



AN ABSTRACT OF THE THESIS OF

Ariel D. Cowan for the degree of Master of Science in Forest Ecosystems and Society presented on June 23, 2015.

Title: Recovering Lost Ground: Effects of Soil Burn Intensity on Nutrients and Ectomycorrhiza Communities of Ponderosa Pine Seedlings.

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Jane E. Smith

Fuel accumulation and climate shifts are predicted to increase the frequency of high-severity fires in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) forests of central Oregon. The combustion of fuels containing large downed wood can result in intense soil heating, alteration of soil properties, and mortality of microbes. Previous studies show ectomycorrhizal fungi (EMF) improve ponderosa seedling establishment after fire but did not compare EMF communities at different levels of soil burn intensity in a field setting. For this study, soil burn intensity effects on nutrients and EMF communities were compared at Pringle Falls Experimental Forest, La Pine, Oregon. Twelve replicate sites were used, each with three treatments: high intensity soil burn (HB), low intensity soil burn (LB), and unburned control (UB). The combustion of downed logs stacked together, simulating a large downed log, was used to apply HB treatments. Broadcast burning of pre-existing ground fuels was used to apply LB treatments. Temperatures lethal to fungi were recorded at 0-cm, 5-cm, and 10-cm depths in HB soils and 0-cm depth in LB soils. Ponderosa pine seedlings planted post-burn were harvested after four months for EMF root tip analysis. We found: a) greater differences in soil nutrients in HB soils compared to LB and UB soils; b) no differences in EMF richness and diversity; c) weak differences in community composition based on relative abundance between UB and burn treatments; and d) EMF composition in HB and LB treatments correlated with low carbon and organic matter contents. These results confirm the combustion of large downed wood can alter the soil environment beneath it. However, an EMF community similar to LB soils recolonized HB soils within one growing season. We theorize that

quick initiation of EMF communities is possible depending on the size of high burn patches, proximity of low and unburned soil, and survival of nearby hosts. The importance of incorporating mixed fire effects in fuel management practices will help to provide EMF refugia for ponderosa pine forest recovery and regeneration.

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Recovering Lost Ground: Effects of Soil Burn Intensity on Nutrients and Ectomycorrhiza  
Communities of Ponderosa Pine Seedlings

by  
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Ariel D. Cowan, Author

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## LIST OF ACRONYMS

EMF	Ectomycorrhizal Fungi
HB	High Soil Burn Intensity
LB	Low Soil Burn Intensity
UB	Unburned
FDR	False Discovery Rate
NMS	Non-metric Multidimensional Scaling
MRBP	Multi-Response Blocked Permutation Procedures
IV	Indicator Species Value
DEEMY	Information System for Characterization and Determination of Ectomycorrhizae

## Recovering Lost Ground: Effects of Soil Burn Intensity on Nutrients and Ectomycorrhiza Communities of Ponderosa Pine Seedlings

### CHAPTER 1 – WILDFIRE PERSPECTIVES: PAST, PRESENT, AND BELOWGROUND

#### *A history of fire and fear*

After years of fire exclusion and increasing drought, forests of the western United States are becoming increasingly susceptible to frequent, large fires of growing severity. As a consequence, we are witnessing a great change to our forests that will impact social and ecological systems for years to come. Reminding ourselves how this situation originated and researching what the increasing threat of fire means for the future of western forests will help us learn how best to manage for it.

Fire is a natural part of western forest ecosystems, yet we have used our fear of fire to dictate our decisions on fire prevention. When considering the threat of forest fires, one common concern is the economic impact of the loss of timber, an important natural resource by which the timber industry and many communities are supported. Fires also threaten understory vegetation, including rare plants, and may create conditions that aid in the spread of invasive plants (Korb et al. 2004; Hebel et al. 2009). Stand-replacing fires can shift plant communities and affect the habitat and population of many other organisms (Hart et al. 2005). The result of losing a population or creating imbalance in an ecosystem is poorly understood and may have cascading consequences for entire plant communities (Pace et al. 1999).

Perhaps the greatest source of our fear of fire is the direct threat it poses to civilization. As human populations expand into previously undeveloped forests, more fires are occurring in the expanding zone we call the Wildland Urban Interface (WUI) (North et al. 2012). Besides the threat to lives and human-built structures, burnt forests are often viewed as unacceptable due to the loss of plant life, aesthetic appeal, and

recreational value. Considering all these concerns, it is not surprising that the United States set out on a campaign to prevent forest fires from the start of the 20<sup>th</sup> century.

In 1905, President Theodore Roosevelt designated the first national forest reserves in the United States and established the U.S. Forest Service to maintain them (Egan 2009). He assigned Gifford Pinchot the difficult task of overseeing the new organization (Pyne 2001; Egan 2009). Pinchot viewed the reserves as forests for the people and aimed to protect the millions of acres of virgin forest from private industry and wildfire.

In 1910, a massive wildfire now known as the “Big Burn” tested this very mission. The fire, caused by a unique combination of intense weather and drought conditions, charred more than three million acres of forest in Washington, Idaho, and Montana in just 36 hours (Pyne 2001; Egan 2009). In addition to destroying vast amounts of forest, several towns were decimated and many lives were lost. The devastation left in the wake of the “Big Burn” helped to pacify political opponents in congress and justified the need for organized management and protection of the country’s forests. As a result, the Forest Service budget and workforce were greatly increased and the anti-fire campaign was born (Egan 2009).

Forest managers today are increasingly concerned with when to fight fires, when to let them burn, and how to prevent large severe fires from occurring. Should we approach reducing fire impacts proactively instead of putting lives at risk and spending billions of dollars to fight each fire? If we seek ways to adapt to wildfires, we might learn to coexist with this inevitable force of nature.

### *Understanding fire*

The “fire triangle” is a symbol used to convey the three main contributors to fire behavior: climate, topography, and fuel (Fitzgerald 2005). Climate and topography are often beyond immediate human control which is why fire management objectives are often aimed at controlling fuel. Fuel refers to vegetation, dead or alive, that can ignite

and carry fire. Needles and twigs are fine fuels that burn quickly but can carry flames to other fuel sources. By contrast, downed and decomposing logs are larger fuels that can burn at higher temperatures for longer periods of time (Busse et al. 2013).

The quantity, quality, and spatial distribution of fuels can influence the intensity and severity of a fire (Busse et al 2005; Busse et al. 2013). Intensity and severity are terms used to classify fire events and the degree of their impacts. Burn intensity often refers to aboveground heat that can be measured in energy flux, flame lengths, temperature, and heat duration (Keeley 2009). Burn severity often refers to both physical and ecological effects. Though usually classified by percentage of tree mortality and organic litter consumption, severity can also be measured by impacts to soil properties and biota (Keeley 2009). As a great example of how fuels contribute to burn intensity and can influence the degree of burn severity, Rhoades and Fornwalt (2015) found treeless openings and overall reduced vegetation fifty years after the burning of slash piles.

Fire regimes can vary greatly by ecoregion and must be considered when comparing past, present, and future fire trends. Historical fire regimes describe the typical pattern of fire frequency and severity in an ecoregion before human intervention (Hessburg et al 2005; Ryan et al. 2013). Lightning was often the natural matchstick of historical fire events, igniting forests during the months of summer and fall when vegetation was most dry (Hessburg et al. 2005). On the east side of the Cascade Mountains, fires were more frequent but of lower severity compared to fires on the west side, where higher annual precipitation allowed for dense vegetation communities that would burn at a greater severity every 200+ years (Graham and Jain 2005; Hessburg et al. 2007; Mitchell et al. 2009; Perry et al. 2011). Today, shifting trends in our climate are contributing to longer periods of fire-conducive weather (Hessburg et al. 2005). Extended summers are giving fuel more time to dry out and increase the likelihood of combustion.



Fire also plays a positive role in western forest ecosystems. It is a natural process that shapes and maintains forest structure, providing opportunities for a reset in community succession (Hessburg et al. 2007; Stephens et al. 2009). Fire can promote diversity on the landscape by creating a variety of habitats, sustaining populations of fire-adapted plant species that might otherwise be replaced in the absence of disturbance (Oliver et al. 2015). The burning of organic matter from plant litter and debris allows for the release of nutrients into the soil thus increasing nutrient access for plants and microbes (Neary et al 1999). It also keeps vegetation accumulation in check. Without fire, fuels can accumulate to unnaturally high amounts, setting the stage for increases in intensity and severity when fire does occur (Stephens et al. 2009).

### *Effects on soil environment*

Belowground ecosystem effects are rarely cited as one of society's concerns for fire consequences. In reality, exposure of soils to intense heating from high severity fire can have important implications for ecosystem functioning. Alteration of soil properties and the mortality of soil microbes can affect carbon and nutrient cycling (Neary et al. 1999; Certini 2005). Many organisms, large and small, use soil or the vegetation that grows from it as their habitat or food source. Fire can change soil in ways that make it more susceptible to erosion, nutrient losses, and drought with immediate to long-lasting effects (Certini 2005).

The combustion of large downed wood in direct contact with the soil can increase soil burn severity by increasing the intensity of heat penetrating the soil profile (Busse et al. 2013). This effect is usually visible as red oxidized soil, barren of organic litter, in the footprint of the large downed log (Hebel et al. 2009). In contrast, the combustion of finer fuels creates a black-charred appearance on the soil surface (Hebel et al. 2009).

The visual alteration of soil is the result of changes to its physical and chemical properties (Neary et al. 1999; Hebel et al. 2009; Busse et al. 2010). Some soil nutrients

are volatilized at moderate to high temperatures, making them less accessible for plants and microbes. High burn intensity also reduces soil carbon, water content, and elevates soil pH, creating a less-habitable environment for different organisms (Neary et al. 1999). Lower carbon to nitrogen ratios, less than an approximate ratio of 12:1, might encourage increased competition between fungi and bacteria and exhaust nutrient supply (Strickland and Rousk 2010).

Current fuel management practices in western forests such as prescribed burning often differ from historic fire regimes in terms of seasonality (Heyerdahl et al. 2002; Hatten et al. 2012). Prescribed burns are commonly conducted in the spring to avoid smoke and the risks of a fire escape (Heyerdahl et al. 2002). Precipitation from the months preceding a spring burn reduces fire intensity (Smith et al. 2004; Busse et al. 2010). However, increased soil moisture can amplify the effects of soil burn intensity in some cases. Increased heat transfer in moist soils and long-duration smoldering can heighten damage to belowground microbes (Swezy & Agee 1991). Additionally, roots and soil microbes acclimated to lower temperatures from winter conditions are more vulnerable to dramatic heating from a spring fire (Swezy & Agee 1991).

Another consequence of high intensity soil heating is the formation of water repellent layers. Water repellency can occur when the combustion process breaks organic matter into hydrocarbons that migrate deeper into the mineral soil and recondense on soil particles (Doerr et al. 2009). Carbonization and water repellent layer formation occurs at around 200-280 °C (DeBano 2000). This formation results in decreased water infiltration for plant roots and microbes as well as overall nutrient cycling (DeBano 2000). Additionally, water repellent layers can increase water runoff and erosion (DeBano 2000; Hubbert et al. 2006). Cations and macronutrients such as potassium, phosphorus, sulfur, magnesium, and calcium do not volatilize until 760-1240 °C and so remain in the nutrient rich ash above the water repellent layer where it can easily be washed away (Neary et al. 1999; Knicker 2007). This result can be a serious

problem for forests after severe fire, creating conditions for landslides, loss of topsoil, and water quality issues. Spatial heterogeneity of water repellency can reduce the severity of hydrologic runoff (Hubbert et al. 2006).

Biological thresholds, the temperature extremes at which an organism can no longer survive, vary among different soil microbes. Fungi, for example, cannot tolerate temperatures above ~60 °C (Neary et al. 1999). Guerrero et al. (2005) reported a 60% reduction in bacteria biomass when heated to 100 °C for 15 minutes and a 99.6% reduction when heated to 200 °C for the same length of time. Plant roots are more sensitive and lose viability when heated to about 48 °C (Neary et al. 1999). Root mortality is of particular importance for most of the soil microbe community that rely on root exudates and mutualisms with the living roots of host plants. On the other hand, the death of plant roots can provide a new carbon source for decomposing fungi.

### *Post-fire fungi*

Microbes can specialize in a variety of post-disturbance niches. Pioneer or early-successional species are short-lived but can produce large quantities of propagules that can be the first to colonize burned soil (Nguyen et al. 2012). Unburned soil occupied by the roots of mature or late-successional vegetation will likely harbor late-successional microbes that dominate over early-successional species (Hagerman et al. 2001; Hebel et al. 2009).

Post-fire fungi are typically pioneer species that benefit from the habitat created by fire. They can decompose woody debris and dead roots and aid in soil stabilization by aggregating soil particles with their mycelial strands. Post-fire conditions are conducive to fruiting and spore dispersal (Claridge et al. 2009). The fruiting bodies of fire-adapted fungi provide a food source for other organisms such as insects, rodents and other animals (Claridge et al. 2009). Several insect species of the *Aradus* genus, “flat bugs”, are post-fire specialists that benefit from the fruiting of post-fire fungi (Hjalten et al. 2006).

Adults lay eggs and nymphs feed on the hyphal contents of post-fire fungal fruit-bodies, such as morels (*Morchella* spp.), and help spread their spores (Hjalten et al. 2006).

Post-fire fungi can combat the formation of water repellent layers by aggregating soil. Fire-adapted fungi in Australia have been documented to fruit beneath water repellent layers (Claridge et al. 2009). Small mammals dig up and consume the truffle-like fruiting body, thereby breaking the water repellent layer as well as dispersing the fungal spores (Claridge et al. 2009). Most interestingly, the depression created by the small mammal can serve as a water catchment for rain runoff, providing water directly to the mycelia of the fungus (Claridge et al. 2009). This complex ecological story conveys the adaptive ability of post-fire fungi in a disturbed landscape.

### *Importance of ectomycorrhizal fungi*

Ectomycorrhizal fungi (EMF) are ecologically important soil organisms. They create symbiotic relationships by colonizing the roots of host plants, where they exchange water and nutrients for the plant's carbohydrates (Smith & Read 1997). Ectomycorrhizal fungi are considered essential for conifer seedling growth and survival by aiding in water and nutrient uptake. Some EMF species create hyphal networks in the soil, allowing for the exchange of carbon and nutrients through the linked roots of multiple hosts (Simard et al. 1997).

Ectomycorrhizal fungi are readily affected by fire because they mostly inhabit the organic litter and shallow layers of soil often penetrated by heat. Surviving host trees, also known as "legacy trees," can ensure the survival of EMF species (Buscardo et al. 2011). The absence of legacy trees in severely burned stands results in a lack of food for EMF symbionts (Cairney & Bastias 2007). Resprouting shrubs can also host EMF (Perry et al. 2011) and influence community composition after fire (Buscardo et al. 2011). Additionally, unburned and low severity burn patches of soil can provide a refuge area for orphaned EMF (Smith et al. 2004).

Ectomycorrhizal fungi are a key component of post-fire recovery. They can support fire-adapted plant communities and aid in seedling reestablishment (Oliver et al. 2015). A seedling's first growing season is a critical time for recruitment of EMF. Immediate formation of mycorrhizae may influence the length of time it takes a seedling to break free from competition (Smith & Read 1997). If EMF are adversely affected by high soil burn intensity then the regeneration of host trees might be negatively affected as well. Management objectives that consider the survival of EMF from host shrubs, legacy trees, and refugia patches can help promote post-fire regeneration of western forests (Perry et al. 1989).

### *Conclusions*

Higher fuel loads in western U.S. forests have been attributed to decades of fire exclusion (Stephens et al. 2009). Careful management of forests, with both ecological and silvicultural interests in mind, can help maintain healthy, resilient forests. Prescribed fire is a tool used for managing fuels to reduce the likelihood of large destructive fires (Stephens et al. 2009). Thinning, mowing, and other fuel reduction practices are also useful in reducing the risk of large high severity patches (North et al. 2012). Strategic placement of fuel treatments on a landscape scale is also important for encouraging burn heterogeneity and reducing fire spread (Busse et al. 2013; Campbell & Ager 2013).

Current funding restraints, air quality laws, and liability issues limit prescribed burning and fuel reduction efforts (Donovan and Brown 2007; Ryan et al. 2013). In response to this, federal fire managers are being forced to reconsider how they address pro-active fire management in a political atmosphere where funding is decreasing. Policy and funding changes can allow managers to incorporate fire in their management goals, thereby preventing the buildup of fuel that can lead to large intense fires (Donovan & Brown 2007; Ryan et al. 2013).

Despite the acceptance of the beneficial role of fire in the scientific community, a lack of public awareness still exists. Public perception has a significant influence over our society's ability to tackle the problem of increasing fires (North et al. 2012; Ryan et al. 2013). Although humans ignite and manage fires for their own benefit in many parts of the world, the majority of fires in the western U.S. are aggressively extinguished. An increased public awareness and acceptance of the benefits of fire can encourage politicians to enact policies to support fuel management programs.

Overall, increased funding and public awareness will aid in keeping fire-prone forests economically productive and ecologically functional. Collaborative groups and prescribed fire councils can help address some of these constraints by bringing multiple stakeholders and the community together to make informed fire management decisions. Collaboration between researchers and land management agencies is needed to address complex questions and uncertainties related to fire impacts. Future work is needed to create and maintain resilient forests and mitigate the effects of increasing wildfire intensity.

## CHAPTER 2 – RECOVERING LOST GROUND: EFFECTS OF SOIL BURN INTENSITY ON NUTRIENTS AND ECTOMYCORRHIZA COMMUNITIES OF PONDEROSA PINE SEEDLINGS

### 1. Introduction

Fire provides many benefits to forest ecosystems by clearing accumulated vegetation and releasing nutrients into the soil. Over the past century, humans have actively suppressed wildfires to prevent damage to human infrastructure and natural resources. Fire exclusion has resulted in an unprecedented overgrowth of vegetation. Accumulated vegetation acts as fuel when fire returns to a forest, resulting in hotter and more destructive fires (Kaufmann et al. 2005). Climate shifts in conjunction with fuel accumulation are contributing to an increase in the frequency and size of high severity fires in the ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) forests of central Oregon (Covington 2000; Fitzgerald 2005; Hessburg et al. 2005). This outlook has created a need to comprehend the effects of fire on soil ecosystems and their implications for post-fire ponderosa pine regeneration.

The impact of a fire can be classified by burn severity, the “degree to which a site has been altered or disrupted by fire” (NWCG 2003). The degree of severity can be determined by assessing the physical, chemical, and ecological changes observed post-fire that occur as a direct result of combustion (Keeley 2009). Burn severity is a product of burn intensity, the amount of heat energy measured in temperature and duration of heating (NWCG 2003; Keeley 2009). The term *soil burn intensity* directly refers to the heat absorbed by the ground during a fire. High soil burn intensity can be characterized by surface soil temperatures >400 °C and low soil burn intensity can be characterized by surface soil temperatures <250 °C (Neary et al. 1999). The size, quantity, and quality of the fuel in contact with the forest floor influence the depth and degree of soil burn intensity (Busse et al. 2013).

Large downed wood increases soil burn intensity (Busse et al. 2013) — what role does it play in the post-fire effects and recovery of ponderosa pine forests? In many forest ecosystems decaying downed wood can provide habitat for organisms as well as shade, water, and growing substrate for plants (Maser & Trappe 1984; Franklin et al. 1987; Fukasawa 2012). The natural recruitment of large downed wood in ponderosa pine forests was historically managed and maintained by periodic fire which often consumes the wood (Fitzgerald 2005). In the environment created post-fire, the combustion of large downed wood can create a mineral soil seedbed temporarily void of competing vegetation for ponderosa pine seedlings (White 1985). However, large downed wood may also have negative effects on soil. Greater belowground degradation of some soil properties occurs as a result of high intensity soil burning compared to low intensity soil burning (Neary et al. 1999). Soil nutrients may be volatilized or leached at higher temperatures, making them less accessible for plants and microbes (Neary et al. 1999; Bormann et al. 2008). Water repellent layers, which contribute to soil erosion, can form when temperatures reach the range at which oils in organic matter breakdown and recondense on soil particles (DeBano 2000). High soil burn intensity can also reduce soil water content and elevate soil pH (Certini 2005; Neary et al. 2005), creating a less habitable environment for some plants and microbes.

High soil burn intensity can also directly affect soil microbes, including ectomycorrhizal fungi (EMF) (Holden et al. 2013). Ectomycorrhizal fungi form symbiotic relationships with the roots of host trees and shrubs where water and nutrients are exchanged for the tree's carbohydrates. Through hyphal networks, EMF expand the surface area of soil from which a tree can attain resources. Ectomycorrhizal fungi can also prevent nutrient leaching post-disturbance and supply host trees in depleted soils (van der Heijden et al. 2015). In the absence of an EMF symbiont, some conifer species are unable to germinate and thrive (Miller et al. 1998).



Ectomycorrhizal fungi are particularly sensitive to fire and changes to EMF community composition can be influenced by soil burn intensity. Fungal mortality occurs at temperatures around 60 °C and above (Neary et al. 1999). While the insulating properties of soil help to buffer heat penetration from fire (Neary et al. 2005), the depth can vary depending on soil type, moisture, and other factors (Agee 1996; Smith et al. 2004). Fire-adapted EMF produce spores that can withstand higher temperatures (Baar et al. 1999; Peay et al. 2009). While these spores can serve as sources of inoculum post-fire (Cairney & Bastias 2007), successional dynamics and the length of time needed for recovery are poorly understood. Measures of EMF diversity such as species richness and relative abundance have been used as indicators for recovery of an EMF community (Taylor 2002). The presence of an intact EMF community (similar to pre-disturbance status) can support the growth and survival of the host plants with which they associate (Barker et al. 2012).

Ectomycorrhizal fungi form an obligate association with ponderosa pine roots, forming a partnership that protects trees from drought and root pathogens (Read 1998; Peterson et al. 2004). The first growing season is critical for ponderosa pine seedlings and EMF colonization during this time can determine seedling success (Stendell et al. 1999; Barker et al. 2012). Previous observational and greenhouse studies have shown EMF also improve ponderosa seedling growth and survival after fire but did not investigate community composition among different levels of soil burn intensity in a field setting (Miller et al. 1998; Stendell et al. 1999).

Current research gaps expose the question: Does soil burn intensity alter early EMF recruitment on ponderosa pine seedlings? The goal of this study was to investigate first-growing season effects of high soil burn intensity from the combustion of large downed wood in ponderosa forests. To do this, we conducted an *in-situ* experiment with three treatments: high soil burn intensity (HB), low soil burn intensity (LB), and an unburned control (UB). We expected to find (a) greater differences in soil properties and

nutrient contents in soils subjected to HB treatment in comparison with LB and UB treatments; (b) lower EMF richness and diversity on ponderosa pine seedlings grown in HB soils in comparison with LB and UB soils; (c) greater differences in EMF constancy and relative abundance in HB soils compared to LB and UB soils; and (d) differences in EMF composition in HB micro-sites would correlate with differences in HB soil properties and nutrient contents.

## 2. Materials and Methods

### 2.1. Study area

We conducted our research at the Pringle Falls Experimental Forest on the eastern slope of the Cascade Range in Central Oregon (43°42' N, 121°37' W). Pringle Falls is located about 48 km southwest of the city of Bend, in the Deschutes National Forest (Fig. 1). Operated by the United States Department of Agriculture-United States Forest Service (USDA-FS), the experimental forest is part of a national network of outdoor laboratories dedicated for silviculture, insect, disease, fire, and climate change research (Adams et al. 2008). Originating from a stand-replacing fire in 1845, the forest has experienced over a century of fire exclusion and infrequent thinning (Youngblood et al. 2004). Researchers from the USDA-FS Pacific Northwest Research Station are conducting an operational-scale experiment on Lookout Mountain, a 1,430 ha unit of the Pringle Falls Experimental Forest. The goal of the long term experiment is to study climate change and vegetation dynamics under different management practices (Youngblood et al. 2004; Youngblood 2009). Treatments include the thinning of closed-canopy, high density forests and mastication of slash and understory vegetation.

For our study, we designated a roughly 100 ha area of Lookout Mountain that, as part of the aforementioned study, was thinned from below to 75% of the site occupancy limit for the plant association (Youngblood 2009). The study area ranges from flat to

gently sloping terrain (0-20%, East aspect) at elevations of 1,340 m to 1,440 m. Ponderosa pine (*Pinus ponderosa* Dougl. ex Laws) is the dominant tree species, with a stand density index of 107 and an average basal area of 16.8 m<sup>2</sup> ha<sup>-1</sup> (Youngblood 2009). The dominant shrubs of the understory plant communities are bitterbrush (*Purshia tridentata* Pursh) and snowbrush ceanothus (*Ceanothus velutinus* Dougl. ex Hook) typical of a ponderosa pine/bitterbrush/Idaho fescue (*Festuca idahoensis*) plant association (Simpson 2007). The forest floor is mostly comprised of pine needle litter and averages 3 cm in depth (Smith et al., unpublished data). Soils are 62 to 148 cm in depth (to bedrock) and consist of well- to excessively-drained loamy coarse sands and volcanic ash deposits from the eruption of Mount Mazama 7,700 years ago (Larsen 1976). The soils are classified as Xeric Vitricryands in the La Pine Soil Series (Soil Survey Staff 2014) and the density of pumice components averages 81% (Klug et al. 2002). Hot, dry summers and cold winters typify the climate of the region. The mean annual precipitation is 519 mm, usually arriving in the form of winter snow, and annual temperatures average around 6.4 °C (Youngblood et al. 2004).

## 2.2. Experimental design

We established 20 sites throughout the study area in the summer of 2011. Sites were non-randomly assigned to areas with open canopies for maneuverability of log-lifting machinery. Parallel stacks of large downed logs (mega-log) were constructed to simulate large diameter coarse woody debris that can be found in ponderosa pine ecosystems. Mega-logs were covered and left to cure over the following two years. After an assessment of site conditions in early spring of 2013, we rejected eight of the initial 20 sites as unsuitable for our study based on underlying rocky substrate or insufficient mega-log size. At each of the 12 remaining replicate sites, we established a site center and three micro-sites: high soil burn intensity (HB), low soil burn intensity (LB), and an unburned control (UB). The three micro-sites were located 10 m from the site center, equidistance from one another in a pinwheel design (Fig. 2). HB micro-sites

were assigned to the mega-log locations. We randomly assigned the LB and UB micro-sites to the remaining two sides of the pin-wheel with the flip of a coin. Micro-site basal area, distance and DBH of nearest live *P. ponderosa*, and GPS coordinates are reported in Table 1.

### 2.3. Treatments

HB treatments were created by the complete combustion of mega-logs during a prescribed burn. The dimensions of each mega-log ranged from 1.5-2 m wide, 8-10 m long, and 0.7-1.2 m in height. Individual logs had a decay class of 1-2 (low decay, bark and twigs intact) (Maser et al. 1979). A week before the burn, we used a chainsaw to cut disks from logs to measure their moisture content. Moisture contents of logs are reported in Smith et al. (unpublished data).

To mimic the footprint of a mega-log, we established 2 m wide and 10 m long micro-sites for LB and UB treatments. LB treatments were applied through the broadcast burning of the pre-existing fuels. Fuel measurements (Brown et al. 1982) are reported in Smith et al. (unpublished data). A 2 m wide fire line was constructed around the perimeter of UB micro-sites to prevent fire movement. The construction of the fire lines included removing surface organic matter down to mineral soil from the outer perimeter of UB micro-sites.

A week before the burn, thermocouple probes were installed at a subset of 9 sites to record soil temperatures within LB and HB micro-sites. We used PVC, stainless steel probe, and glass braid Omega 30-gauge, type K thermocouples (Omega Engineering, Stamford, CT) to evaluate fire intensity, a covariate of the study measured in °C. Thermocouples were attached to insulated data loggers (Omega Engineering, Stamford, Connecticut) buried 5 m outside of each site. Data loggers recorded temperatures every 90 seconds at 0, 5, 10, and 30 cm depths under the center of HB micro-sites and at 0 and 5 cm depths under the center of LB micro-sites. Portions of mega-logs were removed to install thermocouples and then replaced. Temperatures

were recorded for a total of 269 hours, beginning four days before the burn and concluding seven days after. Temperature data points were analyzed for mean maximum temperature (Fig. 3) and mean duration above 60°C (Fig. 4), the approximate lethal threshold for fungi (Neary et al. 1999). Analysis of temperature data is reported in Smith et al. (unpublished data).

The USDA-FS Bend-Fort Rock Ranger District conducted the prescribed burn on May 14<sup>th</sup> of 2013. Mega-logs and the surrounding forest floor were ignited using drip torches and fire accelerant (Heat Source Slash Burner, Wildfire Environmental Inc.). Weather conditions for the day were light W to NW winds, clear skies, 12-19°C (dry bulb), and 25-40% relative humidity. Under heading conditions, 10 and 100 hour fuels were the primary carriers of the fire. Flame lengths were 0.6-1.8 m (2-6 ft) with a 12-18 m (40-60 ft) flame zone depth.

#### *2.4. Soil sampling*

We returned to the sites one week after the burn to collect soil samples. After removing the O horizon (where present), mineral soil was collected at 0-10 cm, 10-20 cm, and 20-30 cm depths from 10 of 12 sites. Soil cores were taken from the center of each micro-site (30 soil cores, 90 soil samples) using a 6.35 cm diameter X 30.4 cm length impact coring device containing three 10 cm liners (AMS Inc., American Falls, Idaho). Each soil sample, containing a volume of 179.2 cm<sup>3</sup>, was placed in a plastic zip-lock bag and kept in a cooler on ice until cold room storage at 4°C. Forest floor depth was recorded from the center of each micro-site after the burn.

Soil samples were processed in July of 2013 and were analyzed by the Page-Dumroese Lab at University of Idaho, Moscow, Idaho. Samples were dried, sieved (2 mm mesh), and mechanically homogenized with a Spex Mixer Mill (Metuchen, New Jersey) before being processed as described in Cerise et al. 2013. Analysis included total nitrogen (N), total carbon (C), soil organic matter (SOM), pH, total extractable calcium (Ca), total extractable magnesium (Mg), percent water content (%MC), total bulk density

(BD) and fine-fraction bulk density (FineBD). Total C and N were evaluated by dry combustion at 950°C on a Leco TruSpec CN determinator (St. Joseph, Michigan). Bray phosphorus (Bray-P) samples were analyzed using methods described in Amacher et al. 2003, first undiluted to check Bray-P range then re-analyzed at 1:10 dilutions on over-range samples. The pH neutral 1M ammonium acetate method (Helmke & Sparks 1996) was used to extract Ca and Mg before analysis on a Perkin Elmer 5100PC AA Spectrometer (Waltham, MA, USA). Mineral soil C, N, Bray-P, Ca, Mg, and SOM contents were corrected for rock fragment content and converted from percentages to units of Mg/ha (C, SOM) and kg/ha (N, Bray-P, Ca, Mg) using fine-fraction bulk density values (Andraski 1991; Page-Dumroese et al. 1999; Klug et al. 2002). Total bulk density, fine fraction bulk density, and gravimetric rock-fragment contents are reported in Table 2.

### *2.5. Planting*

Eight days after the burn, we planted ponderosa pine seedlings at each micro-site in all 12 sites. Two-year-old Q-plug +1.5 seedlings were obtained from the same central OR *Pinus ponderosa* stock (PIPO-01-0136-553-89 SB) from a Forest Service-operated nursery in Coeur d'Alene, Idaho. For a pre-planting evaluation, twenty seedlings from the original nursery stock were analyzed for presence of ectomycorrhizal root tips using the methods described in sections 2.6 and 2.7. Approximately thirty seedlings were planted in two rows within each micro-site, 50 cm apart and 50 cm from the micro-site perimeter. Surface woody debris was removed to facilitate planting. Vexar tubes were secured around each seedling to protect them from herbivory.

The weather during planting was seasonably cool and moist, with about 5 cm (2 in) of snow on the second day. Post-planting weather remained cool and moist for about a week, creating ideal conditions for seedling acclimation. Seedling caliper (mm) and height (cm) measurements were recorded from root collar to shoot apex of each seedling after planting. Caliper and height measurements were used to calculate seedling stem volume (cm<sup>3</sup>) and are reported in Fitzgerald et al. (unpublished data).

## 2.6. Seedling assessment

Seedlings were harvested in September 2013, four months after planting. Three randomly selected seedlings were carefully removed from each micro-site using a tree-planting shovel (108 seedlings total). Seedlings were stored at 4°C and examined within one month of harvest. Roots were kept in plastic bags with a small portion of the surrounding soil remaining attached. Prior to examination, roots were separated from each seedling at the root collar and washed in de-ionized water. Roots were then cut into 1.5-2 cm fragments and evenly dispersed on a grid with 2.5 cm<sup>2</sup> spacing. Colonized root tips were obtained from grid squares by means of random selection until a total of 100 root tips was collected from an individual seedling (Jones et al. 2008). Root tips were examined with a 10x stereomicroscope (Zeiss Stemi SV6, Jena, Germany) and grouped by morphological type (morphotype) using methods described in Barker et al. 2012 and the Colour Atlas of Ectomycorrhizae (Agerer 1997). Root tips were then stored in 2x cetyltrimethylammonium bromide (CTAB) at -20°C until molecular analysis. Remaining roots were oven dried at 37°C and weighed to record mean root biomass.

## 2.7. Molecular analysis

Sigma Extract-N-Amp™ kit (Sigma Aldrich, St. Louis, Missouri) was used to extract DNA from one root tip per morphotype from each examined seedling. The extracted DNA was used in polymerase chain reaction (PCR) amplifications of internal transcribed spacer regions (ITS) using primers ITS 1F and ITS 4 (White et al. 1990). We also used ascomycete specific primers ITS 1F – ITS 4A (Larena et al. 1999) and basidiomycete specific primers ITS 1F – ITS 4B (Gardes & Bruns 1993) in separate reactions in an effort to isolate multiple species found on the same root tip (Kennedy et al. 2012; Bogar & Kennedy 2013). PCR reactions contained 0.16 µl GoTaq® (Promega, Madison, Wisconsin), 5 µl of 5X PCR buffer, 2 µl of 10X deoxynucleotide triphosphates (dNTPs), 0.85 µl of MgCl, 0.5 µl of bovine serum albumin (BSA), 1 µl of the DNA template, 0.2 µL

of each primer, and 10.35  $\mu$ l of molecular grade water. PCR cycling parameters consisted of a 2-minute denaturation at 95°C followed by 30 cycles of 94°C for 30 seconds, 50°C for 1 minute, 72 °C for 1.5 minutes, and a final extension of 72°C for 10 minutes. PCR products were visualized under UV light on a 2.5% agarose gel treated with Gel-Red™ (Biotium, Hayward, California). Positive and negative controls ensured target DNA amplification and non-contaminated reagents. When a sample failed to amplify, another root tip from the same morphotype was used for molecular analysis. DNA from PCR products were purified using ExoSAP-IT® (Affymetrix, Santa Clara, California) and quantified for sequencing using a Qubit fluorometer (Invitrogen, Carlsbad, California).

A total of 513 purified PCR samples were sent to the University of Kentucky Advanced Genetics Technology Center (UK-AGTC) for sequencing. Direct sequencing of PCR products was performed by the Sanger reaction using the ABI Big Dye terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, California) on an ABI 3730xl DNA Analyzer. ITS 1F primer was used to sequence PCR products in the forward direction. DNA sequences were compiled and analyzed using Geneious® v6.1.7. Regions with primer motifs of more than 4% chance of error per base were trimmed from sequences. Sequences were assembled into contigs of  $\geq$  99% similarity. We performed sequence identity searches using the Nucleotide Basic Local Alignment Search Tool (nBLAST) from the National Center for Biological Information (NCBI) database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Taxonomic names (taxa) were generally assigned to morphotypes using the following sequence identity criteria:  $\geq$ 98% ID value similarity for species, 96 to 97.9% similarity for genera, 90 to 95.9% similarity for families, and  $\geq$ 80% query cover for all matches. Taxa were also assigned when sequences were found to be consistent with the top ten identity matches. Sequences below 90% identity similarity were named by the closest consensus of taxonomic order. Species identification criteria were set at higher percentages compared to the typical 97% cutoff in order to increase



accuracy of assigned species names (Nilsson et al. 2012). *Wilcoxina mikolae* (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf and *Laccaria proxima* (Boud.) Pat. were removed from sample results due to detection in a pre-planting evaluation of seedling roots.

Identified taxa were assigned one of four exploration types using the web-based information system for characterization and determination of ectomycorrhizae (DEEMY). Exploration types describe differences in EMF hyphal development and the distance mycelia can spread from a colonized root (Agerer 2001; Peay et al. 2011; DEEMY). For simplification purposes, we combined the various exploration types described by Agerer (2001) into four types used for classification of taxa identified in our study. Long distance exploration types have the longest range of mycelial length and have a reported ability to spread up to several decimeters, linking with other compatible mycelia to form a network for nutrient transport (Agerer 2001). Medium fringe exploration types form mycelial and hyphal fans at medium distances while medium smooth types form uniform mycelial strands that are smooth in appearance (Agerer 2001). Short distance exploration types form short hyphae that emanate from the ectomycorrhizal structure (Agerer 2001).

## 2.8. Statistical analyses

Soil nutrient data was analyzed in RStudio v.0.97.318. After investigating whether the soil nutrient data met statistical assumptions, all had normal distributions except for total carbon (C), magnesium (Mg), and calcium (Ca). Data for C, Mg, and Ca were natural log-transformed for use in the linear mixed model and back-transformed for ratio inference in treatment comparison. An extension of a two-way analysis of variance was used that included a repeated measures (autoregressive 1) linear mixed model. Variation between sites, micro-sites, and soil depths was accounted for in the model. Mean soil nutrient differences among treatments and their confidence intervals were estimated from the model. *P*-values and confidence intervals were adjusted using

False Discovery Rate (FDR) adjustments (Benjamini & Hochberg 1995) to account for multiple comparisons. Since detection of differences can be considered difficult for field experiments with a small sample size (Steele & Torrie, 1980), comparisons were considered significant at  $\alpha = 0.1$ .

We compared EMF community composition using richness, Simpson's diversity index, relative abundance, and constancy. PC-ORD v.5.12 was used to calculate richness and Simpson's diversity values based on relative abundance (McCune & Grace 2002). First and second order jackknife estimates were calculated to predict total richness (Palmer 1991). Relative abundance was calculated as the number of root tips colonized by a given taxon in a micro-site divided by the total number of root tips sampled from a micro-site (three seedlings = 300 root tips per micro-site). Constancy was calculated as the percentage of micro-sites in which a taxon occurred. For taxa found present in all three treatments, relative abundance and constancy were compared among treatments in a two-way analysis of variance with a linear mixed model in RStudio v.0.97.318. Differences between treatments and their confidence intervals were estimated from the model. *P*-values and confidence intervals were FDR-adjusted and comparisons were considered significant at  $\alpha = 0.1$ .

For non-parametric multivariate analysis, EMF relative abundance and soil nutrient content were compared among treatments in non-metric multidimensional scaling (NMS) ordinations in PC-ORD v.5.12. The environmental variable matrix included site (block), treatment, pH, %MC, SOM, C, N, Bray-P, Ca, Mg, C:N ratio, and forest floor depth. Using the Sørensen (Bray-Curtis) distance measure, a random starting configuration, and 500 runs with real data, a stable two-dimensional solution was found after 91 iterations and passed a Monte Carlo significance test ( $p = 0.019$ ). Under the default settings, the PC-ORD's Multi-Response Blocked Permutations (MRBP) function was used to conduct pair-wise comparisons among all three treatments. MRBP comparisons were considered significant if both (a) the probability of a smaller or

equivalent  $\delta$  to the observed  $\delta$  was  $p \leq 0.05$ ; and (b) the chance-corrected within-group agreement (A) was  $< 0.1$  (McCune & Grace 2002). Indicator species analysis was used to identify taxa that were detected to be most abundant and constant in a particular treatment (McCune & Grace 2002; Dufrene & Legendre 1997). Indicator values were tested with 1000 randomizations in a Monte Carlo test and were considered significant at  $p \leq 0.05$ .

### 3. Results

#### 3.1. Soil environment

The soil environment conditions we tested did not differ significantly among the three treatments at either 10-20 cm or 20-30 cm depths (Fig. 5). The following soil environment and nutrient results are in reference to the 0-10 cm depth.

Median C was estimated to be 1.7 times greater in UB soils than in HB soils (FDR-adjusted  $p = 0.08$ , DF = 18, CI = 1.02 to 2.71). The mean soil organic matter (SOM) content of HB treated soils was estimated to be  $9.9 \text{ Mg ha}^{-1}$  less than the mean SOM content of UB soils (FDR-adjusted  $p = 0.01$ , DF = 18, CI = 2.51 to 17.25) and  $6.9 \text{ Mg ha}^{-1}$  less than the mean SOM content of LB soils (FDR-adjusted  $p = 0.08$ , DF = 18, CI = 0.35 to 13.4). When compared with UB soils, HB soils had an estimated increase in mean pH by 1.4 units (FDR-adjusted  $p < 0.0001$ , DF = 18, CI = -1.91 to -0.8) while LB soils had an estimated mean pH increase of 0.35 units (FDR-adjusted  $p = 0.04$ , DF = 18, CI = -0.65 to -0.05). High burn soils were estimated to be 1 mean pH unit higher than LB soils (FDR-adjusted  $p < 0.0001$ , DF = 18, CI = -1.5 to -0.52). Overall, HB soils were significantly drier at the time of sampling. Mean moisture content (% MC) was estimated to be 13.2% greater in UB soils compared to HB soils (FDR-adjusted  $p = 0.004$ , DF = 18, CI = 4.59 to 21.74). Although no significant difference in % MC was detected between UB and LB soils, mean % MC of LB soils was estimated to be 9.8% higher than in HB soils (FDR-

adjusted  $p=0.02$ ,  $DF=18$ ,  $CI=2.18$  to  $17.35$ ). The median magnesium (Mg) content of HB soils was estimated to be 0.4 times greater than the median Mg content of UB soils (FDR-adjusted  $p=0.001$ ,  $DF=18$ ,  $CI=0.26$  to  $0.66$ ). High burn soils had an estimated 0.4 times greater median Mg content than LB soils as well (FDR-adjusted  $p=0.001$ ,  $DF=18$ ,  $CI=0.23$  to  $0.66$ ). The median calcium (Ca) content of HB soils was estimated to be 0.36 times greater than the median Ca content of UB soils (FDR-adjusted  $p=0.001$ ,  $DF=18$ ,  $CI=0.2$  to  $0.65$ ). High burn soils had an estimated 0.4 times greater median Ca content than LB soils (FDR-adjusted  $p=0.002$ ,  $DF=18$ ,  $CI=0.23$  to  $0.67$ ). We did not detect differences in mean total nitrogen (N), median C:N ratios, median Bray phosphorus (Bray-P), mean total bulk density (BD) or mean fine fraction bulk density (FineBD) among any of the three treatments.

Forest floor depth differences were detected among all treatments post-burn (Fig. 6). Mean forest floor depth was estimated to be 2.95 cm greater in UB micro-sites compared to HB micro-sites (FDR-adjusted  $p<0.0001$ ,  $DF=18$ ,  $CI=2.02$  to  $3.88$ ), 1.5 cm greater in UB micro-sites compared to LB micro-sites (FDR-adjusted  $p=0.004$ ,  $DF=18$ ,  $CI=0.57$  to  $2.43$ ), and 1.45 cm greater in LB micro-sites compared to HB micro-sites (FDR-adjusted  $p=0.004$ ,  $DF=18$ ,  $CI=0.52$  to  $2.38$ ).

### 3.2. EMF composition

A total of 66 fungal taxa were identified from 414 root tip samples (81% sequence success rate) at all 10 sites (Table 3). We were unable to detect significant differences in EMF taxon richness and Simpson's diversity index (Table 4). First and second order jackknife estimates (Fig. 7) suggest that we failed to capture the total taxon richness of our sites (Palmer 1991). The overall EMF richness we observed was about 50% of the jackknife estimates.

Only 21% of taxa were detected in all three treatments (Table 3). Five taxa were detected in only UB and LB micro-sites. Four taxa were detected in only LB and HB micro-sites while five taxa were detected in both HB and UB micro-sites. A total of nine

taxa were found to be unique to seedlings grown in UB micro-sites. Twenty two taxa were found to be unique to LB micro-sites. A total of seven taxa were found to be unique to HB micro-sites. Mean relative abundances of taxa identified more than two times are reported in Figure 8. Constancy (percent occurrence among micro-sites) of taxa identified more than two times or occurring in more than one treatment is reported in Figure 9.

Out of 414 identified root tips, 70% were classifiable by exploration type using the information available (DEEMY). Unique taxa in LB micro-sites included EMF of all four exploration types, whereas unique taxa of HB micro-sites were comprised of long or unknown exploration types. Unique taxa in UB micro-sites included medium and short exploration types. Taxa identified by exploration type are reported in Table 3. Mean relative abundance of identified exploration types are reported in Figure 10.

Multi-Response Blocked Permutation Procedures (MRBP) results showed EMF composition (relative abundance and constancy) differed by treatment ( $p = 0.01$ ), yet the effect size was small ( $A = 0.03$ ). There was evidence of a difference in taxa composition between HB and UB micro-site (FDR-adjusted  $p = 0.02$ ) with a small effect size ( $A = 0.06$ ). There was also evidence of a difference in taxa composition between LB and UB micro-sites (FDR-adjusted  $p = 0.01$ ) with a small effect size ( $A = 0.07$ ). We were unable to detect differences in taxa composition between LB and HB micro-sites (FDR-adjusted  $p = 0.86$ ,  $A = -0.01$ ). For the Indicator Species Analysis, *Rhizopogon salebrosus* AH Sm. was found to be the only significant indicator taxa and was an indicator for UB micro-sites (FDR-adjusted  $p = 0.01$ , indicator value (IV) = 64.3) (Fig.11).

### 3.3. EMF and soil nutrient correlations

Ordination results, displayed as micro-sites in taxa space, provided two axes (Fig. 11). The two axes explained 61% of the variation in EMF composition among micro-sites based on relative abundance. Points (micro-sites) are closer together the more similar they are in terms of taxa composition. Results from the NMS ordination found

considerable overlap in taxa composition among LB and HB micro-sites and none of the three treatments were completely isolated or dissimilar in taxa composition from one another. Total carbon (C) and soil organic matter (SOM) were the only soil nutrients found to correlate with micro-sites based on taxa composition. Both C and SOM aligned with axis 2 ( $r^2 = 0.3$ ) and increase in content from the bottom to the top of the graph. Most of the unburned micro-sites are in the top half of the graph and therefore are correlated with higher C and higher SOM contents. Alternatively, both LB and HB micro-sites occupy space in the graph correlated with low to moderate C and SOM contents. The proportion of variation in axis 1 ( $r^2 = 0.31$ ) is partially explained by N content and %MC which aligned with both axes, increasing in content toward the top left of the graph. However, N and %MC explained <15% of the variation which may not be biologically significant (McCune & Grace 2002) and therefore both were excluded in the overlay vectors of Figure 11.

## 4. Discussion

### *4.1. Forest floor and mineral soil differences*

In characterizing the soil environment conditions resulting from burn intensity treatments, we found the highest alteration of soil environment with the combustion of downed mega-logs. In particular, the exposure of mineral soil after full consumption of the forest floor at most HB micro-sites greatly contributed to the post-fire soil climate. Larger differences in daytime and nighttime temperatures observed in HB soils (Smith et al., unpublished data) are a product of the lack of insulation from daily solar radiation and nighttime cold air (Neary et al. 1999; Massman et al. 2008; Dobre et al. 2014). In addition to direct evaporation from fire, open-air exposure of mineral soils can also explain increased moisture losses observed in HB micro-sites. Moisture and temperature extremes, though ephemeral, can create an inhospitable environment for

microbes living in the shallow layers of soil and certainly deviates from the climate of their pre-burn habitat.

As hypothesized, HB soils differed from UB and LB soils in several of the variables we measured though only at shallow depths. A steep vertical temperature gradient (Fig. 3) resulting from the insulating properties of soil (DeBano 2000) restricted soil nutrient effects to 0-10 cm. Increases in ash contributed to an increase in mineral soil pH (Neary et al. 1999; Choromanska & DeLuca 2001), which can support biological recovery (Knicker 2007) but can also directly affect nutrient availability (Agee 1996). Despite this finding, a greater release of macronutrients Ca and Mg was observed in HB micro-sites. Calcium and Mg have high ion potentials which lower the probability of post-fire leaching (Agee 1996), possibly explaining the increase in Ca and Mg.

Similar to previous studies (Fernandez et al. 1997; Knicker 2007), high intensity soil burning resulted in decreases in C and SOM, both of which begin to break down at around 100 °C (Agee 1996). The dramatically higher temperatures we observed in HB micro-sites volatilized C and organic matter-derived nutrients, thus depriving fungal communities of important sources of nutrition. A decrease in C can lower C:N ratios, though the observed C:N ratios in HB soils were still within the typical range for mycorrhizal growth (C:N of ~3:1-51:1) (Strickland & Rousk 2010). Carbon and SOM degradation can also produce water repellent layers which may have formed just above the 5 cm depth at HB micro-sites where temperatures reached 175-280 °C (DeBano 2000). Low burn micro-sites did not reach water repellency-producing temperatures and therefore may have maintained an erosion-resistant and water-absorbent habitat for EMF.

Changes in soil nitrogen from burning are often complex (Agee 1996; Monleon et al. 1997). We believe the large variability in N content in our results was responsible for our inability to detect N differences among treatments. Some of the variation may be attributed to the timing of our soil sample collection. Collection of samples one week

after the burn may not have provided sufficient time for ash and released nutrients to leach into the mineral soils. This issue may have been more pronounced in LB soils where the forest floor was not completely consumed and could have temporarily intercepted ash and delayed nutrient release (Hart et al. 2005). Precipitation, which would have aided in nutrient filtration into the mineral soil, did not occur in the time between the burn and soil sampling. Because of a time lag between sampling and soil analysis, we were unable to obtain accurate ammonium and nitrate values. One possible explanation for our inability to detect differences in Bray-P may have been due to phosphorus bound by cations and pH, rendering P insoluble (McDowell et al. 2003). We also acknowledge that soil nutrient contents were likely different at the time of seedling harvest four months post-burn. However, nutrient contents assessed one week after the burn were valuable in determining differences among treatments and may influence the development of soil microbe communities (Hart et al. 2005).

#### *4.2. EMF community differences*

Our results suggest that EMF can recolonize HB soils within one growing season despite effects on soil properties. Due to our inability to detect differences among treatments, we reject our hypothesis of reductions in EMF richness and diversity in HB micro-sites. By comparison, our community composition results were complicated. We were able to detect a pattern that distinguished unburned from burned communities, though composition differences had a small effect size (magnitude of differences between groups) and therefore may not have been biologically significant. Whereas differences in C and SOM correlated with taxa composition (Fig. 11), a large proportion of the variation in EMF composition remained unexplained. Differences in C and SOM were therefore not enough to differentiate HB and LB EMF composition four months after burn treatments.

The similarities in HB and LB communities may be due to the broadcast burning that occurred around all micro-sites. However, we argue that these conditions mimic



realistic fire events in which adjacent surface flames ignite large downed logs. We also acknowledge that the technique of using morphotyped groupings of root tips for molecular identification may have limited our ability to detect rare EMF species (Sakakibara et al. 2002). Despite this limitation, and the typically high spatial variability in EMF communities (Grogan et al. 2000), we were able to identify dominant and ubiquitous taxa among the three treatments. High burn and LB micro-sites had a fairly gradual relative abundance curve while UB micro-sites had distinct dominant taxa resulting in a steeper relative abundance curve (Fig. 8). This finding suggests that UB soils have fewer and more dominant taxa, whereas burned soils may have a more leveled playing field in which multiple species can compete in soil space. Alternatively, sampling heterogeneity may have increased with burn treatment, posing a challenge in our ability to detect EMF composition differences.

#### *4.3. The role of refugia and dispersal mechanisms*

Frequent low fires historically occurred in central Oregon ponderosa pine forests (Fitzgerald 2005; Graham et al. 2005; Hessberg et al. 2005) and it is likely that the local EMF community is adapted to soil heating at shallow depths. However, the lethal temperatures we recorded in the top 10 cm of HB soils penetrated deeper than lethal temperatures recorded in LB soils and directly affected where most EMF live (Swezy & Agee 1991, Cromack et al. 2000). If the extreme temperatures we observed likely removed the EMF community, then how did HB micro-sites become recolonized with an EMF community most similar to LB micro-sites?

In the process of post-fire recovery, sterilized soil becomes an open playing field for various microbes. Who is first to arrive? From the air, high spore producing fungi have the first advantage, a larger concentration of their spores landing on the disturbed soil surface. Both long distance and short distance spore dispersal can contribute to recolonization (Galante et al. 2011; Horton et al. 2013). Alternatively, mycelia from relict populations might spread into high intensity burned soil from nearby unburned or low

intensity burned soil refugia. Root exudates, chemical compounds released by plant roots, can attract nearby mycelia and trigger spore germination (Grayston et al. 1997).

Categorizing EMF by exploration type gives us insight into how dispersal modes can influence post-fire succession and why certain taxa may have been present or absent from a treatment. Of all the taxa we identified, *Rhizopogon salebrosus* was found on most of our seedlings, yet we identified it in greater abundance and constancy in UB micro-sites. In general, *Rhizopogon* species have the ability to spread via mycelia over longer distances than many other fungi (Simard et al. 1997; Peay et al. 2011). They specialize in colonization of disturbed habitat, are widely distributed throughout the soil, and are prolific producers of hypogeous sporocarps (truffles) (Molina et al. 1999; Taylor & Bruns 1999). Small mammals consume *Rhizopogon* truffles and disperse their spores (Claridge et al. 2009). *R. salebrosus* spores can germinate faster than many other fungi and can remain viable in the soil for decades, awaiting detection of host's roots and optimal growing conditions (Kennedy et al. 2009). These functional characteristics may explain why *R. salebrosus*, when present in nearby LB and UB soil, can colonize seedlings grown in HB soils. The abundance of *R. salebrosus* in our UB micro-sites might also be explained as a response to disturbance from thinning operations that occurred at all sites two years prior. However, Garcia et al. (*in press*) found *R. salebrosus* to be one of three dominant EMF species in soil sampled from nearby pine forest regions in central Oregon.

In addition to *Rhizopogon spp.*, *Suillus spp.* can also disperse by spores and have the ability to produce long-distance mycelia (Ashkannejhad & Horton 2006; Peay et al. 2011). Ashkannejhad and Horton (2006) found that *Rhizopogon* and *Suillus spp.* are dispersed by deer and can dominate pine seedlings in an early successional habitat. *Russula spp.*, which typically produce short-distance mycelia, were found in UB and LB micro-sites but not in HB micro-sites. Overall, long distance types were detected in greater abundance in all three treatments. Nevertheless, this may be a product of low

root densities in this forest system where long distance exploration types may be better adapted (Peay et al. 2011). We assume dry ponderosa pine forests have low root densities compared to most other forest types because of low basal area, vegetation density, and soil moisture (Litton et al. 2003; Siegel-Issem et al. 2005). Our study area may have also had low root densities because of thinning treatments that occurred two years prior which potentially selected for long distance EMF. Additionally, lethal temperatures to plant roots, 48 °C (Neary et al. 1999), may have lowered surface root densities in both burn treatments. Besides these considerations, we theorize that long distance EMF found to be abundant in UB soils were able to colonize LB and HB soils before less-abundant short distance EMF. Colonization by long distance EMF may provide an explanation for similarities in EMF composition among all three treatments.

Heat-resistant spores and sclerotia may have an advantage in the recolonization of burned soils (Izzo et al. 2006). Although Izzo et al. (2006) refer to *Rhizopogon*, *Cenococcum*, and *Wilcoxina* as EMF with heat-resistant propagules, spores were found to lose viability around 75 °C and were not tested at the temperatures reached in our HB micro-sites. *Cenococcum geophilum* was found to be present in low abundance at all micro-sites. This species is drought tolerant and capable of producing sclerotia that may be more fire-resistant than spores (Massicotte et al. 1992). This form of dispersal could explain why a short exploration type EMF was detected in all three treatments.

Another possible explanation is that EMF inoculum came from below. In the deep layers of soil – where fire merely warmed the soil habitat – any spores that filtered down over time and through water transport may have sprung to life. Although uncommon, mycelia from deep root ectomycorrhizas can rise through the soil column to inoculate seedling roots (Rosling et al. 2003; Tedersoo et al. 2003; Genney et al. 2006). Ascomycetes, such as Pezizomycetes 1 and 2, were found in both burn treatments and may have originated from deeper in the soil profile. Heat-stimulated fungi can fill functional roles in water-limited post-fire soils where other fungal species are no longer

present (Persiani & Maggi 2013) and can support post-fire plant communities (Oliver et al. 2015). Similarly, drought-adapted EMF can extend their mycelia into further depths of the soil in search of moisture where they may inadvertently survive a fire event (Genney et al. 2006). It should also be noted that greenhouse studies using burned soil taken from the field would not have the advantage of these alternative sources of inoculum experienced in natural systems. Through trial and error, a species may fill the void where EMF competition has released. Like most ecological stories, the answer is likely a combination of complex interactions.

#### *4.4. The role of large downed wood*

The mega-log combustion used in our HB treatments mimicked the size and shape of exposed soil that we would expect to see from the combustion of a large downed log. Because of the sheer amount of fuel in one space, the long duration of plant lethal temperatures (Neary et al. 1999; Busse et al. 2013) created a gap in vegetation. The reduction in plant competition from high intensity soil burning would be advantageous for ponderosa pine seedlings in dry ponderosa pine forest ecosystems (Graham & Jain 2005). Despite the obvious downsides of forest floor consumption and alteration of the soil environment, exposed soil can also provide a natural seedbed. The area previously occupied by a downed log can offer direct access to the mineral soil and a release of macronutrients for a seedling's first year (Monleon et al. 1997). This may buy the seedling enough time to grow a taproot to the moisture below.

If the quantity and spatial distribution of fuels result in large continuous areas of high intensity burned soil, maintaining both EMF refugia and new seedbeds may be sacrificed. We hypothesize that regeneration success depends on the size of high burned soil areas and proximity to low and unburned soils. Not surprisingly, we see this situation in nature. Wildfires typically produce a mosaic of burn severities across a landscape (Hessburg et al. 2005; Ryan et al. 2013). If a seedling growing in high intensity burned soil is colonized by EMF, it can obtain essential nutrients from beyond that patch

of soil (Peay et al. 2011). Fleming (1983) and Buscardo et al. (2011) reported tree seedling colonization by EMF species from an already established mycorrhizal network via mature trees nearby. Ponderosa pine seedlings growing in high intensity burned soils may have a similar advantage if a surviving mature ponderosa pine is in the vicinity. Even in high severity wildfires with areas of 100% tree mortality, there is variation in the localized effect on the fungal community (Rincon & Pueyo 2010). In those cases, the mortality of all hosts may create a larger effect on EMF communities than the changes to the soil environment itself — unless the multiple forms of inoculum can endure until a host seed germinates or a seedling is planted. This example is a reminder of the need to distinguish impacts from high severity crown fires, where the majority of heat can remain aboveground (Hartford & Frandsen 1992; Keeley 2009), to fires with high downed fuel loads where large pulses of heat may penetrate the soil.

#### *4.5. Assumptions and limitations*

An assumption of this study is that the EMF communities observed after the experimental burn reflect the EMF communities that would be present after a natural wildfire. This assumption is necessary because of the unpredictable nature of wildfires and the necessity of a controlled experiment to arrive at causal inferences. An additional assumption is that nursery EMF growing with the seedlings before planting may be out-competed once they are transplanted in the field. Few EMF species are found in nurseries because of the use of fertilizer, and any transplanted EMF are quickly out-competed by species from the surrounding native soils (Dahlberg & Stenström, 1991). As mentioned in the methods, EMF species *Wilcoxina mikolae* and *Laccaria proxima* were found on a subset of our nursery seedlings and were thus removed from our sampling results.

Because of feasibility issues related to mega-log construction and prescribed burning restrictions, our study was limited by a small sample size and sample area which should be considered if the study is to be replicated. It would also be interesting to

compare effects of a fall burn and/or spring harvesting of seedlings, as well as the quantification of all colonized root tips. Likewise, a similar study could be conducted where tree thinning had not previously occurred, though the thinning treatment was comparable to conditions typically created prior to prescribed burning in this region and resembled historic stand structure.

## 5. Conclusions

As expected, HB treatments had a greater effect on soil properties and nutrient contents than LB and UB treatments. Despite this effect, we could not detect differences in EMF richness and diversity. We were able to detect small shifts in EMF composition but it remains unclear whether these differences are biologically or functionally significant. Composition differences correlated with changes to soil properties and nutrient contents, but only explained differences between EMF communities in burned and unburned micro-sites. Both burn treatments may have increased the patchiness of EMF taxa, exacerbating our ability to distinguish patterns in the EMF communities. When considering the process of recovery and recolonization, the area of ground that received high intensity burn treatments (similar to the area of large downed log combustion) was small enough to be recolonized within one growing season. The proximity of unburned and low burned soils may also have aided in the speed of this process as sources of inoculum.

The detrimental impacts of severe fire on soil microbes are often used as an argument for reducing fuels and fire severity. However, this argument must be qualified in order to acknowledge the complexity of fire effects and time scale of recovery. Should we stress the importance of burn severity patch size rather than the severity itself? These findings demonstrate the advantage to maintaining low burn and unburned patches. These patches can serve as refugia from which ectomycorrhizal fungi may recolonize. If such recolonization can occur, not all areas previously occupied by large downed logs will result in long-term sterilization, but instead may aid in maintaining

ectomycorrhizal diversity through disturbance and ecological succession. In turn, intact EMF communities could contribute to ponderosa pine forest regeneration success and resilience in the face of an increasing frequency of severe fires.

Prescribed burn treatments in ponderosa pine forests can aim to minimize the area of severely burned patches and promote patches of low and unburned soil for preserving EMF refugia. Thinning treatments can reduce fuel loads and encourage spatial heterogeneity to reduce areas of continuous high burn severity. Although our scope is limited to our study area, forest managers and other researchers may apply these results to fuels management in other dry interior ponderosa pine forests of the western United States. More research must be done to understand post-fire EMF community assembly and its role in forest recovery. We hope this study will stimulate *in-situ* research on recolonization origins and other stages of EMF succession in post-fire ponderosa pine regeneration.

## 6. References

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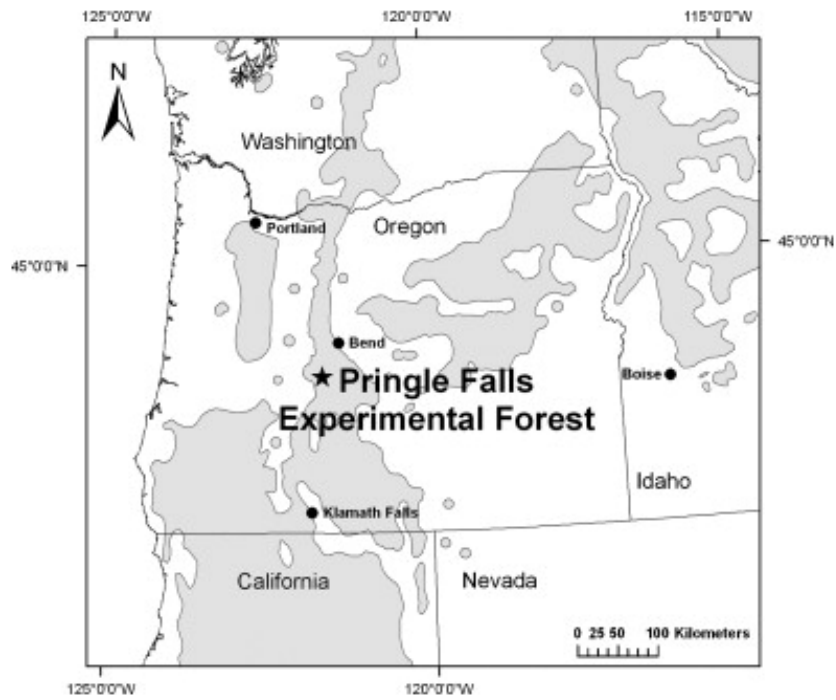


Fig. 1. Pringle Falls Experimental Forest location. Ponderosa pine distribution shown in grey shaded areas (Youngblood 2004).

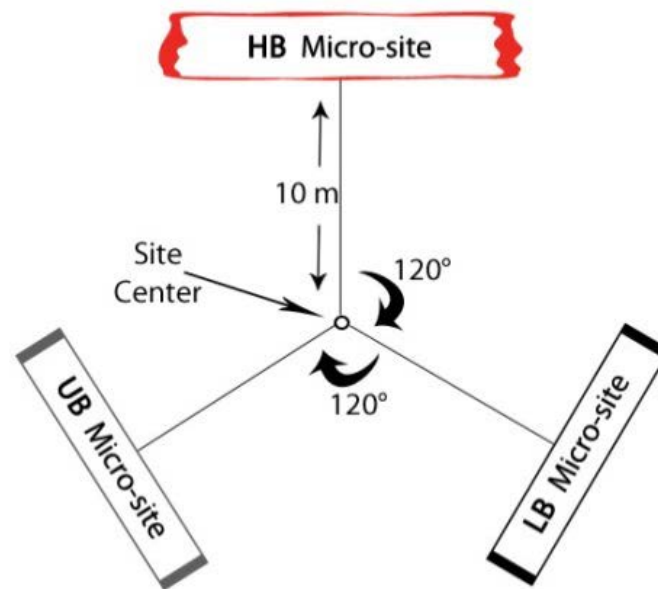


Fig. 2. Site design showing three micro-sites located 10 m from the site center. HB = high soil burn intensity, LB = low soil burn intensity, and UB = unburned control ( $n=12$  sites).

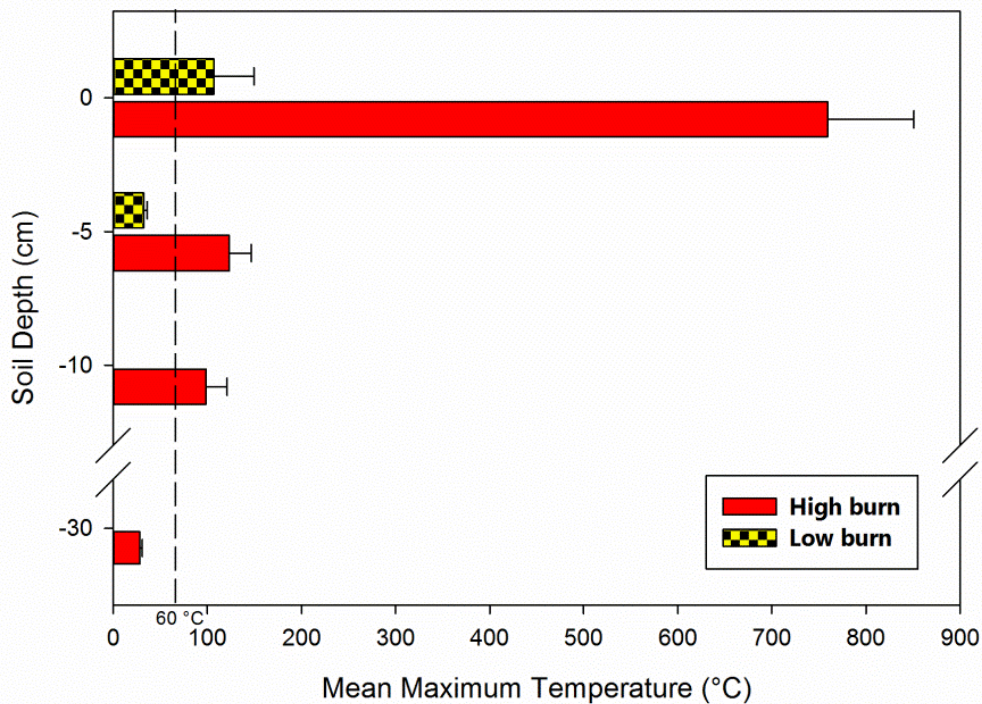


Fig. 3. Mean maximum temperature ( $^{\circ}$ Celsius) recorded under low intensity (Low burn) and high intensity (High burn) soil burn treatments at 0, 5, 10, and 30 cm depth from micro-site center ( $n=9$  sites per treatment). Approximate lethal temperature threshold for fungi is  $60^{\circ}$ C (Neary et al. 1999). Temperatures of 10 and 30 cm depths in Low burn treatment were not recorded. Error bars denote  $\pm 1$  standard error.

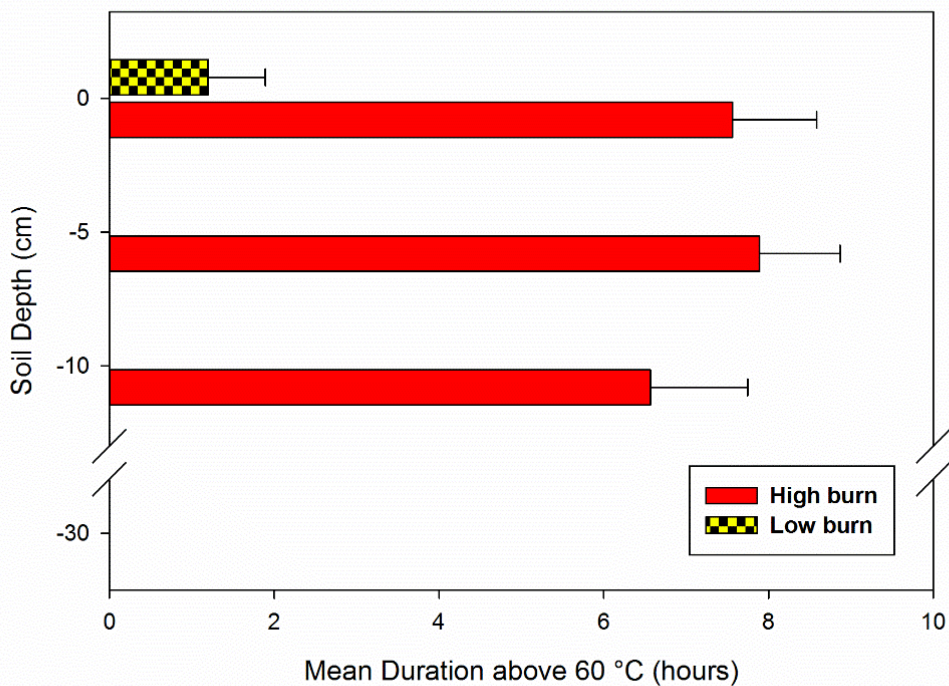


Fig. 4. Mean duration (hours) of heat above 60 °C recorded under low intensity (Low burn) and high intensity (High burn) soil burn treatments at 0, 5, 10, and 30 cm depth from micro-site center ( $n=9$  sites per treatment). Approximate lethal temperature threshold for fungi is 60 °C (Neary et al. 1999). Heat durations of 10 and 30 cm depths in Low burn treatment were not recorded. Error bars denote  $\pm 1$  standard error.

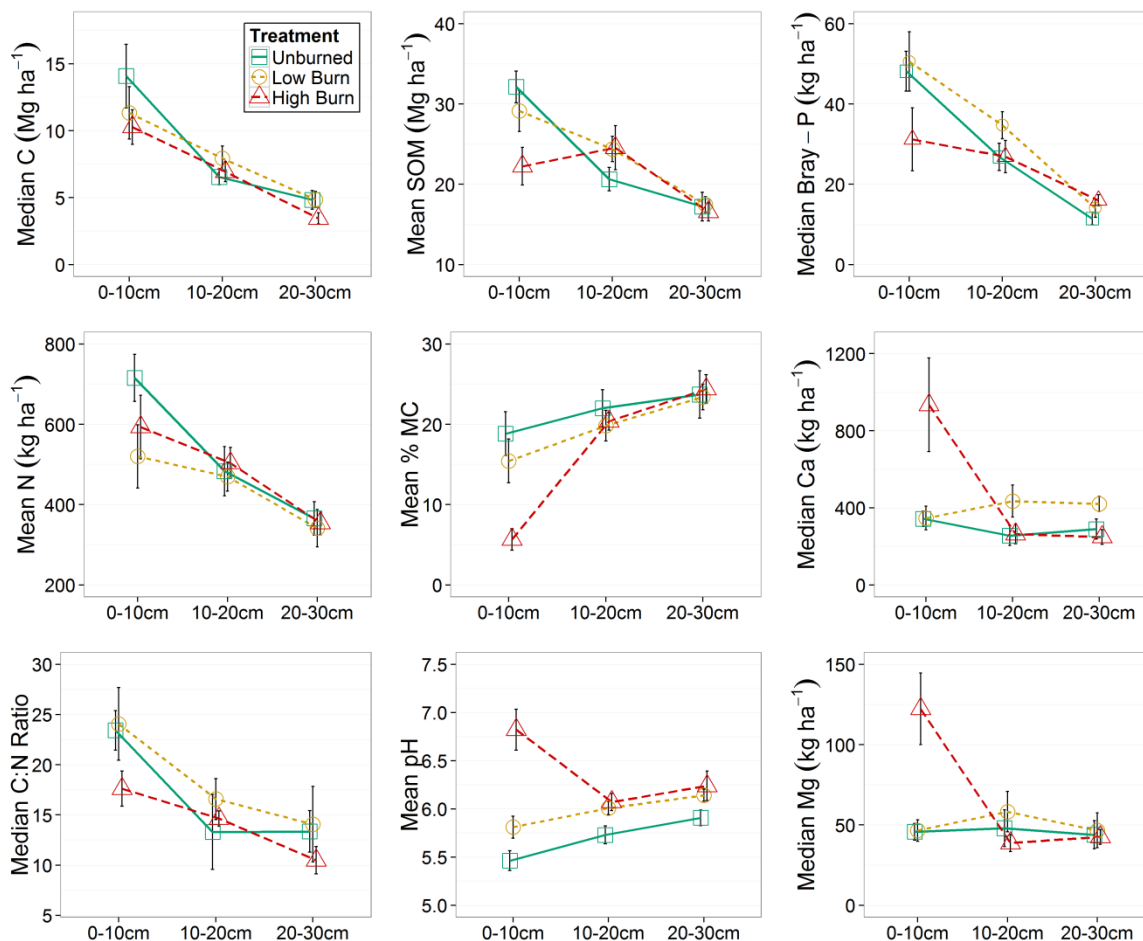


Fig. 5. Post-burn soil properties and nutrient contents. Median carbon (C), mean nitrogen (N), median C:N ratio, mean soil organic matter (SOM), mean percent moisture content (% MC), mean pH, median Bray phosphorus (Bray-P), median calcium (Ca), and median magnesium (Mg) from the center of unburned, low soil burn intensity (Low Burn), and high soil burn intensity (High Burn) micro-sites at all three soil depths ( $n = 10$  samples per treatment per depth). Error bars denote  $\pm 1$  standard error.



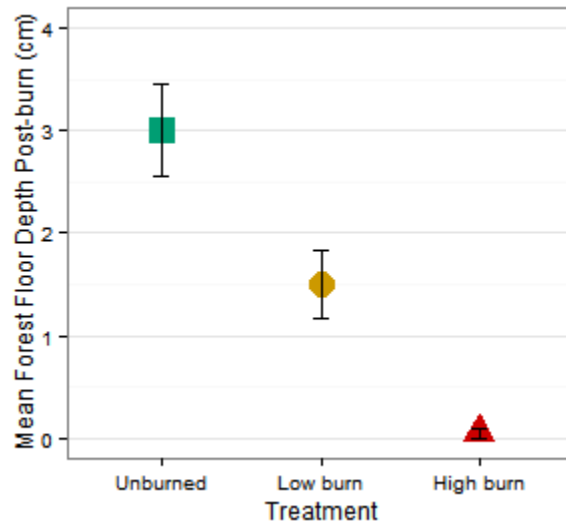


Fig. 6. Mean forest floor depth post-burn from the center of unburned, low soil burn intensity (Low Burn), and high soil burn intensity (High Burn) micro-sites ( $n = 10$  samples per treatment). Error bars denote  $\pm 1$  standard error.

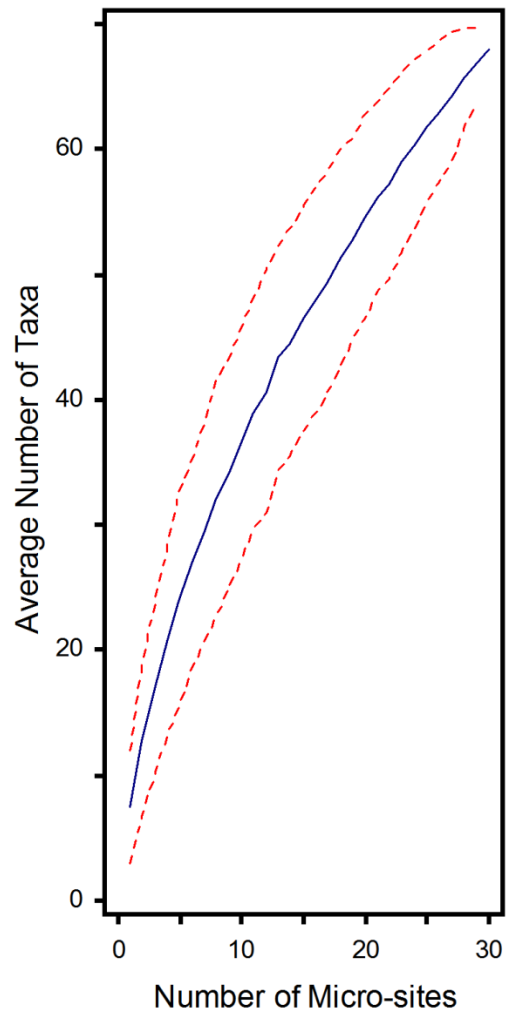


Fig. 7. Taxa area curve of average number of taxa identified by number of micro-sites. Band of confidence intervals outlined in red dashed lines. Continuous increase in curve, without leveling, demonstrates incomplete identification of all taxa present. First-order jackknife estimate = 103.77 taxa, Second-order jackknife estimate = 129.28, N=66 taxa in 10 microsites per treatment.

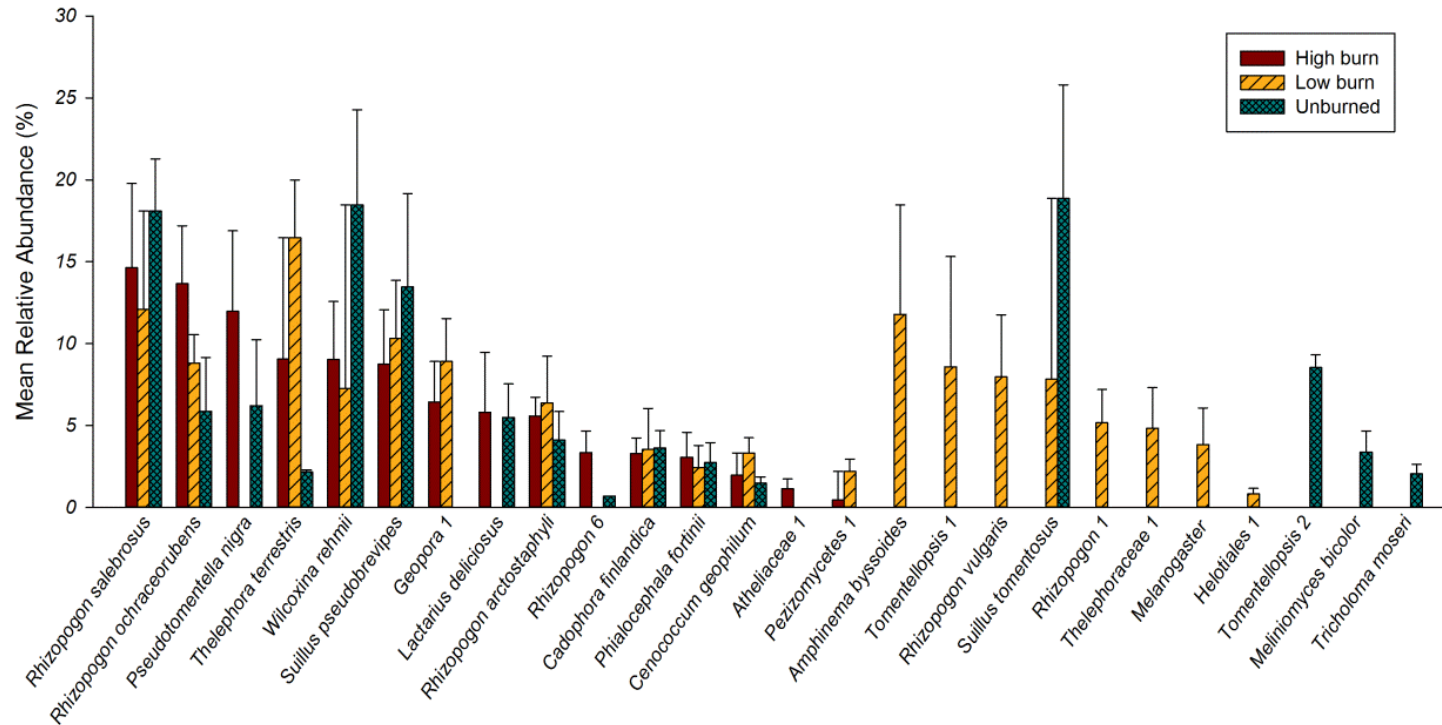


Fig. 8. Percent mean relative abundance for taxa (operational taxonomic units (OTUs)) occurring more than once in at least one of three treatments ( $n = 66$  taxa in 12 sites). Error bars denote  $\pm 1$  standard error.

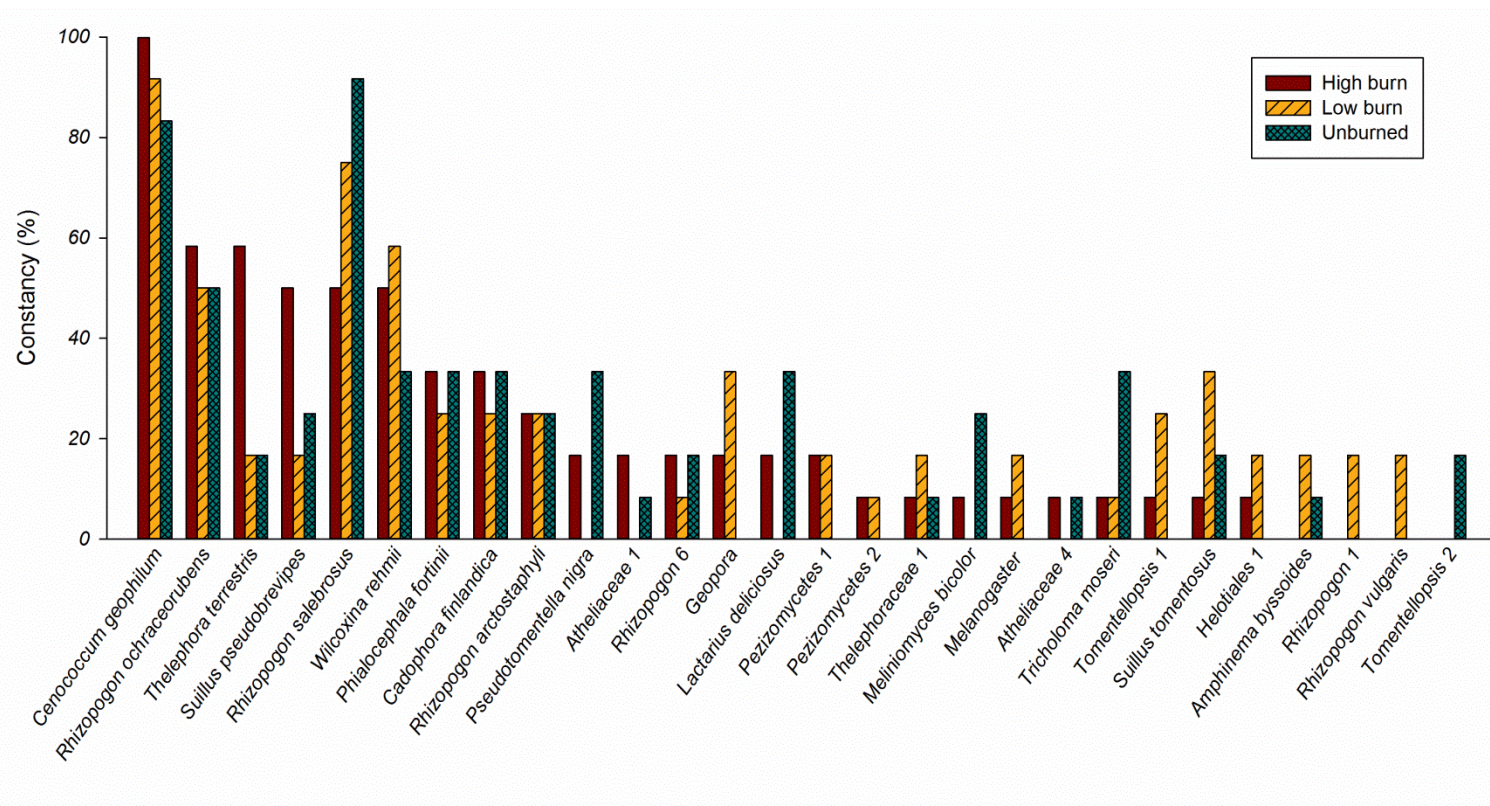


Fig. 9. Percent constancy for taxa (operational taxonomic units (OTUs)) occurring in more than one of three treatments or more than once in at least one of three treatments ( $n = 66$  taxa in 12 sites).

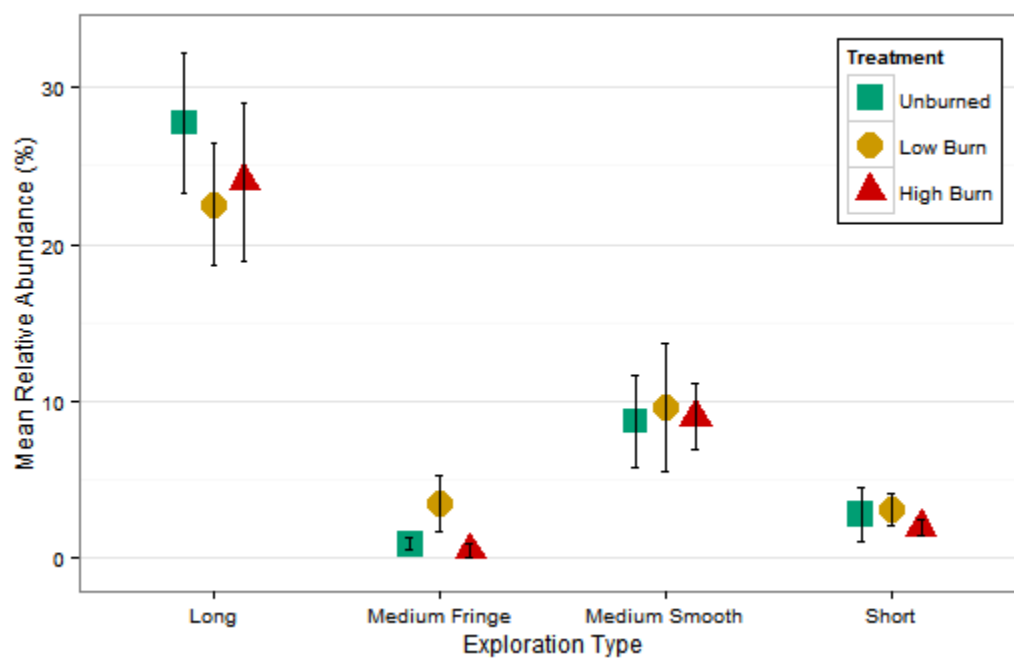


Fig. 10. Mean relative abundance of taxa identified by hyphal exploration types “long”, “medium fringe”, “medium smooth”, and “short” based on the exact or most closely related taxa from information available on DEEMY ([www.deemy.de](http://www.deemy.de)) or Peay et al. 2011. Error bars denote  $\pm 1$  standard error.  $n=36$  taxa in 12 sites (70% of identified root tips).

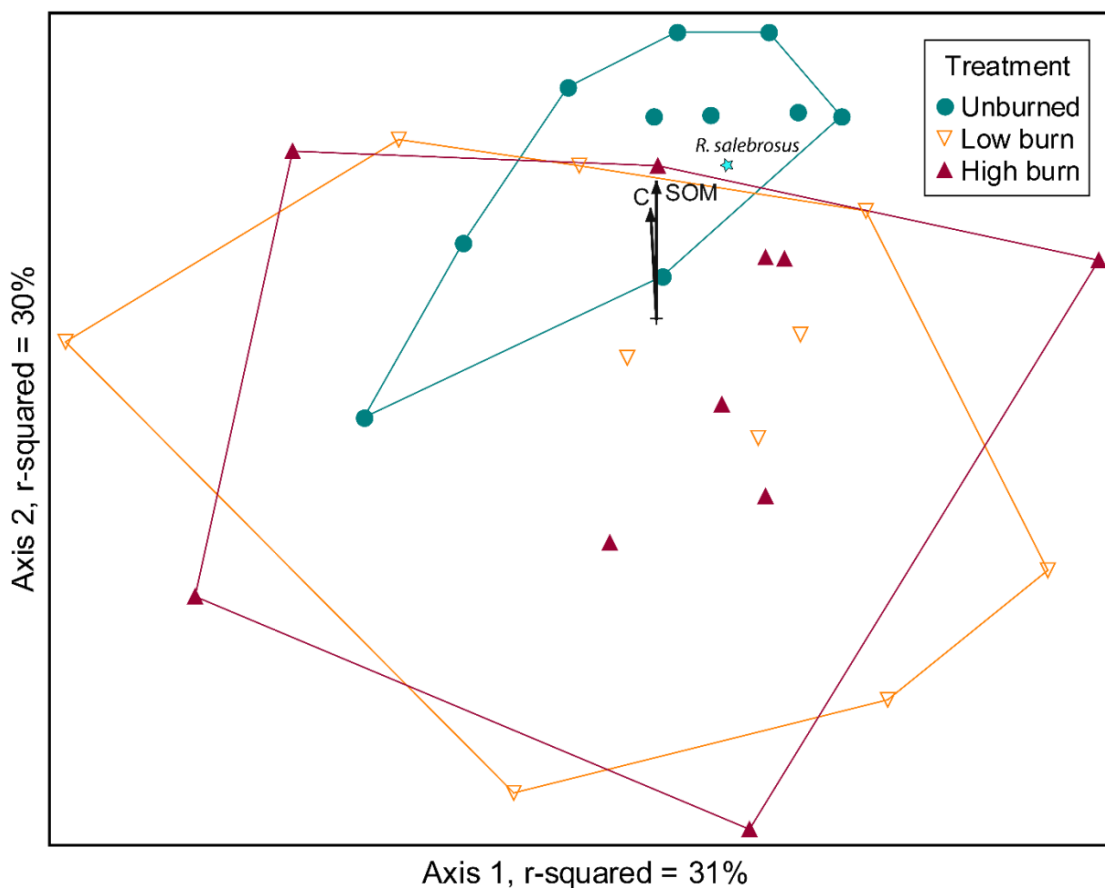


Fig. 11. Graphic results of Non-metric Multidimensional Scaling (NMS) ordination according to relative abundance of ectomycorrhizal taxa ( $n = 66$  taxa in 10 sites and 13 environmental variables). Empty triangles represent unburned micro-sites, filled circles represent low soil burn intensity micro-sites, and filled diamonds represent high soil burn intensity micro-sites. Lines connecting points outline region of graph where micro-sites of the same treatment are located.  $R^2$  is the proportion of variance (in the Sørensen distance matrix) among micro-sites explained by the axes. Nutrient contents correlated with species composition are displayed as overlay vectors in the direction of increasing correlation ( $r^2$  cutoff = 0.2). Blue star represents *Rhizopogon salebrosus*, significant indicator of unburned micro-sites (FDR-adjusted  $p = 0.0136$ , IV = 64.3).

Table 1. Site characteristics including Global Positioning System (GPS) coordinates, distance to nearest live *Pinus ponderosa* (PIPO), Diameter at breast height (DBH) of nearest live PIPO, and basal area of high intensity burn (HB), low intensity burn (LB), and unburned (UB) micro-sites.

Site	GPS Coordinates*	Micro-site	Distance to nearest live PIPO <sup>†</sup> (m)	DBH of nearest live PIPO <sup>†</sup> (m)	Basal Area (m <sup>2</sup> /ha)
43-3	43.785839°42' N, 121.658913°37' W	HB	15.1	1.9	40
		LB	18.6	1.7	30
		UB	3.8	1.9	50
43-4	43.785461°42' N, 121.658836°37' W	HB	10.0	1.5	50
		LB	1.8	2.1	40
		UB	8.7	1.8	40
43-5	43.785545°42' N, 121.658165°37' W	HB	8.0	2.3	40
		LB	13.9	2.3	30
		UB	8.5	1.9	60
43-6	43.784889°42' N, 121.657806°37' W	HB	5.5	1.2	60
		LB	7.6	1.7	90
		UB	6.0	1.9	70
43-7	43.785248°42' N, 121.65905°37' W	HB	12.0	1.8	50
		LB	5.6	1.7	60
		UB	9.1	1.9	30
44-1	43.783512°42' N, 121.648964°37' W	HB	10.0	1.3	40

		LB	11.6	1.3	50
		UB	2.1	1.3	50
44-5	43.784374°42' N, 121.647789°37' W				
		HB	11.9	1.9	60
		LB	3.9	2.0	50
		UB	10.2	2.3	70
44-6	43.783539°42' N, 121.645241°37' W				
		HB	8.2	1.5	40
		LB	4.7	1.3	40
		UB	4.0	1.7	40
44-7	43.783882°42' N, 121.644829°37' W				
		HB	8.2	1.5	40
		LB	11.0	1.9	40
		UB	12.7	1.6	20
44-8	43.783497°42' N, 121.648109°37' W				
		HB	11.2	1.8	50
		LB	4.5	1.8	60
		UB	8.5	1.6	60
44-10	43.787769°42' N, 121.646942°37' W				
		HB	10.7	1.6	40
		LB	2.0	1.5	60
		UB	14.7	1.9	30
44-11	43.78775°42' N, 121.646088°37' W				
		HB	6.1	1.7	30
		LB	13.8	1.1	30
		UB	2.5	1.1	30

\*GPS Datum = WGS84

†PIPO = *Pinus ponderosa*



Table 2. Mean total bulk density ( $\text{Mg m}^{-3}$ ), mean fine fraction bulk density ( $\text{Mg m}^{-3}$ ), and mean gravimetric rock-fragment content (%),  $\pm 1$  standard error, by soil depth in high intensity burn (HB), low intensity burn (LB), and unburned (UB) micro-sites.

<b>Treatment</b>	<b>Depth</b>	<b>Total Bulk Density (<math>\text{Mg m}^{-3}</math>)</b>	<b>Fine Fraction Bulk Density (<math>\text{Mg m}^{-3}</math>)</b>	<b>Gravimetric Rock-fragment Content (%)</b>
HB	0-10cm	0.70 $\pm$ 0.05	0.69 $\pm$ 0.05	9.40 $\pm$ 0.62
HB	10-20cm	0.80 $\pm$ 0.02	0.80 $\pm$ 0.02	9.80 $\pm$ 0.63
HB	20-30cm	0.76 $\pm$ 0.02	0.75 $\pm$ 0.03	9.60 $\pm$ 0.64
LB	0-10cm	0.59 $\pm$ 0.02	0.57 $\pm$ 0.02	9.90 $\pm$ 1.26
LB	10-20cm	0.78 $\pm$ 0.02	0.78 $\pm$ 0.03	9.80 $\pm$ 0.74
LB	20-30cm	0.73 $\pm$ 0.03	0.73 $\pm$ 0.03	10.70 $\pm$ 0.75
UB	0-10cm	0.62 $\pm$ 0.05	0.61 $\pm$ 0.05	9.90 $\pm$ 0.50
UB	10-20cm	0.80 $\pm$ 0.03	0.80 $\pm$ 0.03	11.90 $\pm$ 0.66
UB	20-30cm	0.78 $\pm$ 0.02	0.78 $\pm$ 0.02	12.50 $\pm$ 1.45

Table 3. List of taxa identified by Sanger sequencing analysis by treatments found within high intensity burn (HB), low intensity burn (LB), and unburned (UB) micro-sites, accession number of closest sequence match, maximum percent identity match, and number of sequences per taxa.

Treatment	Taxa	Accession # of Closest Match	Max ID (%)	# of Sequences	Exploration Type <sup>‡</sup>
HB, LB, UB	<i>Cadophora finlandica</i>	AF486119	99.8	13	-
	<i>Cenococcum geophilum</i>	JQ711879	99.6	47	Short
	<i>Meliniomyces bicolor</i>	AY394885	99.2	6	-
	<i>Phialocephala fortinii</i>	JQ711965	98.5	19	-
	<i>Rhizopogon arctostaphyli</i>	AF377167	99.9	14	Long*
	<i>Rhizopogon</i> sp. 6	AF062934	97.6	6	Long*
	<i>Rhizopogon ochraceorubens</i>	AF071440	99.9	31	Long*
	<i>Rhizopogon salebrosus</i>	HQ914300	99.8	76	Long <sup>#</sup>
	<i>Suillus pseudobrevipes</i>	JN858073	99.4	20	Long*
	<i>Suillus tomentosus</i>	FJ845441	99.7	8	Long*
	<i>Thelephora terrestris</i>	JQ711981	99.4	16	Medium smooth
	Thelephoraceae sp. 1	AJ889968	90.4	4	Medium smooth*
	<i>Tricholoma moseri</i>	EU726334	99.8	8	Medium fringe*
	<i>Wilcoxina rehmsii</i>	AF266708	99.1	32	-
UB and LB	<i>Alpova trappei</i>	AF074920	100	2	-
	<i>Amphinema byssoides</i>	JQ711820	99.6	3	Medium fringe
	Russulales sp. 3	GQ249401	71.6	2	-
	<i>Tomentella</i> sp. 1	AJ534914	95.6	2	Medium smooth <sup>#</sup>
	<i>Tomentellopsis</i> sp. 1	AM086447	95.9	8	Medium smooth*
LB and HB	<i>Geopora</i>	FR694202	95	6	-
	Helotiales sp. 1	EU557316	93.2	5	-
	Pezizomycetes sp. 1	JQ758928	89.6	9	-
	Pezizomycetes sp. 2	JQ759758	89.7	2	-
HB and UB	Atheliaceae sp. 1	AJ889936	91.9	3	-
	Atheliaceae sp. 4	JN943925	93.6	2	-
	<i>Lactarius deliciosus</i>	EF685051	98	8	Medium smooth <sup>#</sup>
	<i>Pseudotomentella nigra</i>	AF274770.1	98.3	6	Medium smooth*

	<i>Suillus quiescens</i>	GQ249402	99.8	2	Long*
<b>HB</b>	Atheliaceae sp. 2	KC152068	94.8	1	-
	Atheliaceae sp. 3	JX434677	97.6	1	-
	Boletales sp. 3	KC346854	87.4	2	-
	Boletales sp. 6	AF377159	78.8	1	-
	Corticiaceae sp. 1	KC007143	90.8	1	-
	<i>Rhizopogon</i> sp. 4	AF058307	95.4	1	Long*
	<i>Rhizopogon</i> sp. 5	AF071440	95	1	Long*
<b>LB</b>	Agaricales sp. 1	AY573537	83.9	3	-
	Boletales sp. 2	AJ515410	73	1	-
	Boletales sp. 4	EF458011	79.1	1	-
	Boletales sp. 5	HQ914289	86.8	1	-
	<i>Cortinarius</i> sp. 1	GQ159878	96.5	1	Medium fringe*
	Inocybaceae sp. 1	HQ604260	88.3	1	Short*
	<i>Lactarius scrobiculatus</i>	EU597079	98	1	Medium smooth <sup>#</sup>
	<i>Lyophyllum</i> sp. 1	JF908338	97.5	1	Medium fringe*
	<i>Melanogaster</i> sp. 1	JN022499	97.6	3	Long*
	<i>Rhizopogon</i> sp. 1	AF377173	96.7	3	Long*
	<i>Rhizopogon</i> sp. 2	AF058315	95	1	Long*
	<i>Rhizopogon</i> sp. 3	AF377172	95.7	1	Long*
	<i>Rhizopogon vulgaris</i>	DQ822823.1	99	4	Long
	Russulaceae sp. 1	JQ711835	91.7	1	-
	Russulales sp. 1	JQ902094	85.6	2	-
	Sebacinales sp. 1	JQ711842	72.4	1	Short*
	Thelephorales sp. 1	AY010275	85.6	2	-
	Thelephorales sp. 2	AB831842	89.9	1	-
	<i>Thelephora atra</i>	DQ974772	98	1	Medium smooth*
	<i>Tomentellopsis</i> sp. 3	HM190009	97	1	Medium smooth*
<i>Tricholoma</i> sp. 1	HM590873	95.4	1	Medium fringe*	
<i>Tomentella</i> sp. 2	JX145393	96	1	Medium smooth <sup>#</sup>	
<b>UB</b>	<i>Amanita aprica</i>	KF561972	99.7	1	Medium smooth*
	Atheliales sp. 1	JQ711824	83.7	2	-
	Boletales sp. 1	GQ267488	74.1	1	-

<i>Cortinarius casimiri</i>	GQ159814	99.8	1	Medium fringe*
<i>Russula cascadiensis</i>	EU526006.1	98	2	Short*
<i>Russula turci</i>	JQ711961	98.2	1	Short
Russulales sp. 2	EF685050	79.8	1	-
<i>Tomentellopsis</i> sp. 2	AJ410761	96.2	3	Medium smooth*
<i>Tricholoma ustale</i>	AF458435	99.8	1	Medium fringe*

†Source: DEEMY information system; <[www.deemy.de](http://www.deemy.de)>

\*Based on most closely related taxa if information was available

#Source: Peay et al. 2011

- Unknown

Table 4. Estimated mean differences in taxa (OTU) richness and Simpson's diversity compared among all three treatments. Estimated mean differences (Estimate),  $\pm 1$  standard error (Std. Error), degrees of freedom (DF), FDR-adjusted  $p$ -values, lower and upper confidence intervals (CI) are reported.

	Estimate	Std. Error	DF	FDR-adjusted $p$ -value	Lower CI	Upper CI
<b>Richness</b>						
Low burn vs Unburned	-1.00	0.99	18.00	0.43	-3.59	1.59
High burn vs Unburned	0.80	0.99	18.00	0.43	-1.79	3.39
Low burn vs High burn	1.80	0.99	18.00	0.25	-0.79	4.39
<b>Simpson's Diversity</b>						
Low burn vs Unburned	-0.06	0.06	18.00	0.46	-0.21	0.09
High burn vs Unburned	0.0	0.06	18.00	0.53	-0.11	0.19
Low burn vs High burn	0.10	0.06	18.00	0.32	-0.05	0.25

$n = 66$  taxa in 12 micro-sites per treatment