al.*, 2018), with seedling tray as a random effect. A one-way ANOVA was then used to test for significant differences in seedling survival by inoculation treatment, followed by Tukey's *P*-adjustments for pairwise comparisons. Coefficients and 95% confidence intervals, expressed as logits in the raw output, were exponentiated and divided by the resulting value plus one to generate survival probabilities.

Performance of outplanted seedlings in beetle-killed forests

Outplanted seedlings were assessed for survival and height after 14 months. Cluster (as defined above) and inoculation treatment, and their interaction were included as two-level explanatory factor in survival and height models. To test if inoculation treatment influenced outplanted seedling survival, I used a generalized linear mixed effects logistic regression model with the function *glmer* in the package *lme4* (Bates *et al.*, 2015). Here, I included plot within site as a random effect to account for within-site variation and included the cluster into which sites were grouped as a predictor along with cluster × inoculation treatment. The mixed effect model was tested against a generalized linear logistic regression model with the *glm* function in the package *lme4*, constructed in the same manner, but without the random effects term. Resulting Aikake's Information Criterion (AIC) values were used to confirm the generalized linear mixed model as the more optimal model (AIC = 331 for mixed effects model, AIC = 468 for the generalized linear model). A two-way ANOVA was then used to test for significant differences in seedling survival by inoculation treatment, or interaction effects between inoculation treatment

and cluster. Probability coefficients and 95% confidence intervals were then calculated from the output logit values in the same manner as for the seedling survival in the growth chamber.

To test if inoculation treatment influenced outplanted seedling height, I used a linear mixed effects model with the function *lme* in the package *nlme*. This model was constructed identically to the survival generalized mixed model, with plot within site as a random effect. A linear model was also created using the *gls* function in the *lme4* package, without the random effects term. AIC values indicated the mixed model as the more optimal model (AIC = 294 for mixed effects model, AIC = 330 for linear model). A one-way ANOVA was then used to test for significant differences in seedling height by inoculation treatment. Marginally significant interactions were found between inoculation treatment and cluster ($p = 0.064$). As such, an additional linear mixed model was constructed for each cluster, followed by multi-way ANOVAs and pairwise comparisons with Tukey's method for *P*-adjustments.

Results

Understory vegetation survey

Understory vegetation consisted of *Rosa accicularis*, *Cornus canadensis*, *Linnaea borealis*, *Chamaenerion angustifolium*, *Lathyrus ochroleucus*, *Aralia nudicaulis*, *Ledum groenlandicum*, *Rubus ideaus*, *Fragaria virginiana*, and *Pyrola asarifolia* across most sites. Sites characterized by greater silt content were inhabited by *Rubus pubescens*, *Viburnum edule*, *Calamagrostis canadensis*, *Lonicerum involcrata*, *Gymnocarpium dryopteris*, *Ribes lacustre, Rubus parviflorus*, *Equisetum sylvaticum*, and *Oplopanax horridus*. Sites characterized by greater sand content were inhabited by *Vaccinium vitis-idaea*, *V. myrtilloides*, *Maianthemum canadense*, *Arctostaphylos uva-ursi*, *Shepherdia canadensis*, *Amealnchier alnifolia*, and *Galium boreale*. See *Statistics*, below, for clustering of sites by understory vegetation communities and indicator species analysis at each of these site clusters.

Abiotic soil conditions of field sites: nutrients and texture

Soils differed among study field sites (see Table 1 for descriptive statistics). Of the nutrients, NO_3^- had the highest coefficient of variation (115%) across all field sites, and silt was the most variable textural fraction of the soil (Table 1). Texture and nutrients (% sand, silt, clay, and PO_4^{3-}, NO_3^-, and NH_4^+) were important vectors differentiating sites as indicated by PCA (Fig. 3). Two main groupings emerged (explained variation of PCA axis 1 = 66.5%, PCA axis 2 = 16.9%); one group of field sites had soils high in PO_4^{3-} and which were sandier in texture (herein referred to as "Sandy Sites"), and the other group of field sites had soils high in NH_4^+ and which were dominated by clay and silt fractions (herein referred to as "Loamy Sites").

Biotic soil conditions of field sites: fungal communities

A total of 13,628,373 sequences amplified across all field sites. Denoising and quality filtering reduced this value to 6,401,928 sequences. After rarefaction, 1,090,752 sequences remained, and 1091 exact sequence variants (ESV) of fungi were assigned to sequences. A total of 3251 sequences were unassigned after taxonomic classification and were omitted from further analysis.

The frequency of sequences per ESV across sites was deemed an appropriate conservative estimate of soil fungal abundance, as opposed to sequence counts. There was a moderately strong relationship ($r = 0.63$; $P = 0.001$) between the average frequency of ESVs across sites and the average sequence count (Fig. 4). The ESV classified as *Geminibasidium* sp. 1 was a notable outlier, which may be due to a higher count of ITS copies, although there is little documentation of this relatively recently identified taxon. Ascomycota was the most dominant phylum across all sites, comprising 49.88% of all ESVs, followed by Basidiomycota (38.07%), Mortierellomycota (9.03%), and Zygomycota (1.36%). Other basal or cryptic phyla (Zoopagomycota, Rozellomycota, Olpidiomycota, Chytridiomycota, Entomophtoromycota, Blastocladiomycota, and GS19) accounted for 0.39% of all ESVs across sites. Of the 1091 ESVs, 1.27% of taxa were unidentified at the phylum level (Table 2).

Soil fungal community composition differed by site, as indicated by NMDS (stress = 0.16, r^2 of stress = 0.79; Fig. 5). Silt, clay, and NH_4^+ vectors passed directly through Loamy Sites, and PO_4^{3-} and sand vectors were shown to pass directly through the center of Sandy Sites, whereas NO_3^- was orthogonal to the rest of these soil variables. Sites, positioned by the composition of soil fungal communities in ordination space, were found to group into two

clusters by k-means clustering of compositional similarity (Fig. 6). Specifically, sites were algorithmically weighted by similarity within cluster versus between clusters, and any more than two clusters were deemed not to have enough within-cluster similarity and between-cluster dissimilarity. This is noted by the "elbow" effect of Figure 4. Differences in the fungal community composition between these clusters, Loamy Sites (n= 14 sites) and Sandy Sites (n=16 sites), were confirmed by perMANOVA ($F_1 = 8.479$, $P < 0.001$). Field sites comprising Sandy Sites and Loamy Sites also matched those differentiated by soil abiotic properties (Fig. 5). That is, the separation of field sites by fungal community composition aligned with the separation of field sites based on soil abiotic properties. Soil nutrients and texture of the two groups of field sites are shown in Tables 3 & 4.

The most abundant taxa were represented nearly equally between Sandy Sites and Loamy Sites. A rank abundance curve (Fig. 7) showed a natural break at the 52nd most abundant ESV. Plotting of the 52 most abundant ESVs (Fig. 8) and all 1091 ESVs (Fig. 9) by relative abundance indicated that many of the most abundant taxa were represented almost equally among the two clusters, yet as relative abundance of ESVs decreased, representation of each ESV generally became less equal. The percent of ESVs classified to the phylum and order levels in Loamy Sites and Sandy Sites are listed alongside those of all sites in Table 2.

Soil abiotic properties significantly explained variation among sites in fungal community composition, yet proportions of live and dead standing trees did not, as indicated by indirect gradient analysis and vector fitting (Appendix 1). Of soil properties, percent sand explained the greatest amount of variation in fungal community composition across study sites ($r^2 = 0.76$, $P = 0.001$), followed by percent silt ($r^2 = 0.74$, $P = 0.001$), percent clay ($r^2 = 0.72$, $P = 0.001$), PO_4^{3-} ($r^2 = 0.68$, $P = 0.001$), NH_4^+ ($r^2 = 0.40$, $P = 0.002$) and NO_3^- ($r^2 = 0.37$, $P = 0.001$). Loamy and

Sandy Sites also differed significantly by all soil abiotic variables except NO_3^-, as revealed by Welch's t-sample *t*-tests (Appendix 2).

In total, 143 ESVs were found to be indicator species: 95 in Loamy Sites, and 48 in Sandy Sites. Indicator ESVs were also assigned to guilds; indicators of Loamy Sites included saprotrophic genera such as *Alatospora* (two ESVs), *Scutellina* (three ESVs), *Hypholoma*, *Pholiota*, *Gymnopilus*, *Odontia*, and *Hypochnicium* (two ESVs). Ectomycorrhizal indicators of Loamy Sites included genera such as *Tomentella* (two ESVs), *Russula*, *Alpova*, *Thelephora*, *Spaerosporella*, *Cortinarius*, *Wilcoxina*, *Rhizopogon, Hebeloma* (two ESVs)*, Clavulina*, *Lactarius* (two ESVs)*, Laccaria, Tylospora*, *Trichiloma*, and those in the family Inocybaceae (five ESVs). Pathotrophic fungal indicators in Loamy Sites included the genera *Cystodendron*, *Ramularia* (two ESVs), *Volutella*, and *Galerina*. Other indicators of Loamy Sites included dark septate endophytes belonging to the genera *Oidiodendron* (also Ericoid mycorrhizal), *Mollisia*, and *Phialocephala*. Saprotrophic indicator ESVs of Sandy Sites included those in the genera *Paratritirachium*, *Geminibasidium*, *Umbelopsis* (two ESVs), *Luellia*, *Plectania*, *Pseudoplectania*, *Rhinocladiella*, *Lycoperdon*, *Cladophialophora*, *Basidiodendron*, *Trechispora*, *Perenniporia*, *Mycena*, and *Scopuloides*. Ectomycorrhizal indicator ESVs in Sandy Sites belonged to the genera *Russula* (three ESVs), *Tomentellopsis*, *Tricholoma* (two ESVs), *Sarcodon*, and those in the family Inocybaceae (two ESVs). Indicator ESVs classified as pathotrophs included those in the genera *Phacidium* and *Devriesia*. Dark septate endophytes such as *Leptodontium* and *Capronia* were also found to be indicator ESVs of Sandy Sites. Only two indicator ESVs were found to be within the 52 most abundant taxa, *Geminibasidium* sp. 1 (ESV982) and *Paratritirachium* sp. 1 (ESV1045). Both taxa were found to be indicators of Sandy Sites, and are recently identified to be heat- and xerotolerant genera. Across all sites,

saprotrophic fungal diversity was greater than ectomycorrhizal fungal diversity (Welch's $t(57.7) = -7.176$, $P = 1.514e^{-9}$). Ectomycorrhizal and saprotrophic diversity was greater in Loamy Sites (Welch's $t(23.2) = 3.267$, $P = 0.003$ for ectomycorrhizal; Welch's $t(26.6) = 2.677$, $P = 0.012$ for saprotrophic) than in Sandy Sites, yet the diversity of pathotrophic fungi did not differ between these clusters (Welch's $t(27.5) = -0.537$, $P = 0.595$; Fig. 10).

Performance of inoculated seedlings in the growth chamber

Prior to outplanting, seedling survival was assessed among the three inoculation treatments: inoculum sourced from beetle-killed *P. contorta* forests or undisturbed *P. contorta* forests, and no soil inoculum. After four months growing under controlled conditions of the growth chamber, seedlings receiving soil inoculum sourced from beetle-killed *P. contorta* forests had lower probability of survival ($P = 0.009$; 18.8%, upper/lower 95% CI: 36/6.7) than seedlings receiving no soil inoculum or inoculum from undisturbed sites (Appendix 3). The probability of survival for seedlings receiving soil inoculum sourced from undisturbed *P. contorta* pine forests (83.8%, upper/lower 95% CI: 94.7/65.3) did not differ from that of seedlings receiving no inoculum (69.2%, upper/lower 95% CI: 88.9/43.8).

Performance of outplanted seedlings in beetle-killed forests

Of the 722 seedlings outplanted in the field to sites categorized as 'Loamy Sites' and 'Sandy Sites' (see Fig. 5 for distribution of field sites based on fungal community composition, Table 2 for fungal ESV distributions in 'Loamy' and 'Sandy Sites', and Tables 3 & 4 for average abiotic soil conditions), 72.3% of seedlings survived. After 14 months following outplanting, the probability of survival did not differ among inoculation treatments ($P = 0.365$) or between the

Loamy and Sandy Sites ($P = 0.163$; Appendix 4). Seedlings receiving soil inoculum from beetle-killed *P. contorta* forests had a probability of survival of 59% (upper/lower 95% CI: 76.3/38.7) in Loamy Sites, and 81% (upper/lower 95% CI: 92.2/60.6) in Sandy Sites. Seedlings receiving soil inoculum from undisturbed *P. contorta* forests had a probability of survival of 67% (upper/lower 95% CI: 82.6/46.3) at Loamy Sites, and 76% (upper/lower 95% CI: 90/53) at Sandy Sites. Seedlings receiving no soil inoculum had a probability of survival of 66% (upper/lower 95% CI: 84.2/41.9) at Loamy Sites, and 85.3% (upper/lower 95% CI: 94.8/64.7) at Sandy Sites.

The effects of soil inoculation on seedling height depended on site type (inoculation × Cluster; $F_{2, 52} = 3.08$, $P = 0.054$). Main effects of soil inoculation treatment and site type were not significant (inoculation treatment $F_{2, 52} = 0.247$, $P = 0.782$; Cluster $F_{1, 13} = 0.026$, $P = 0.873$; Appendix 5). Height of outplanted seedlings differed by soil inoculation treatments only when planted to Sandy Sites ($F_{2, 22} = 6.751$, $P = 0.005$; Appendix 6; Fig. 11). In Sandy Sites, seedlings receiving soil inoculum from beetle-killed *P. contorta* forests had a mean height of 6.0 (± 0.4) cm, and seedlings receiving soil inoculum from undisturbed *P. contorta* forests had a mean height of 5.3 (± 0.4) cm, which was not significantly different from seedlings receiving no inoculum (Fig. 11). Height of seedlings receiving no soil inoculum (mean ± cm) was similar to those receiving inoculum from beetle-killed *P. contorta* forests (Appendix 7). There were no height differences among seedlings receiving soil inocula from different origins in 'Loamy Sites' ($F_{2, 30} = 0.182$, $P = 0.8343$; Fig. 11; Appendix 6).

DISCUSSION

Fungal community composition across heterogeneous soils in beetle-killed forests

Soil fungi in beetle-killed *Pinus contorta* forests differed in species composition indicating that within a single landscape-level disturbance, heterogeneity in belowground communities occurs. Specifically, I found two main groups of fungi: those inhabiting soils with greater ammonium and silt content ('Loamy Sites'), and those inhabiting soils with higher phosphate and sand content ('Sandy Sites'). Soil texture, phosphate, ammonium, and nitrate explained significant variation in fungal community composition, yet proportions of live and dead standing trees did not. These results indicate success in capturing a range of soil conditions while minimizing variation among sites due to canopy tree mortality. Aside from nitrate, the same measured abiotic properties differentiated soils and fungal communities. Texture can influence available water holding capacity and water regime (De Jong & Shields, 1988), which can subsequently influence fungal community composition (Kennedy *et al*., 2015). In addition to community composition, diversity of some fungal functional guilds also differed between the two soil types.

Saprotrophic fungal diversity and species indicators across beetle-killed forests

Once trees die, we expect the abundance and diversity of saprotrophic fungi to increase (Štursová *et al.*, 2014; Saravesi *et al.*, 2015), and under some conditions, to replace EcM fungi (Gagdil & Gadgil, 1971; Fernandez & Kennedy, 2016). In the current study, I found that the diversity of saprotrophic fungi was greater than the diversity of EcM fungi, across all beetle-killed sites. However, changes in fungal communities are not uniform across these sites; underlying site variation influences the composition of these communities. Specifically,

saprotrophic diversity was greater in Loamy Sites characterized by greater amounts of ammonium, than in Sandy Sites with greater amounts of phosphate. Previous studies have linked increased ammonium to greater saprotroph abundance and richness (Štursová *et al.*, 2014 Saravesi *et al*., 2015). Saprotrophic fungi are known to mineralize organic N into forms such as ammonium, and their higher diversity in Loamy Sites may be driving the mineralization of organic N to ammonium in soils of these sites (Leake *et al.*, 2002).

Saprotrophic fungi belonging to the genera *Geminibasidium* and *Paratritirachium* were indicators of Sandy Sites. *Geminibasidium* and *Paratritirachium* are two recently described 'Unidentified Saprotroph' genera that are known to be heat- and xerotolerant (Nyugen *et al.*, 2013; Nyugen *et al.*, 2014). *Geminibasidium* and *Paratritirachium* were the only two taxa in this study to be both indicators and abundant across all sites (Fig. 8; *Geminibasidium* is ESV982, *Paratritirachium* is ESV1045). Given their status as indicators of Sandy Sites and their abundance in those sites, elucidating the biology and ecology of these taxa may be beneficial to understanding their roles in forest ecosystems, especially in the context of *P. contorta* disturbance. Additionally, most indicator taxa assigned as saprotrophs were listed as 'Undefined Saprotroph', and therefore warrant further investigation as to their preferred substrate and possible functions in beetle-killed *P. contorta* forests.

Ectomycorrhizal fungal diversity and species indicators across beetle-killed forests

In contrast to saprotrophic fungi, when trees die, we expect EcM fungi to decline (Štursová *et al.*, 2014; Treu *et al*, 2014; Karst *et al.*, 2015; Saravesi *et al.*, 2015; Pec *et al.*, 2017). I found that, similar to saprotrophic diversity, a greater diversity in EcM fungi characterized Loamy Sites compared to Sandy Sites. Though I cannot disentangle the effects of nutrients from

texture on EcM diversity in the survey, indicator species suggest some EcM fungi respond to differences in nutrients between Loamy and Sandy Sites. In fact, most EcM fungi indicative of Loamy Sites have been found to increase in richness or abundance across gradients of, or when fertilized with inorganic nitrogen (e.g. ammonium). For instance, I found *Tylospora* to be an indicator of Loamy Sites with high ammonium, aligned with earlier studies showing that this genus occurs in high ammonium soils in boreal forests (Taylor *et al.*, 2000; Toljander *et al.*, 2006, Sterkenberg *et al.*, 2015). Furthermore, I found both *Thelephora* and *Hebeloma* as indicator EcM fungi of Loamy Sites, which have been demonstrated to utilize ammonium in culture and to increase uptake to plants (Finlay *et al.*, 1992). I also found *Laccaria* and *Tomentella* as indicator EcM at Loamy Sites. *Thelephora*, *Hebeloma*, *Laccaria*, and *Tomentella* been found to be nitrophilic and increase in richness along inorganic N gradients (Lilleskov *et al.*, 2001; Lilleskov *et al.*, 2011; Sterkenberg *et al.*, 2015). The abundance of *Laccaria*, *Lactarius* and *Tomentella* have also been noted to increase in sites where ammonium was added to forest soils (Peter *et al.*, 2001; Corrales, 2017). While I found one species of *Tricholoma* indicative of Loamy Sites, two species within this genus were also found in Sandy Sites with low ammonium. *Tricholoma* has been documented as being generally nitrophobic along N deposition gradients (Van der Linde *et al.*, 2018; Lilleskov *et al.*, 2019), though in rare cases it has been found in high N sites (Kårén & Nylund, 1997; Lilleskov *et al.*, 2002). This corroborates the general preference of *Tricholoma* for low ammonium sites, except for *Tricholoma atroveolaceum* in Loamy Sites. *Tomentellopsis*, an indicator in the current study of Sandy Sites with low ammonium and high P, was found to decrease in abundance along an ammonium gradient in boreal humus layers (Sterkenberg *et al.*, 2015).

Within a few genera, some species showed opposing preferences for soil conditions. For instance, I found some ESVs of *Inocybe* to represent Loamy Sites (four ESVs) and others, Sandy Sites (two ESVs). Previous studies have found the genus *Inocybe* to be an ecological generalist. In both fertilized (N and P) and unfertilized plots of *Populus trichocarpa* and *Populus tremula* x *tremuloides*, species of *Inocybe* produced many sporocarps (Baum & Makeschin, 2000). Additionally, Sterkenberg *et al.* (2015) found *Inocybe* to be associated with ammonium in humus layers of boreal forests, corroborating my finding of species of *Inocybe* indicative of Loamy Sites. In addition to *Inocybe*, species in the genus *Russula* also showed opposing preferences. I found four different *Russula* spp. to be indicators, one of Loamy Sites and three of Sandy Sites. Species within *Russula* have been demonstrated to have divergent responses to N deposition (Wallenda & Kottke, 1998; Lilleskov *et al.*, 2011; Van der Linde *et al.*, 2018; Lilleskov *et al*., 2019), and along ammonium gradients (Sterkenberg *et al.*, 2015). Members of the genus *Russula* are reported to differ in the amount and branching of extrametrical hyphae, possibly influencing their ability to explore and acquire nutrients from the soil (Determination of Mycorrhizae database, Ager & Rambold, 2004; Hobbie & Agerer, 2010; Suz *et al.*, 2017). The variability in exploration type within this genus might indicate different foraging strategies adapted by *Russula* species in beetle-killed *P. contorta* forests of differing nutrient availability.

While I found many indicator EcM fungi to corroborate earlier studies showing soil nutrient preferences, several species coincided with those often dominating after stand-replacing disturbances, though almost exclusively in Loamy Sites. The only indicator EcM fungi characteristic of post-disturbance soils that I found at both Loamy and Sandy Sites was *Inocybe* spp., which may increase in abundance after fire (Visser, 1995) clearcutting (Barker *et al.*, 2013), and bark beetle outbreaks (Pec *et al.*, 2017). Indicator EcM fungi such as *Rhizopogon*, *Wilcoxina*

mikolae, and *Tomentella* (Baar *et al.*, 1999), *Thelephora*, *Laccaria*, and *Tylospora* (Glassman *et al.*, 2015), can establish disturbance-resistant propagules as spores or sclerotia, and frequently colonize seedlings after fire (Baar *et al.*, 1999; Glassman *et al.*, 2015) and clearcutting (Barker *et al*, 2013). As these taxa are characteristic of forest soils after a variety of disturbances, their absence from Sandy Sites suggests that soil characteristics impart greater influence over EcM fungal community composition than disturbance type.

Surprisingly, I found *Cortinarius fluvescens* and *Russula foetens* as indicators of Loamy Sites, while I found *R. antracina, R. farinipes*, and *R. turci* as indicators of Sandy Sites. After fire, clearcut harvest, and within the first four years after beetle-kill by *D. ponderosae* (Treu *et al.*, 2014; Pec *et al.* 2017), *Cortinarius* and *Russula* spp. rarely, if ever, were present. Spores are not typically an effective dispersal for Russulaceae (Deacon & Fleming, 1992). However, evidence exists that some members of *Russula* can potentially be opportunistic saprotrophs. Some *Russula* possess extracellular phenoloxidases (Agerer, 2001) and genes for exocellulase and lignin-degrading class II peroxidase (Bödeker *et al.*, 2009; Štursová *et al.*, 2012), which explains the persistence of *Russula* on dead or declining host trees (Bruns, 2005). *Cortinarius* may too exploit organic layers with increased litter input, as many *Cortinarius* can produce similar phenoloxidases (Bödeker *et al.*, 2009; Bödeker *et al.* 2014), potentially allowing them to persist in humus layers after beetle attack.

Performance of inoculated seedlings in the growth chamber

Lower performance of seedlings inoculated with soil from beetle-killed stands agree with results from a greenhouse experiment imposing similar inoculation treatments (Karst *et al.*, 2015). However, whereas I measured survival, Karst *et al.* (2015) measured growth of inoculated

seedlings. Another important difference in the studies was how seedlings responded to lack of inoculum. In the current study, seedlings receiving no inoculum did not significantly differ in survival from seedlings receiving inoculum from undisturbed stands. Karst et al. (2015) found that pine seedlings receiving no inoculum had the lowest growth. In the current study, I fertilized all seedlings, and this difference in methods may have increased survival of seedlings receiving no inoculum.

Performance of outplanted seedlings in beetle-killed forests

If seedling performance (measured as survival, height, biomass) in the field differs by inoculation treatments, we may infer that tailored inoculum can influence outplanting success. Alternatively, if seedling response to inoculation is muted compared to their response to variation in site types, we may infer that inoculation serves no purpose in outplanting success. In this sense, results of outplanted seedling performance may aid in triaging approaches to establish pine seedlings in beetle-killed stands.

Survival of outplanted seedlings did not differ by inoculation treatments, or between Loamy and Sandy Sites. The probability of survival of seedlings receiving inoculum sourced from beetle-killed *P. contorta* forests increased substantially after outplanting (18.8% to 58.8% in Loamy Sites and 81% in Sandy Sites). This may be due to any combination of influences of inoculation treatments (e.g. root-associating fungi or bacteria), abiotic or biotic characteristics of field sites (e.g. resident fungi in field sites, nutrient content), or environmental variation (e.g. temperature, precipitation) for which I did not account. I have yet to harvest outplanted seedlings, and as such, the direct or indirect influences of inoculations and site variables on seedling survival may yet emerge.

In contrast to survival, heights of outplanted seedlings differed by inoculation treatments, but only in Sandy Sites. I found that seedlings receiving soil inoculum from beetle-killed forests were taller than those inoculated with soil from undisturbed forests and seedlings receiving no inoculum. Fungi inhabiting inoculum from beetle-killed stands may have been selected for in sandy soil while establishing in the growth chamber, subsequently imparting greater benefit to seedlings when outplanted into sites with similar characteristics. Alternatively, site conditions may have selected for or against inoculum fungi, thereby modifying seedling performance. Nevertheless, this begs the question of what fungal taxa are forming such relationships with these seedlings. Seedlings are still currently in field soils and will be harvested to identify these taxa in question, their colonization, as well as any further changes in survival, height, and biomass. Seedling responses to inoculations after outplanting may not only aid in triaging sites for rehabilitation efforts, but also in understanding interactions between soil fungi, pines, and their environment.

Synthesis

The pronounced differences in soil fungal community composition and function across study sites indicate that disturbances over a wide geographic range have varying belowground consequences. Though the outcome of this insect outbreak appears uniform at the tree canopy level, caution should be used when predicting outcomes of fungal community structure and function within these forests. In particular, I demonstrate that the composition of fungal communities after beetle outbreaks diverges based on physical and chemical properties of soils. Additionally, I demonstrate that fungal indicators of Loamy Sites, with high ammonium and likely soil moisture, predominately corroborate previous studies in the context of soil physical

and chemical properties, and other forest disturbances. Therefore, caution should be used when inferring the fungal community compositional and functional consequences of regional-scale disturbances that may vary in soil physical and chemical properties.

Furthermore, my results may have implications for the progression of these forests. In naturally occurring small scale disturbances, nutrient fluxes and above- and belowground succession are well understood (Dickie *et al.*, 2013) relative to *D. ponderosae* outbreaks outside of its native range. Soil fungi play vital roles in both nutrient cycling (Read & Perez-Moreno, 2003; Fernandez & Koide, 2012; Clemmensen *et al.*, 2013) and as mutualists that can influence seedling performance (Smith & Read, 2008). Shifts in community composition and function, as mediated by soil texture and nutrients, have consequences for subsequent generations of forests after disturbance (Wiemken *et al.*, 2006; Clark & St Clair, 2011; Karst *et al.*, 2014). As such, I stress the further examination of above- and belowground succession of these beetle-killed forests across soil physical and chemical properties, with paired, mature undisturbed *P. contorta* forest sites. By characterizing fungal communities in mature forests and monitoring the succession of beetle-killed forests, we may elucidate the potential divergence of forests from their successional pathways, or the resilience of forests to disturbance. Seedlings grown with tailored soil inoculum differ in performance when outplanted into beetle-killed forests, but site variation plays a vital role in modifying the outcomes. Therefore, my results stress the importance of further exploring the outplanting of seedlings to forests varying in soil biotic and abiotic properties, as the success of inoculations is subject to site variation of these essential site characteristics.

Tables

Table 1. Abiotic soil properties of beetle-killed *Pinus contorta* forests (n=30) in west central Alberta, Canada.

	Nutrients (mg/kg)			Texture (%)		
	Phosphate (PO_4^{3-})	**Ammonium (NH_4^+)**	**Nitrate (NO_3^-)**	**Clay**	**Silt**	**Sand**
Mean (± SE)	21.59 (± 2.80)	5.78 (± 0.74)	0.38 (± 0.08)	12.67 (± 1.58)	29.49 (± 3.71)	57.85 (± 5.18)
Minimum	1.98	0.93	0.00	2.34	4.15	19.64
Maximum	60.08	16.38	1.94	29.32	56.23	91.35
Coefficient of Variation (%)	71.03	70.14	114.57	68.24	68.91	49.09

Table 2. Taxonomic distribution of exact sequence variants (ESVs) comprising phyla and orders of soil fungi in beetle-killed *Pinus contorta* forests (n = 30) in west central Alberta, Canada, as well as in Loamy Sites (n = 14) and Sandy Sites (n = 16) determined by ESV community cluster analysis.

Phylum	Order	% ESV All sites	% ESV Loamy Sites	% ESV Sandy Sites
Ascomycota		49.88	48.22	51.49
	Helotiales	19.99	21.56	18.48
	Chaetothyriales	4.80	2.88	6.65
	Hypocreales	3.53	3.01	4.03
	Pezizales	2.84	3.59	2.11
	Capnodiales	2.23	2.01	2.46
	Eurotiales	1.96	1.47	2.42
	Thelebolales	1.72	1.42	2.01
	Pleosporales	1.70	1.33	2.07
	Venturiales	1.61	1.38	1.84
	Coniochaetales	1.47	1.41	1.53
	Sordariales	1.34	1.70	0.98
	Saccharomycetales	0.82	1.00	0.64
	Chaetosphaeriales	0.73	0.89	0.58
	Xylariales	0.39	0.41	0.36
	Hysteriales	0.18	0.05	0.31
	Other Ascomycota[1]	1.41	1.16	1.65
	Unidentified	3.16	2.95	3.36
Basidiomycota		38.07	39.45	36.74
	Agaricales	12.08	13.89	10.33
	Thelephorales	3.22	3.70	2.76

	Atheliales	2.72	3.04	2.42
	Filobasidiales	2.67	2.63	2.71
	Russulales	2.50	2.23	2.76
	Polyporales	1.65	1.82	1.50
	Sebacinales	1.65	1.17	2.11
	Leucosporidiales	1.43	1.67	1.19
	Cantharellales	1.24	1.32	1.17
	Geminibasidiales	0.91	0.65	1.17
	Sporidiobolales	0.79	0.41	1.15
	Boletales	0.73	1.02	0.45
	Tritirachiales	0.65	0.08	1.21
	Trechisporales	0.61	0.53	0.70
	Cystofilobasidiales	0.58	0.56	0.60
	Other Basidiomycota[2]	1.56	1.83	1.30
	Unidentified	3.06	2.90	3.22
Mortierellomycota	Mortierellales	9.03	9.85	8.24
Mucoromycota[3]		1.36	0.79	1.90
Other basal or cryptic phyla[4]		0.39	0.48	0.29
Unidentified		1.27	1.20	1.33

[1]Orders included: Mytilinidales, Lecanorales, Geoglossales, Dothideales, Phacidiales, Myrmecridiales, Lecanoromycetes (order, *Incertae sedis*), Orbiliales, Rhytismatales, Lulworthiales, Archaeorhizomycetales, Taphrinales, Glomerellales, Microascales, Togniniales, Phaeomoniellales, Tubeufiales, Botryosphaeriales, Peltigerales, Minutisphaerales, Pezizomycotina (order, *Incertae sedis*), Diaporthales, Dothideomycetes (order, *Incertae sedis*), Myriangiales, Caliciales, Trapeliales, and GS35,of the Phylum Ascomycota.

[2]Orders included: Auriculariales, Microbotryomycetes (order, *Incertae sedis*) Hymenochaetales, Tremellales, Agaricostilbales, Entylomatales, Tremellodendropsidales, Jaapiales , Erythrobasidiales , Hysterangiales, Gomphales, Platygloeales, Cystobasidiales, Amylocorticiales, Gloeophyllales, Exobasidiales, Cycstobasidiomycetes (order, *Incertae sedis*), Trichosporonales, and Ustilaginales, of the Phylum Basidiomycota.

[3] Orders included: Umbelopsidales, Endogonales, and Mucoromycotina (order, *Incertae sedis*), of the Phylum Mucoromycota.

[4] Order included: Zoopagales of Phyllum Zoopagomycota; orders GS04, GS11, and unidentified taxa of Phylum Rozellomycota; order Olpidales and unidentified taxa of Phylum Olpidiomycota;orders Rhizophylyctidales, Rhizophydiales, Spizellomycetales, and unidentified taxa in Phylum Chytridiomycota; order Basidiobolales of Phylum Entomophthoromycota; order Blastocladiales of Phylum Blastocladiomycota, and Phylum GS19.

Table 3. Soil properties of beetle-killed *Pinus contorta* forests (n = 14) in west central Alberta, Canada of Loamy Sites as identified by greater percent of silt than sand or clay, and higher ammonium as compared to Sandy Sites. *Pinus contorta* seedlings were outplanted into nine of these sites.

	Nutrients (mg/kg)			Texture (%)		
	Phosphate (PO_4^{3-})	**Ammonium (NH_4^+)**	**Nitrate (NO_3^-)**	**Clay**	**Silt**	**Sand**
Mean (± SE)	9.64 (± 1.30)	7.51 (± 1.07)	0.46 (± 0.10)	20.96 (± 1.11)	48.94 (± 1.63)	30.10(± 2.05)
Minimum	1.98	1.74	0.00	16.15	32.90	19.64
Maximum	18.86	16.38	1.18	29.32	56.23	50.67
Coefficient of Variation (%)	50.31	53.20	84.15	19.76	12.48	25.44

Table 4. Soil properties of beetle-killed *Pinus contorta* forests (n = 16) in west central Alberta, Canada of Sandy Sites as defined by greater percent of sand than silt or clay, and higher phosphate as compared to Loamy Sites. *Pinus contorta* seedlings were outplanted into six of these sites.

	Nutrients (mg/kg)			Texture (%)		
	Phosphate (PO_4^{3-})	**Ammonium (NH_4^+)**	**Nitrate (NO_3^-)**	**Clay**	**Silt**	**Sand**
Mean (± SE)	32.05 (± 3.39)	4.27 (± 0.89)	0.31 (± 0.12)	5.41 (± 0.76)	12.47 (± 2.55)	82.12 (± 3.17)
Minimum	1.98	0.93	0.00	2.34	4.15	19.64
Maximum	13.73	0.93	0.03	2.34	4.15	41.82
Coefficient of Variation (%)	60.08	15.29	1.94	13.07	45.43	91.35

Figures

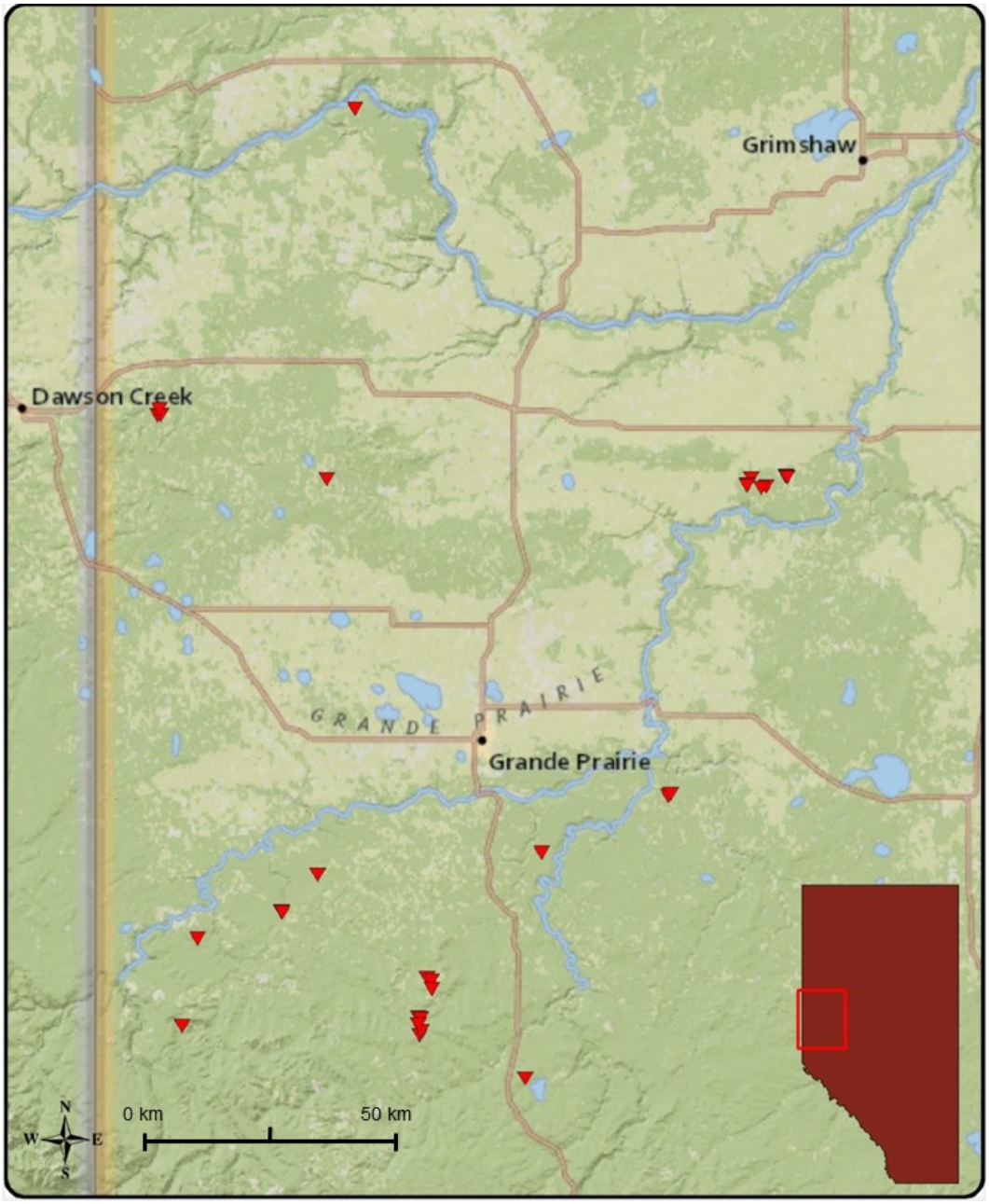

Figure 1. Map of field sites in beetle-killed lodgepole pine (*Pinus contorta*) forests (n=30) of west central Alberta, Canada. Sites are represented by triangles.

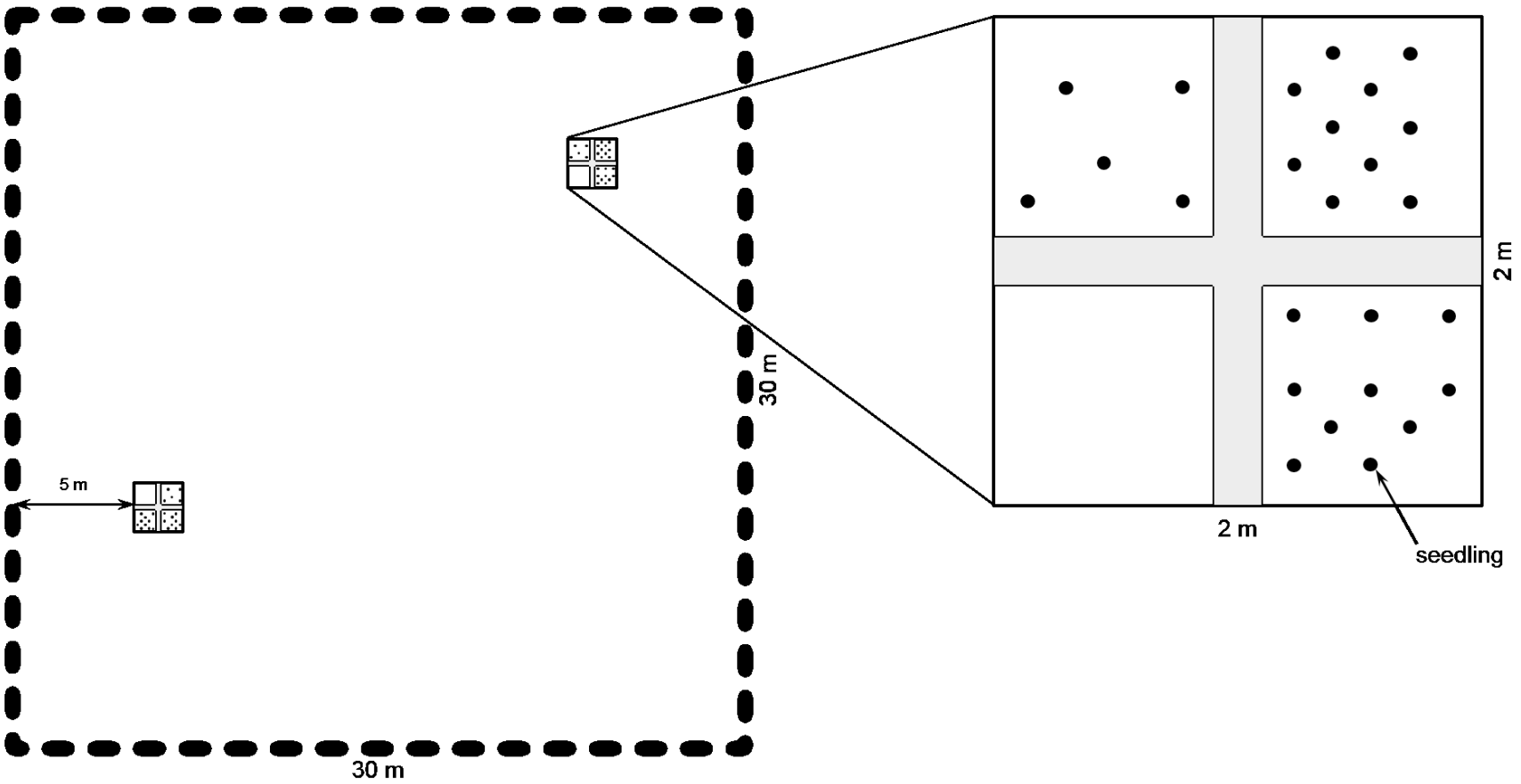

Figure 2. Representative site design for outplanting of seedlings (n = 722) into beetle-killed lodgepole pine (*Pinus contorta*) forests (n = 15) of west central Alberta, Canada. Plots measuring 2 m x 2 m were established within 30 m x 30 m sites (dashed line) surveyed in summer 2016. Subplots were divided by trenches (gray crossed bars).

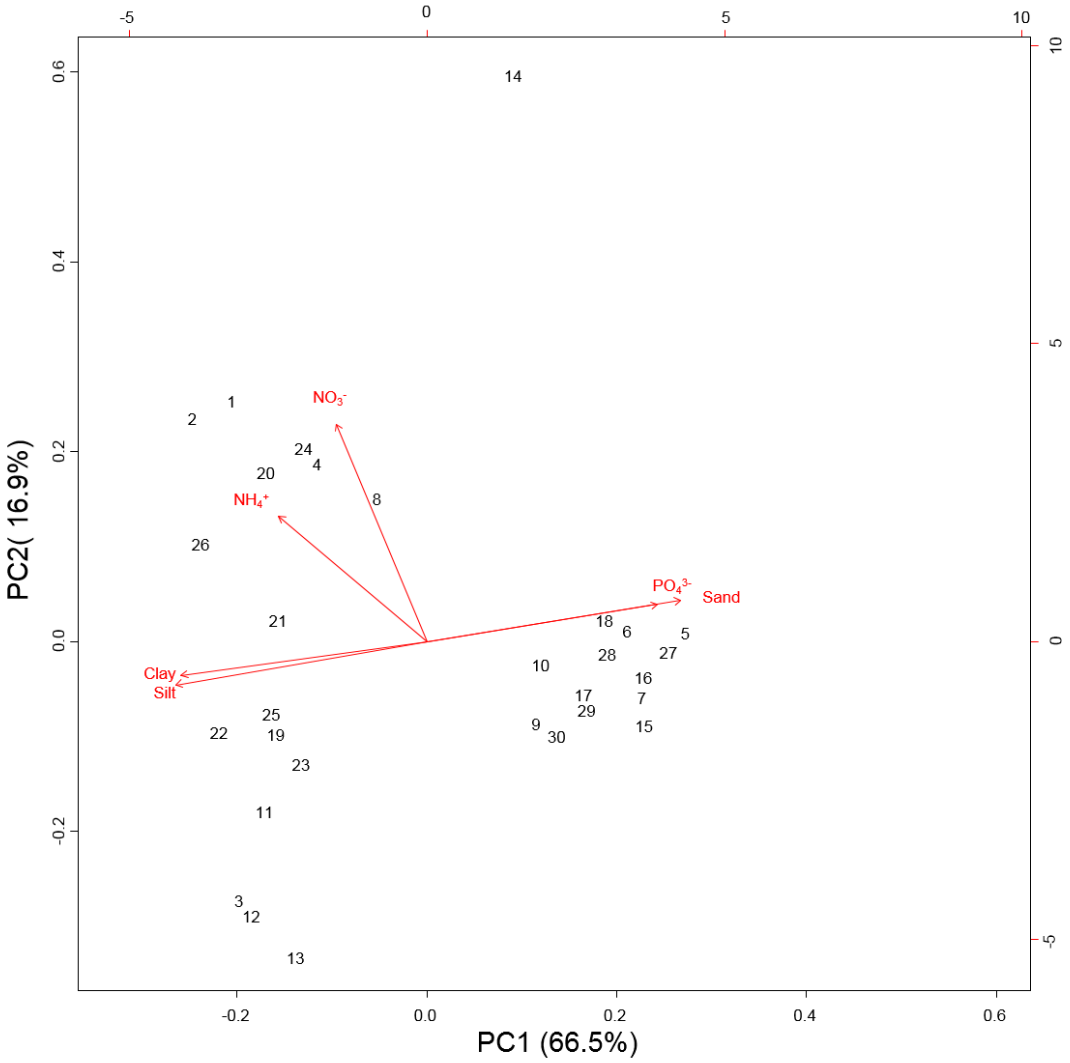

Figure 3. Results of principal component analysis showing variation of nutrients (phosphate, PO_4^{3-}; ammonium, NH_4^+; nitrate, NO_3^-) and texture (percent sand, silt, clay) of soils (red vectors) across beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. All sites are within 95% confidence interval, demonstrating an absence of any outlier sites. Numbers represent field site identifiers.

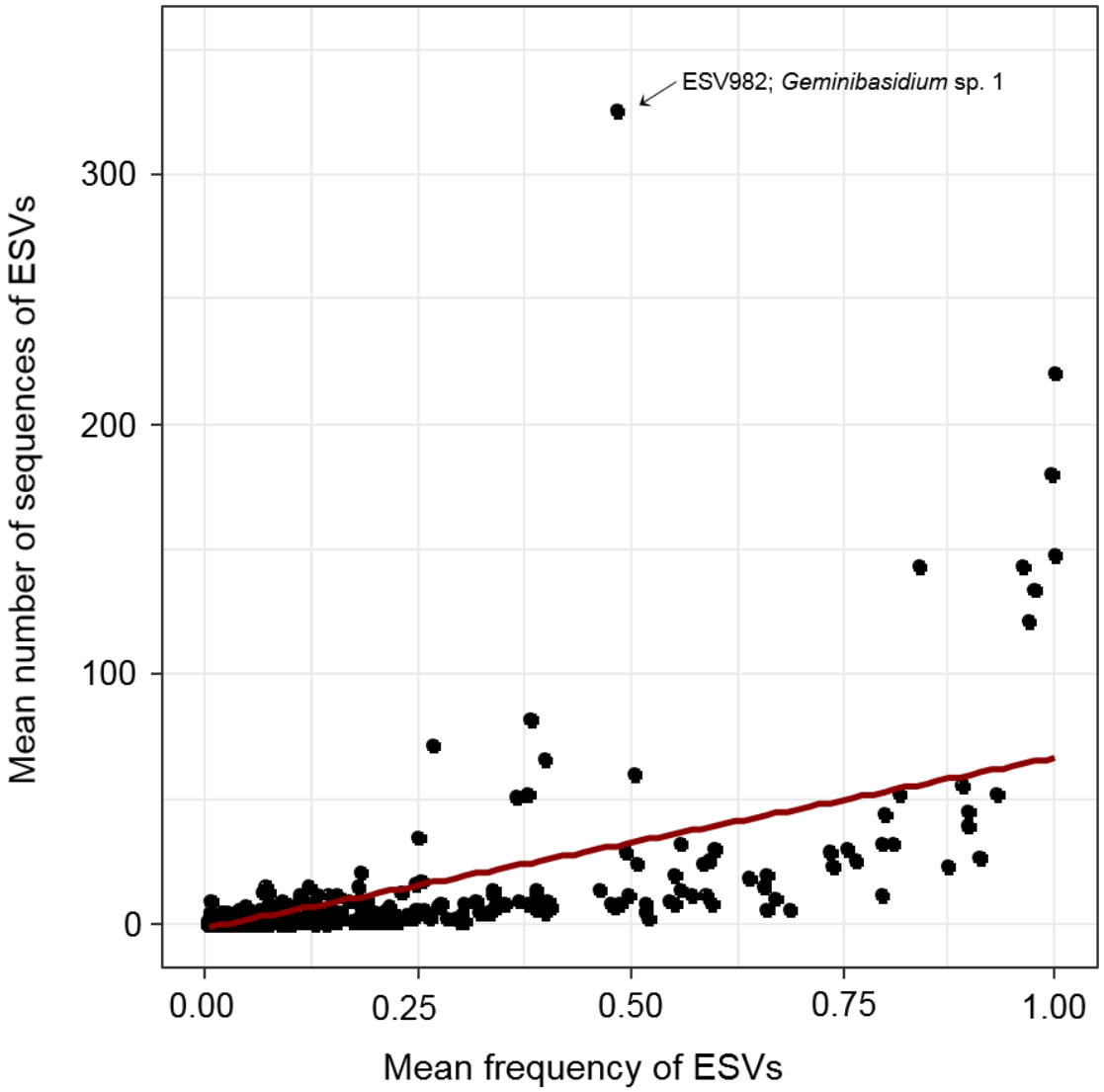

Figure 4. Correlation between mean frequency and mean number of fungal exact sequence variants (ESVs) (n = 1,091) across soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Frequency is calculated from presence and absence values of ESVs in each of 10 soil cores and averaged by site. Pearson's r = 0.63; $P < 0.001$.

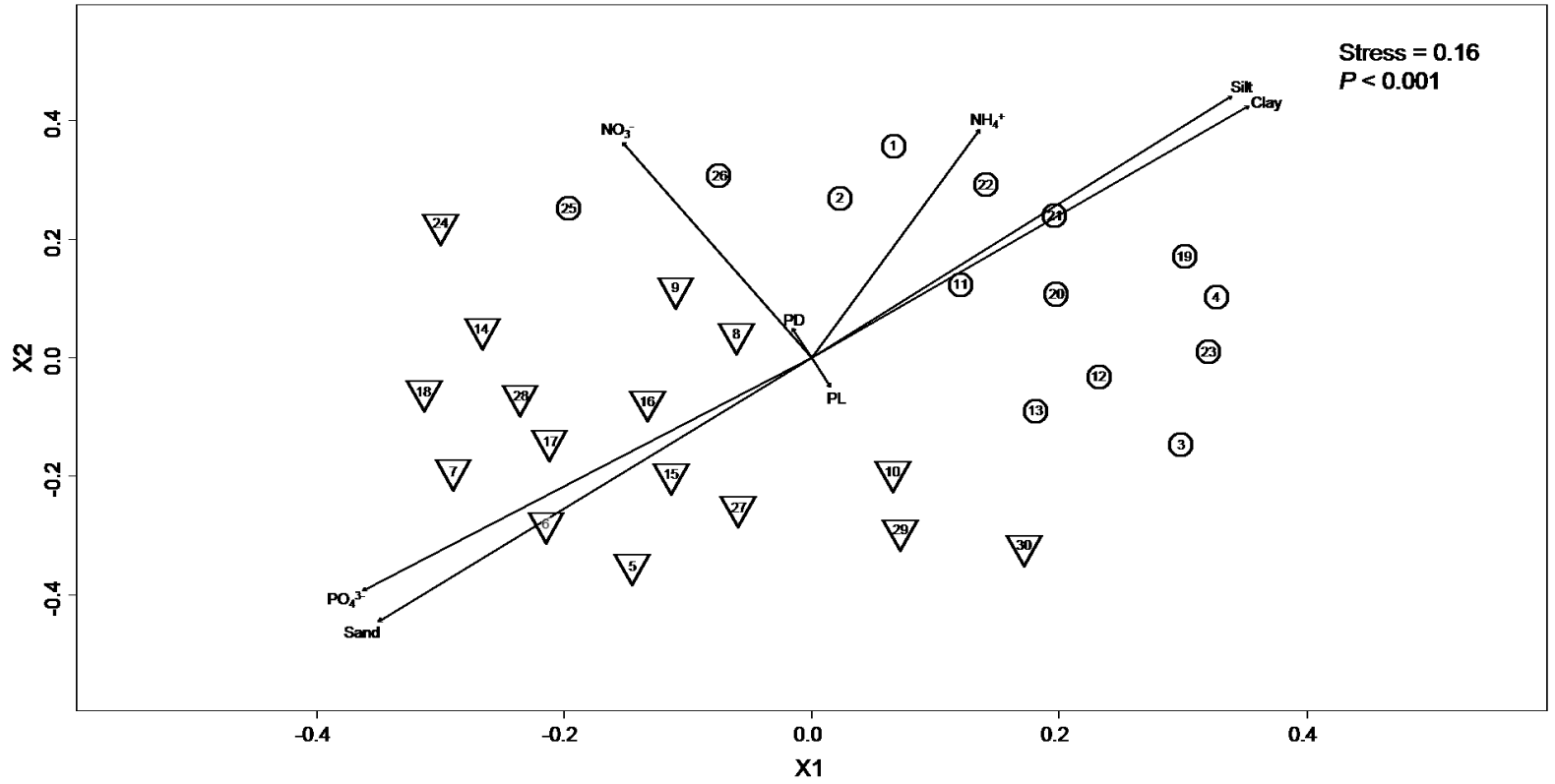

Figure 5. Non-metric multidimensional scaling (NMDS) ordination of fungal communities from soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Shapes around site identifiers represent clusters; the circled sites on the right represent Loamy Sites, and sites in triangles represent Sandy Sites. Predictors are included as black vectors. 'PD' represents the proportion of dead standing trees to total trees, while 'PL' represents the proportion of live trees to total trees (m^2 ha^{-1}).

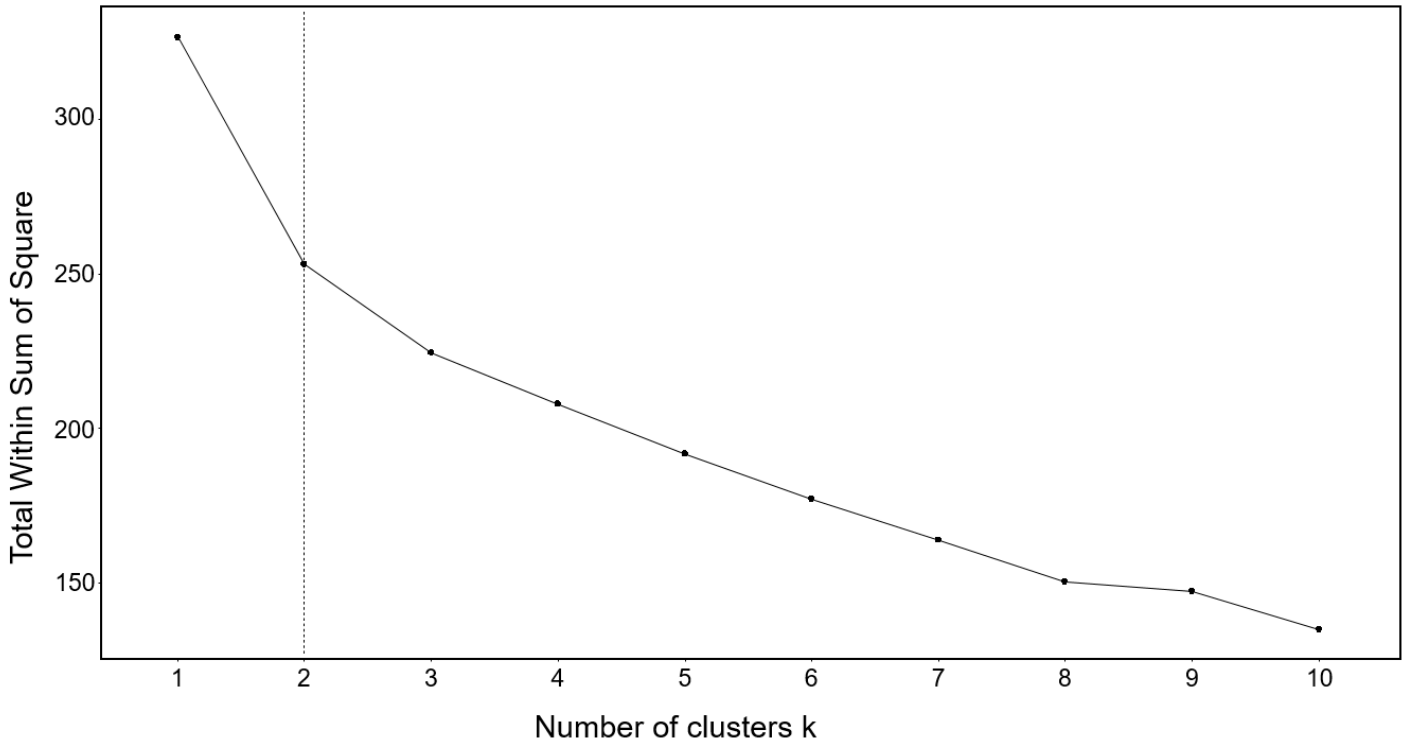

Figure 6. Optimal number of clusters for sites, based on k-means clustering of similarities among fungal communities across soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Optimal number of clusters is indicated by a dotted vertical line.

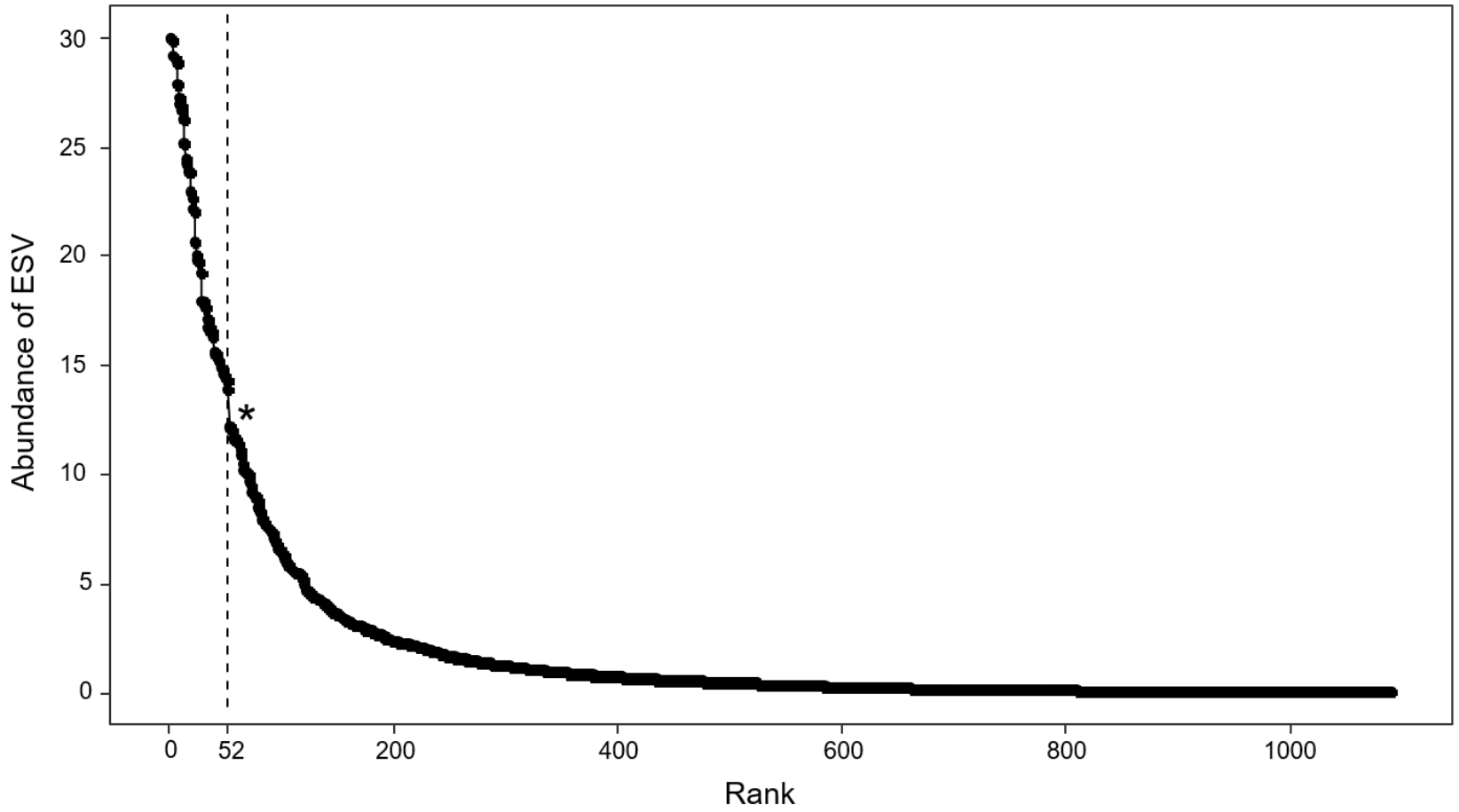

Figure 7. Rank abundance curve of fungal exact sequence variants (ESV) (n = 1091) amplified from soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Abundance is measured by averaging frequency values of each ESV across all sites.

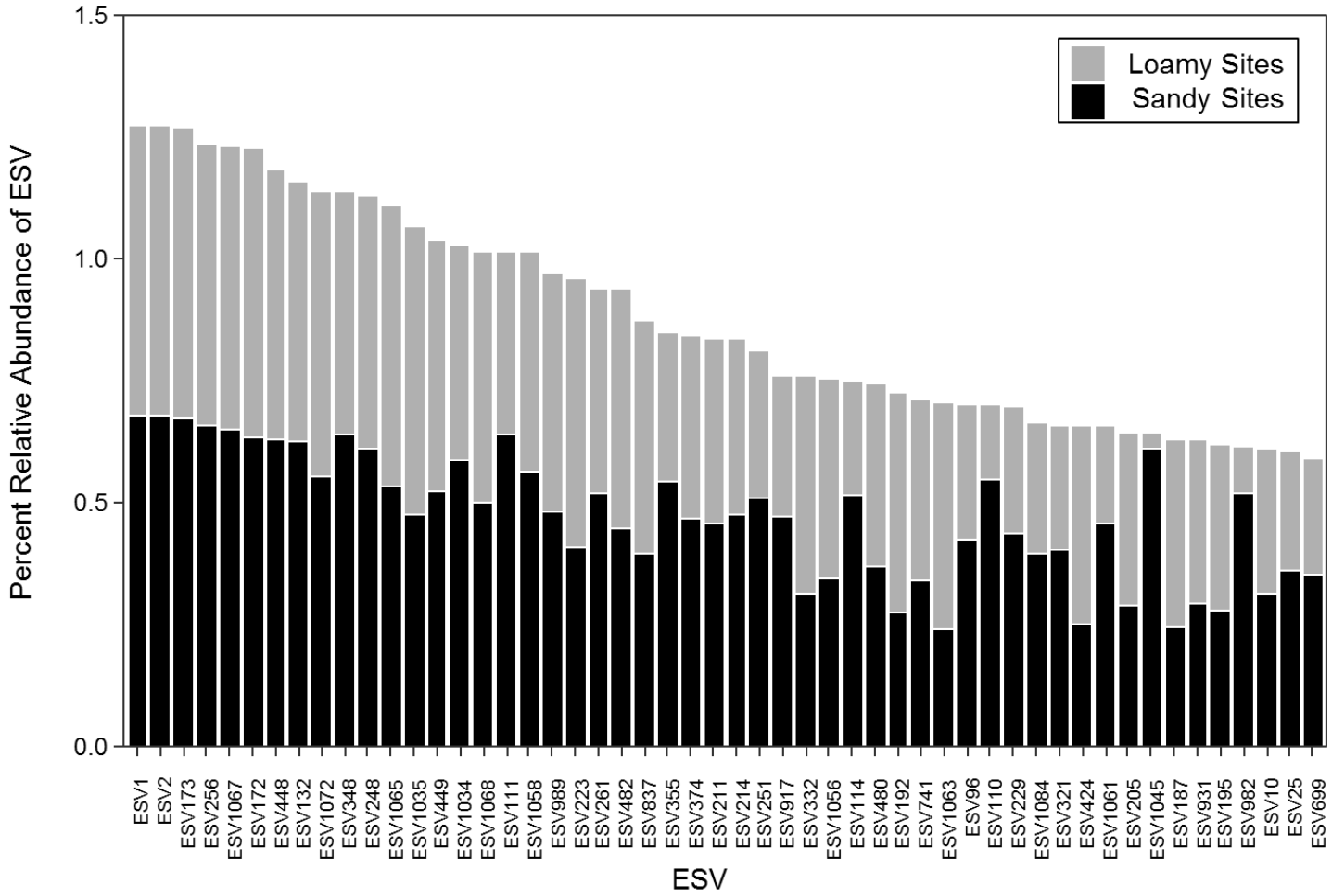

Figure 8. Percent relative abundance of the most abundant fungal exact sequence variants (ESVs) (n = 52) amplified from soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Exact sequence variants were selected based on a natural break in a rank abundance curve (Fig. 7). Gray columns represent the percent relative abundance of ESVs of Loamy Sites, while black columns represent the percent relative abundance of ESVs of Sandy Sites.

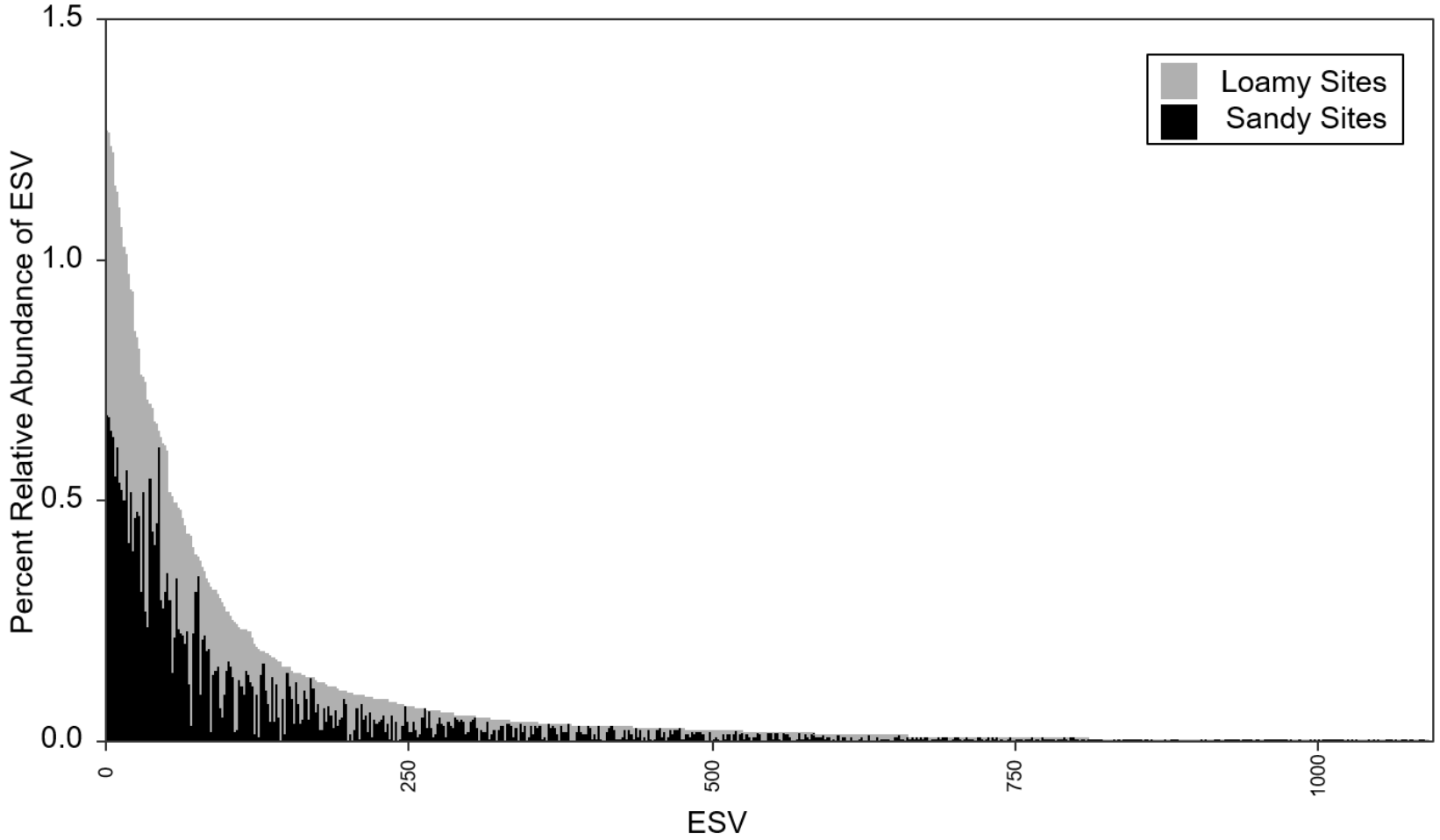

Figure 9. Percent relative abundance of fungal exact sequence variants (ESV) (n = 1091) amplified from soils in beetle-killed *Pinus contorta* forests (n = 30) of west central Alberta, Canada. Gray columns represent the percent relative abundance of ESVs of Loamy Sites, while black columns represent the percent relative abundance of ESVs of Sandy sites.

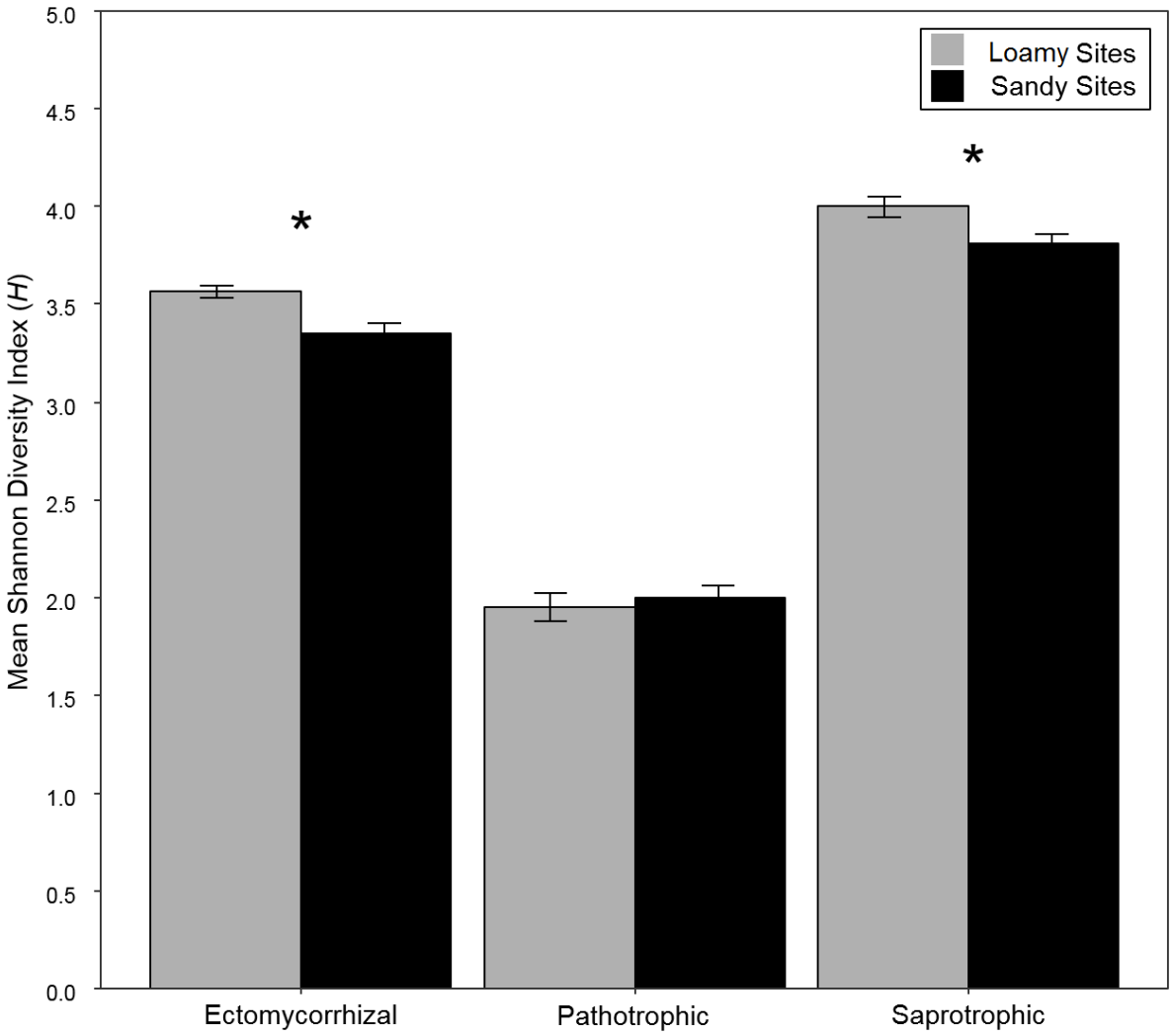

Figure 10. Diversity indices of ectomycorrhizal, pathotrophic, and saprotrophic fungal ESVs between Loamy (n = 14) and Sandy (n = 16) Sites in beetle-killed *Pinus contorta* forests of west central Alberta, Canada. Asterisks represent significant ($P < 0.05$) differences in mean diversity among clusters.

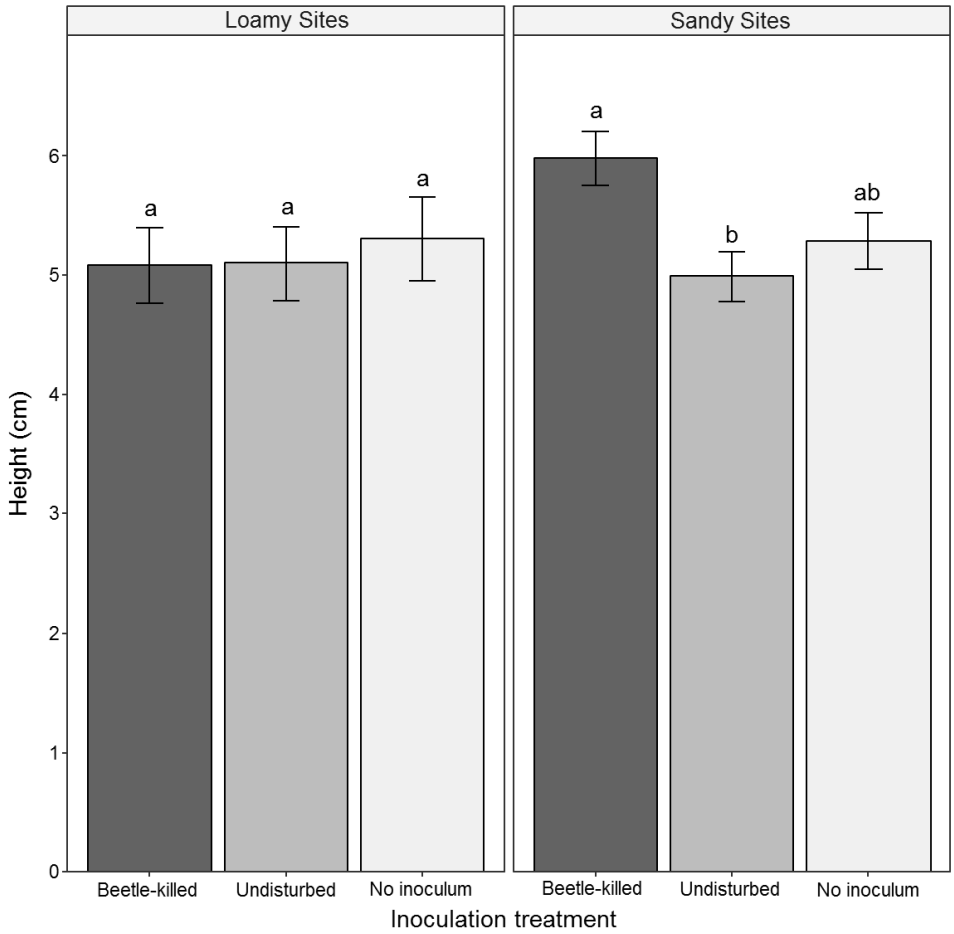

Figure 11. Mean *Pinus contorta* seedling heights (± SE) by soil inoculation treatments and site clusters (Loamy Sites, n = 6; Sandy Sites, n = 9) 14 months following outplanting to beetle-killed forests in west central Alberta, Canada. Letters indicate significant ($P< 0.05$) differences among soil inoculation treatments within clusters.

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Appendix

Appendix 1. Soil abiotic variable and proportion of live trees to total trees ('PL') and of dead tre es to total trees ('PD') vector fitting to NMDS scores of sites positioned by fungal community co mposition. Soils were collected from in beetle-killed *Pinus contorta* forests in west central Alberta, Canada (n = 30).

Predictor	r^2	***P*-value**
Sand	0.76	0.001
Silt	0.73	0.001
Clay	0.72	0.001
Phosphate (PO_4^{3-})	0.67	0.001
Ammonium (NH_4^+)	0.39	0.001
Nitrate (NO_3^-)	0.36	0.002
PL	0.00	0.925
PD	0.00	0.925

Appendix 2. Soil abiotic variable Welch's *t*-tests between Loamy Sites (n = 14) and Sandy Sites (n = 16) beetle-killed *Pinus contorta* forests in west central Alberta, Canada.

Predictor	**Df**	***t*-value**	***P*-value**
Sand	25.0	-13.783	$3.33e^{-13}$
Silt	24.9	12.054	$6.59e^{-12}$
Clay	23.5	11.58	$3.237e^{-11}$
Phosphate (PO_4^{3-})	19.2	-6.168	$5.974e^{-06}$
Ammonium (NH_4^+)	26.3	2.33	0.027
Nitrate (NO_3^-)	27.9	0.977	0.336

Appendix 3. Logistic binomial regression analysis of *Pinus contorta* seedling survival of seedlings (n=2053) inoculated with soil sourced from beetle-killed (n = 744) and undisturbed (n = 675) *Pinus contorta* forests in west central Alberta, Canada, and seedlings grown without soil inoculum (n=634). Seedlings were assessed for survival after 4 months in the growth chamber. Values computed using Wald's Type III Chi-square (X2) tests. Coefficient estimates and standard error (SE) are represented in logit scale.

	DF	X^2	***p*-value**
Inoculation source treatment	2	9.520	0.009

	Estimate	**SE**	**z-value**	**p-value**
Beetle-killed	-1.4625	0.5020	-2.919	0.003
Undisturbed	0.8093	0.5839	1.386	0.165
No Inoculum	1.6412	0.5631	2.915	0.003

Appendix 4. Logistic binomial regression analysis of *Pinus contorta* seedling survival of outplanted seedlings (n = 722) inoculated with soil sourced from beetle-killed (n = 347) and undisturbed (n = 260) *Pinus contorta* forests in west central Alberta, Canada (n = 30), and seedlings grown without soil inoculum (n=115). Seedlings were assessed for survival after 14 months in beetle-killed *Pinus contorta* forests (n = 15). Values computed using Wald's Type III Chi-square (X2) tests. Coefficient estimates and standard error (SE) are represented in logit scale.

	DF	X^2	***p*-value**
Inoculation	2	2.015	0.365
Cluster	1	1.944	0.163
Inoculation x Cluster	2	2.761	0.251

Appendix 5. Two-way analysis of variance (ANOVA) of seedling heights (cm) outplanted to beetle-killed *Pinus contorta* seedling forests in west central Alberta, Canada (n = 15) among inoculation treatments and between site clusters (n = 2 clusters; Loamy Sites, n = 9; Sandy Sites, n = 6).

	DF (num)	DF (den)	F-value	*p*-value
Inoculation	2	52	0.247	0.781
Cluster	1	13	0.026	0.873
Inoculation x Cluster	2	52	3.082	0.054

Appendix 6. Analysis of variance (ANOVA) of outplanted seedling heights (cm) among inoculation treatments at each cluster (Loamy Sites, n = 9; Sandy Sites, n = 6) in beetle-killed *Pinus contorta* forests in west central Canada (n = 15).

	DF (num)	DF (den)	F-value	*p*-value
Loamy Sites	2	30	0.182	0.834
Sandy Sites	2	22	6.751	0.005

Appendix 7. – Pairwise comparisons and least square means (± SE) of outplanted *Pinus contorta* seedling height (cm) after analysis of variance (ANOVA) of inoculation treatments in 'Sandy Sites' (n = 9) in beetle-killed *Pinus contorta* forests in west central Canada (n = 15). *P*-values adjusted using Tukey's method.

Comparisons

Inoculation source treatment		Mean difference (± SE)	DF	t-ratio	*p*-value
Beetle-killed	**Undisturbed**	0.69 (± 0.29)	22	2.398	0.0632
Beetle-killed	**No Inoculum**	0.99 (± 0.29)	22	3.429	0.0065
Undisturbed	**No Inoculum**	0.30 (± 0.29)	22	1.031	0.5656

Least Square Means

Inoculation source treatment	Least square means (± SE)	DF	Lower 95% confidence interval	Upper 95% confidence interval
Beetle-killed	5.97 (± 0.35)	5	5.07	6.88
Undisturbed	5.28 (±0.35)	5	4.37	6.19
No Inoculum	4.99 (±0.35)	5	4.08	5.89