

# Recovery of ectomycorrhizal fungus communities fifteen years after fuels reduction treatments in ponderosa pine forests of the Blue Mountains, Oregon



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## ABSTRACT

Managers use restorative fire and thinning for ecological benefits and to convert fuel-heavy forests to fuel-lean landscapes that lessen the threat of stand-replacing wildfire. In this study, we evaluated the long-term impact of thinning and prescribed fire on soil biochemistry and the mycorrhizal fungi associated with ponderosa pine (*Pinus ponderosa*). Study sites were located in the Blue Mountains of northeastern Oregon where prescribed fire treatments implemented in 1998 and thinning treatments in 2000 included prescribed fire, mechanical thinning of forested areas, a combination of thinning followed by fire, and an untreated control. Soil sampling for this study occurred in 2014 and included four replications of each treatment for a total of 16 experimental units. Differences among treatments in Bray-P, total C and N, and pH were likely driven by the thinning treatments and the resultant deposition of residual slash following harvesting or the consumption of slash by prescribed fire. Similar litter depths across treatments suggest that litter depth stabilizes over time in these forests. After more than a decade of recovery, mycorrhizal fungi in dry inland forests dominated by ponderosa pine that were subjected to fire returned to levels similar to the untreated controls. The results of this study demonstrate the resiliency of these forests to disturbances associated with restoration treatments, providing managers increased flexibility if maintaining abundant and persistent fungal communities for healthy soils is an objective.

## 1. Introduction

Fire suppression, among other factors, has contributed to the alteration of previously fire-adapted ecosystems to those more at risk of stand replacing wildfire (Covington and Moore, 1994). In the last two decades, fire suppression costs have been rising exponentially as the amount of forested land that annually burns has increased (Covington and Vosick, 2016). In effect, fire suppression is producing more severe and larger fires by allowing fuel beds to increase relative to historical levels. In response to the rising cost of suppression at a receding benefit of that effort, the potential benefits of proactive actions to reduce wildfire severity have received increased attention. Increased emphasis on restoring fire adapted forest structure, along with appropriate management response to wildfires (FLAME Act 2009) and public education of the ecological benefits of fire, has facilitated heightened interest about the potential for prescribed fire and manual removal of woody materials from forested areas to decrease fire severity.

Historically, forests dominated by ponderosa pine (*Pinus ponderosa*

Lawson & C. Lawson) extended throughout much of the western United States (Oliver and Ryker, 1990; Graham and Jain, 2005). These forests were characterized by frequent fires that removed understory vegetation and promoted cohorts of trees clumped in densities as low as 20–57 trees ha<sup>-1</sup> (Covington and Moore, 1994). This sparse distribution allows increased levels of light to reach the forest floor, and when combined with frequent fire, promotes an understory dominated by grasses and herbaceous plants. Historically, low severity fire consumed fuels on the forest floor ranging from fine fuels and litter on a single fire occurrence to coarse woody debris over multiple fires. Germinating shrubs and shade-tolerant seedlings were also removed this way, creating an open understory. These “janitorial services” were removed with the advent of fire suppression, resulting in a shift in plant community structure from herbaceous and shade intolerant shrub to shade tolerant shrub and fire-susceptible tree species such as grand fir (*Abies grandis* (Dougl. ex. D. Don) Lindl.) and white fir (*Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr) (Moore et al., 1999). In-growth of this community changed fire interactions within these landscapes, bolstered

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competitive stress for water and nutrient sources, and left trees more susceptible to attack from pathogens such as bark beetles (Youngblood et al., 2004). Fires that were historically confined to the forest floor became able to climb a vegetative “ladder” that allows fire to enter the crown layer of the forest canopy and move quickly through the forest, resulting in tree mortality outside the historic range (Binkley et al., 2007). To mitigate risks of future stand-replacing wildfires, forest managers have accelerated the use of fuel reduction methods (e.g. prescribed fire and thinning), but do so with little comparative knowledge of their short and long-term environmental effects.

In the Blue Mountains of eastern Oregon, at the original site of the nationwide Fire and Fire Surrogate study (FFS) network (<http://www.fs.fed.us/ffs/sites.htm>), fuels reduction with low-intensity prescribed fire and mechanical thinning/removal treatments were studied at the operations scale, a novel approach at the time. A limitation of previous research was that many studies were conducted at scales relatively easy to measure, but not applicable beyond a single site (McIver et al., 2000). Research at the first FFS project area, known as Hungry Bob, incorporated research considerations including wildlife, insects, economics, forest pathology, vegetation, fuels, soils, and soil microbial communities (Youngblood et al., 2006; McIver et al., 2013).

The objective of the FFS study was to determine the ability of fuels reduction treatments to transition fires from those of high severity and often stand-replacing, to those of low severity. Fire severity is a secondary metric related to fire intensity. Fire intensity relates to the energy released by fire (temperature, flames height, duration of heat pulse), while fire severity relates to the consequences of the fire intensity (amount of vegetation killed, amount of fuels consumed, damage to roots) (Keeley, 2009). High severity fires may burn at soil surface temperatures exceeding 300 °C (Smith et al., 2016) and cause partial to total vegetation mortality aboveground and complete or near complete loss of belowground soil microbes in the top 15 cm of the soil profile (Rundel, 1983; Hebel et al., 2009; Reazin et al., 2016; Smith et al., 2017). In contrast, low severity fires typically experience temperatures below 100 °C at the surface and remove mostly smaller shrubs and small diameter trees, leaving larger trees and soil microbes below the top 5 cm intact (Agee, 1973; Cowan et al., 2016).

Soil microbes tend to congregate in the upper portion of the soil profile where nutrient concentrations are highest (Oliver et al., 2015), decrease in frequency with increasing depth (Anderson et al., 2014), and respond quickly to ecosystem disturbances such as fire (Smith et al., 2004, 2005, 2017; Barker et al., 2013; Kageyama et al., 2013; Reazin et al., 2016). However, their long-term response to restoration treatment disturbances, exacerbated by over a century of fire suppression, is largely unknown. Forests in which low-intensity fire has been excluded have diminished release of nutrients bound in accumulated surface leaf litter, duff, and woody debris (Monleon and Cromack, 1996; Monleon et al., 1997). This pool of nutrients, localized in the upper soil layer and diminishing with depth, creates a gradient that is presumably followed by microbial presence and abundance with higher levels of microbes near the surface and lower levels with increasing depth (Hart et al., 2005b). This accumulated duff and organic layer also contributes to increased soil heating in the event of a fire (Ryan, 2002). In a low-severity fire, flames typically have a low duration in any one place, translating into temperatures that barely penetrate the soil, leaving the microbial community intact. Deep organic layers at the soil surface provide fuel that increase the duration of time heat radiates into the soil (Busse et al., 2013; Smith et al., 2016), and have the potential to heat soil to temperatures lethal to soil microbes, communities of which can remain altered after more than a decade (Klopatek et al., 1990).

Soil microbes are directly responsible for the survival of forest trees. Mycorrhizal fungi expand the functional root network of the tree by growing over an increased surface area, making it possible to access water and nutrients that non-colonized tree roots would be unable to access on their own. In many forested systems, the primary limiting nutrients to tree growth are nitrogen (N) and phosphorus (P).

Mineralization (mobilization) of these nutrients is often facilitated by other soil microbes in the interstitial space between soil particles (Binkley and Fisher, 2012) and are then absorbed and transported via the mycelial network to the fungal symbiont's host tree(s). In exchange for soil nutrients, the host tree provides carbon (C) to the ectomycorrhizal fungi (EMF) in the form of sucrose, which is then converted to glycogen by the fungi (Smith and Read, 2010). Additional services of EMF to the host tree include physical and chemical protection from antagonistic/pathogenic fungi (Smith and Read, 2010), and connection and transfer of C among other host trees through *common mycorrhizal networks* (i.e. shared mycelial connections among trees) (Molina and Trappe, 1982; Molina et al., 1992; Simard et al., 2015).

While there is a large pool of research on mycorrhizal fungus responses to experimental changes to their environment, few studies investigate beyond a few growing seasons. Indeed, there is a paucity of long-term research on mycorrhiza responses to fire and fire-mitigation, especially when considering the extent of the fire-prone landscapes of western North America (Bastias et al., 2006; Dooley and Treseder, 2012; Holden et al., 2013; Oliver et al., 2015; Overby et al., 2015). It could be expected that after forestry operations to reduce fire risk (mechanical thinning and prescribed fire), adverse effects on fungal populations could occur. Smith et al. (2005) found this outcome, concluding that following restoration activities, fungal abundance and diversity were significantly decreased two years following thinning and one year following subsequent burning treatments. Some evidence suggests that long-term, short rotation burning can alter the belowground EMF to a depth of up to 10 cm (Bastias et al., 2006; Hart et al., 2005a). What is less well known is the response of mycorrhizal fungi to restoration practices and the length of time for which initial damage to the fungal communities persists.

The objective of this study was to assess the EMF community in the dry-climate region of eastern Oregon where fuels reduction treatments were implemented at the Hungry Bob study site over 15 years ago. Specifically we ask, did the impact of mechanical thinning, prescribed burning, or a combined effect of the two as compared to untreated forest stands have a transient or long-term effect? Additionally, is there a spatial component by depth to the EMF communities' variation in diversity, abundance, and composition within the top 10 cm of soil among each treatment type? Also investigated were differences among treatments in soil nutrients C, N, and P; pH and soil bulk density; as well as the recovery of litter - which contributes to nutrient cycling near the soil surface.

## 2. Materials and methods

### 2.1. Study area

Samples were collected from the Hungry Bob study area located on the Wallowa Valley Ranger District (Wallowa-Whitman NF) within the 12,000 ha “Waipiti Ecosystem Restoration Project” (Matzka, 2003). The Hungry Bob project area ([www.fs.fed.us/ffs/docs/hb/pubs.html](http://www.fs.fed.us/ffs/docs/hb/pubs.html)) is located between the Crow Creek and Davis Creek drainages (45°38'N, 117°13'W), about 45 km north of Enterprise, OR, and was designed as an experimental site for operations-scale harvest units. The area covers approximately 9400 ha (50 km<sup>2</sup>) and was established for the purpose of evaluating the economic and environmental impacts of restoration timber harvesting and prescribed underburning (Youngblood et al., 2008).

Soils found at the study site are generally derived from ancient Columbia River basalts, which form steep topographies interspersed with numerous plateaus, draws, and ridges. In addition, the soil has received ash from pre-historic eruptions of ancient Mount Mazama and other volcanos in the Cascade Mountains to the west (Powers and Wilcox, 1964). While there was some ash deposition from the Mount St. Helens eruption in 1980, it was less than 1.25 cm (Tilling et al., 1990) and investigation of soil at the time of sampling did not reveal an

obvious amount of ash in the upper soil profile. Soil depth varies from deep to shallow depending on aspect and elevation, and soil profiles include Typic Vitrixerands from the Olot series, Vitrandic Argixerolls from the Melhorn and Larabee series, Lithic Ultic Haploxerolls from the Fivebit series, and Lithic Haploxerolls in the Bocker Series (Youngblood et al., 2006). Soil textures of the surface mineral soil horizons were loam and ashy silt loam on the deeper more productive soils and very cobbly silt loam on the shallower soils. In this area, soils generally support plant communities dominated by ponderosa pine and sometimes mixed with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), grand fir, and lodgepole pine (*Pinus contorta* Douglas ex Loudon var. *latifolia* Engelm. ex S. Watson). Elevation at the study area ranges from 1040 m to 1480 m (Youngblood et al., 2008). Annual temperatures average 7.8 °C. Annual precipitation averages 50 cm, including an average of 66 cm occurring in the form of snow. The two distinct periods of precipitation occur annually, with snow occurring in November through February and rain occurring in March (Youngblood et al., 2006).

Previous to the treatments, the forests of the Hungry Bob area were impacted by over a century of wildfire-suppression as well as timber harvesting and grazing pressure. Timber harvest early in the 20th century resulted in removal of most of the larger trees, and forests in the area are generally in the stem-exclusion phase (O'Hara et al., 1996). Trees found at the study site are predominately second-growth (harvesting occurred as recently as 1986) and exhibit the following characteristics: tree diameters average 33.5 cm dbh, stand basal areas average 26 m<sup>2</sup> ha<sup>-1</sup>, with an average SDI of 206, and post-harvest tree density of 26 ha<sup>-1</sup>, with some residual large diameter trees that are 100–200 years old (Matzka, 2003). The understory consists of the ponderosa pine and the Douglas-fir with snowberry plant associations (Johnson and Clausnitzer, 1992), with understory vegetation dominated by snowberry (*Symphoricarpos albus* (L.) S.F. Blake), white spirea (*Spirea betulifolia* Pall.), pinegrass (*Calamagrostis rubescens* Buckley), Idaho fescue (*Festuca idahoensis* Elmer) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh)). The forest floor is covered in a layer of primarily ponderosa pine needles that averages 3 cm in depth.

## 2.2. Experimental design

From a set of 44 potential stands, 37 were determined to be the most suitable for experimental units in regard to topography and stand structure (Youngblood et al., 2006). Of these, 16 units were randomly selected for operational experimentation. Experimental units varied from 10 to 20 ha in size. Four replications of each of the four treatment types were randomly assigned to units for a completely randomized study design. The treatments were: control-untreated (no action), thinning-single entry from below, prescribed fire- single underburning of forest floor, and thin + burn-thinning from below followed by prescribed underburning of forest floor.

Thinning was conducted in 1998 via mechanical harvesting systems. Burning was scheduled for 1999 but due to unfavorable weather conditions was delayed until the fall of 2000. Restoration treatments were designed to create stands of trees that would adhere to or exceed the “80/80 Rule”, where if a head fire (moving with the wind direction) were to pass through treated stands, 80% of the trees would survive under 80th percentile weather conditions (Weatherspoon and McIver, 2000). For thinning treatments, silvicultural prescriptions for the stands prioritized establishment of irregular gaps and clumps of fire-resistant ponderosa pine into the dominant and co-dominant size classes. Trees were marked for harvesting by focusing on smaller diameter trees, leaving behind larger trees to establish dominant and co-dominant classes. Treatments were designed to reduce total basal area from ~25 to ~16 m<sup>2</sup> ha<sup>-1</sup> (Youngblood et al., 2006). Felling, limbing, and bucking of trees was accomplished with tracked, single-grip harvesters; yarding was ground based with a wheeled forwarder that used logging residuals left on site to minimize soil compaction.

Burn treatments had individual burn plans developed to address site specific characteristics of fuels and terrain (Youngblood et al. 2006). Burning included both backing and strip head fires depending on which would accomplish low flame heights. Ignition was from hand-carried drip torches, beginning in the early afternoons. Thin + Burn treatments followed protocols described for thinning (above) in 1998 and burning (above) in 2000, with the added caveat that burning procedures had to be modified to account for full combustion of logging residuals. As referenced in Youngblood et al. (2006), units randomly assigned to the no-treatment class were later determined to be dissimilar enough to the treated units (in regard to site characteristics and disturbance history) that they should not be considered “true” controls. Therefore, the use of “control” in referring to the no-treatment units is in reference to the original experimental design.

## 2.3. Plot establishment and re-establishment

Individual experimental units were located within stands of trees previously established as part of the operations-scale design. Some experimental units comprised the entirety of the stand, but in most cases they encompassed a portion of a larger stand. In all cases the experimental unit was treated the same as the rest of the stand. Prior to any treatment, plot networks were established for later sampling by creating grids of points 50 m apart using a staff compass and no less than 50 m from stand edge to address edge-related issues. At time of establishment, points were recorded with GPS units and marked with either wooden stakes in control and thin-only units (to prevent damage to harvest equipment and reduce costs) or metal posts in burn-only and thin + burn units (to relocate plots immediately following treatment). At the time of re-sampling for the current study, some plots were not readily relocated and were re-established within each stand. Using previous GPS coordinates and aerial photo map overlays, plot networks were surveyed until plots from original sampling were found, marked with flagging, and new GPS points recorded.

## 2.4. Soil sampling

Within each experimental unit, three plots had been randomly located at the time of the original sampling in June 1998 (Smith et al., 2005) and were re-sampled for the current study in June 2014 (see Hart, 2017, Fig. 2 for schematic of sampling location and design). Within each 0.2 ha circular plot, a randomly pre-determined ponderosa pine tree over 20 cm DBH was used for sampling. In cases where that tree had died or could not be located, the nearest ponderosa pine to plot center was used. A line was followed from the base of each tree due east until the edge of the canopy (dripline) was reached. At tree dripline, a serrated knife was used to cut along the edges of a 20 × 20 cm square template through the O-horizon of the soil. Once the square had been cut, the litter layer (Oi) was removed manually, leaving the duff layer (Oe) intact on the soil surface. Litter depth was measured to the nearest 0.5 cm (Table 1). Two separate soil cores were taken at each plot for analysis of soil chemistry and ectomycorrhizas, respectively. A slide-hammer with a 5 cm × 15 cm coring head lined with three 5 cm sleeves was used to extract soil (AMS Inc., American Falls, Idaho). Each core was separated into 0–5 cm and 5–10 cm depth classes (samples); the lower 10–15 cm depth was not collected. Soil was placed into sealed plastic bags and stored on ice until brought to the lab for cold storage at 4 °C.

## 2.5. Soil chemistry analysis

Soil chemical analysis was performed by the Central Analytical Laboratory (CAL) at Oregon State University. Prior to analysis, soils were dried at 105 °C for 24 h, sieved to 2 mm, homogenized in a coffee grinder, and 40 g samples were packaged in coin envelopes. The soil bulk density values were based upon the total dry weight of each soil

**Table 1**

Effects of management treatments and soil depth on live root biomass, ectomycorrhizal species richness, soil properties, and nutrient content. Means are listed with standard errors in parentheses. Within a row, bold indicates a difference among treatments or between depths, means with a common lowercase letter are not different at  $\alpha \leq 0.05$ . NA = not applicable.

Response variable	Treatment				Depth	
	Control	Thin	Burn	Dual	Upper (0–5 cm)	Lower (5–10 cm)
Root biomass <sup>1</sup> (g)	0.06 (0.03)a	0.05 (0.01)a	0.05 (0.01)a	0.07 (0.01)a	0.05 (0.01)a	0.06 (0.01)a
Species richness <sup>1</sup>	3.13 (0.79)a	3.75 (0.92)a	2.75 (0.92)a	4.34 (0.98)a	4.06 (0.57)a	2.93 (0.67)a
C (%)	5.59 (1.03)a,b	<b>8.96 (2.19)a</b>	<b>5.07 (0.91)b</b>	<b>4.24 (0.69)b</b>	<b>8.49 (1.10)a</b>	<b>3.44 (0.24)b</b>
N (%)	0.29 (0.05)a,b	<b>0.39 (0.07)b</b>	0.28 (0.04)a,b	<b>0.24 (0.04)a</b>	<b>0.40 (0.04)a</b>	<b>0.19 (0.01)b</b>
C:N	18.95 (0.87)a,b	<b>21.57 (1.37)a</b>	<b>17.53 (1.11)b</b>	<b>17.89 (0.54)b</b>	<b>20.36 (0.84)a</b>	<b>17.61 (0.60)b</b>
Bray-P (mg kg <sup>-1</sup> )	<b>40.14 (2.66)a</b>	<b>64.52 (3.45)b</b>	<b>38.14 (5.34)a</b>	<b>46.90 (6.73)a</b>	<b>54.77 (3.79)a</b>	<b>40.08 (3.81)b</b>
pH	5.84 (0.05)a,b	<b>5.57 (0.07)a</b>	5.76 (0.07)a,b	<b>6.05 (0.05)b</b>	5.80 (0.08)a	5.81 (0.05)a
Bulk density (g cm <sup>-3</sup> )	0.18 (0.06)a	0.15 (0.05)a	0.19 (0.05)a	0.19 (0.13)a	<b>0.15 (0.05)a</b>	<b>0.21 (0.03)b</b>
Litter depth (cm)	3.31 (1.4)a	3.24 (0.43)a	2.92 (0.95)a	2.67 (0.93)a	NA	NA

<sup>1</sup> Root biomass and Species richness are per soil sample.

core, which also included the remaining mineral soil particles that were equal to or greater than 2 mm in size, as well as the < 2 mm size fraction that was used for the soil chemical analyses. Samples were analyzed for total C and total N using a dry combustion process utilizing an Elementar vario MACRO Cube. Bray-phosphorus (Bray-P) was determined using sodium bicarbonate extraction following protocols by Olsen et al. (1982) and modified by Horneck et al. (1989). pH was determined using a 1:2 soil to water ratio on an ATI Orion PerpHecT LogR Meter Model 350 following Horneck et al. (1989).

## 2.6. Fine root processing of mycorrhizas

After being removed from the freezer, each sample was first soaked in water for no more than 12 h at 4 °C to loosen soil particles. Samples were then rinsed with tap water through a 2 mm sieve to remove soil. Fine roots were isolated and kept in petri dishes for examination via stereomicroscopy. Roots were examined with a stereomicroscope (Zeiss Stemi SV6, Jena, Germany), at 40x or higher. Fine roots were examined for presence of mycorrhizal colonization and were classified by morphotype following criteria as described in the Colour Atlas of Ectomycorrhizae (Agerer, 1997). Ectomycorrhizas with slight differences in appearance were separated into several morphotypes and later pooled for data analysis if warranted by identification with molecular methods. Group morphotypes were kept in individual PCR tubes and stored in 2x cetyltrimethylammonium bromide (CTAB) at 0 °C until molecular analysis. On average, two root tips from each morphotype were placed in each 5 ml tube, with only enough CTAB added to sufficiently immerse the tips. Ectomycorrhizas of each morphotype from a given core sample were lyophilized and weighed to the nearest 0.0001 g.

## 2.7. Molecular analysis

From each group morphotype, one root tip was selected for DNA extraction and amplification so that in the event that PCR products could not be visualized with UV light, the other tip could be used for extraction. Each root tip was pulverized with a micro-pestle and then DNA was extracted using a Sigma Extract-n-Amp kit™ (Sigma Aldrich, St. Louis, Missouri). The extracted DNA was then amplified via PCR of the internal transcribed spacer region (ITS) using primers ITS 1F (Gardes and Bruns, 1993) and ITS 4 (White et al., 1990). Individual PCR reactions contained 17.65 µl of molecular grade water, 0.16 µl GoTaq® (Promega, Madison, Wisconsin), 7.5 µl of 5 × PCR Buffer, 3 µl of 10x deoxynucleotide triphosphates (dNTPs), 0.85 µl of MgCl<sub>2</sub>, 0.4 µl of bovine serum albumin (BSA), 0.3 µl of each primer (50 µM), and 0.5 µl of DNA template. PCR of the internal transcribed spacer region cycling parameters consisted of a 2-min denaturation at 95 °C followed by 30 cycles of 94 °C for 30 s, 50 °C for 1 min, 72 °C for 1.5 min, and a final

extension of 72 °C for 10 min. 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 uncontaminated reagents. When a sample failed to amplify, another root tip from the same morphotype was used for molecular analysis. DNA from PCR products was purified using ExoSAP-IT® (Affymetrix, Santa Clara, California) and quantified for sequencing using a Qubit® 1.0 fluorometer (Invitrogen, Carlsbad, California).

Purified PCR samples were shipped to the University of Kentucky Advanced Genetics Technology Center (UK-AGTC) for sequencing. Direct sequencing of PCR products was performed by 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 (Biomatters Limited). Regions with primer motifs of more than 4% chance of error per base were trimmed from sequences. Once trimmed, sequences of high similarity (> 99%) were placed into identical groupings (contigs). Operational taxonomic units (OTUs) were defined as those sequences having ≥ 98% similarity to each other. Sequences were analyzed for taxonomic placement and further trimming using the blastn suite of the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (NCBI) database (Altschul et al., 1997). The morphotyped root tips were given names to the lowest taxonomic level possible based on BLAST results and the following criteria: “uncultured/environmental sample sequences” were excluded and the query term “voucher” was specified to limit searches. OTUs with ≥ 98% identity over at least 500 base pairs were assigned to the species or species group level; at the genus level, OTUs generally had an (85%) 90% to 97% identity, identities at the family level were variable depending on the context of the matches but were generally in the 90% to 95% range, and OTUs with identities in the 80% to 94% range were assigned to an order if the top 5 matches were consistent at that level. When sequences within a soil core sample were the same species, they were combined to create “contigs” for subsequent root weight abundance measurements.

## 2.8. Statistical analysis

Univariate soil nutrient data, species count data, and species abundance data were analyzed in RStudio v.0.99.491. Analysis of statistical assumptions yielded data that were all normally distributed except for total C and total N which were naturally log-transformed for statistical analysis and then back-transformed for ratio inference of treatment comparisons. An extension of a two-way analysis of variance was used that included a repeated measures (autoregressive 1) linear mixed model except for litter data that did not have a repeated measures element. Variation from sample units and depth were accounted

for in the models. Mean soil nutrient differences and median ratios of differences for log-transformed data and the subsequent confidence intervals were estimated from the model.

Non-parametric multivariate comparisons of species abundance and soil nutrient data were conducted using PC-Ord™ software version 6.2 (McCune and Mefford, 2009). Non-metric multidimensional scaling (NMS) (Kruskal, 1964) ordinations were used to provide graphical representations of variation in community structure among treatments while accounting for soil nutrient data. Species with only 1 occurrence were removed to increase the chance of detecting trends. The analysis used Sørensen (Bray-Curtis) (Mather, 1976) distance measures, run in auto-pilot, penalizing for ties, in “slow and thorough” mode, used 250 runs of real data and 249 runs of randomized data, to produce an NMS output with a stable 2-axis solution that had real and random stress values of 14.52 and 19.8, respectively, and passed the Monte Carlo significance test ( $p = 0.012$ ). Multi-response Permutation Procedure (MRPP) (Mielke, 1991) was used to test for differences among communities based on treatment type. MRPP analysis provides a  $p$ -value for a test of the hypothesis of no difference between groups and an  $A$  statistic (chance corrected within-group agreement) that represents the effect size on homogeneity of random chance, with  $A = 0$  meaning that group homogeneity equals that expected by chance while  $A = 1$  means that all sample units within each group are identical. Indicator Species Analysis (Dufrene and Legendre, 1997) was performed to assess if any of the taxa in the study were indicative of a particular treatment type using PC-Ord (McCune and Mefford, 2009).

Species accumulation curves were generated using the sample-based estimator of EstimateS v. 9.1 (Colwell, 2013). Cowell et al. (2012) introduced linked rarefaction and extrapolation curves and these were implemented in EstimateS v. 9.1. Extrapolation returns the expected number of species in  $t$  pooled samples, given the reference sample. We had a reference sample size of 12 and extrapolated to 36 samples which is within the range recommend by Cowell et al. (2012). Data from the three soil cores from each of the four treatment replications were used to generate the curves ( $3 \times 4$  provides the reference sample size of 12). Samples 13–36 in the ectomycorrhizal fungus species accumulation curves present the extrapolation in the model based on 1000 randomizations of the data. EstimateS v. 9.1 was also used to obtain the diversity measures for Fisher’s alpha, Simpson’s inverse (reciprocal), and Shannon’s indices and to calculate evenness.

### 3. Results

#### 3.1. Soil physical properties

For bulk density, there was no evidence of a treatment level effect ( $F_{3,12} = 1.93$ ,  $p = 0.17$ ), but there was significant evidence of an effect of depth ( $F_{1,12} = 24.48$ ,  $p < 0.01$ ) (Table 1). There was no evidence of an interaction between treatment type and depth ( $F_{3,12} = 0.75$ ,  $p = 0.54$ ). Mean upper layer soil bulk density was estimated to be  $0.06 \text{ g/cm}^3$  less than lower layer ( $p = 0.03$ ,  $DF = 12$ ,  $CI = -0.08$  to  $-0.02$ ). There was no evidence that litter depth differed among treatments ( $F_{3,12} = 0.36$ ,  $p = 0.78$ ) (Table 1).

#### 3.2. Soil chemical properties

There were significant differences in total soil C among the treatments ( $F_{3,12} = 4.02$ ,  $p = 0.03$ ) and between depths ( $F_{1,12} = 136.02$ ,  $p > 0.01$ ) (Table 1) but no evidence of an interaction between treatment type and depth ( $F_{3,12} = 2.71$ ,  $p = 0.09$ ). There were no statistical differences in C among treatments compared to the control (Tables 1 and 2). Among treatments, mean soil C content was estimated to be 1.77 times higher in the Thinning treatment compared to the Burn treatment ( $p = 0.02$ ,  $DF = 12$ ,  $CI = 1.21$  to  $2.56$ ) and 2.03 times higher in the Thinning treatment compared to the Dual treatment ( $p \leq 0.01$ ,  $DF = 12$ ,  $CI = 1.41$  to  $3.02$ ).

**Table 2**

Associated overall ANOVA  $p$ -values and Fisher’s PLSD  $p$ -values for comparisons of differences among treatment means of those response variables showing significant differences in Table 1 ( $n = 4$ ). Between-treatment comparison  $p$ -values of  $\alpha \leq 0.05$  are shown in bold.

Comparison	$p$ -value
<i>C</i> ( $p = 0.003$ )	
Control vs Thin	<b>0.02</b>
Control vs Burn	0.51
Control vs Dual	0.11
Thin vs Burn	<b>0.004</b>
Thin vs Dual	<b>0.0004</b>
Burn vs Dual	0.33
<i>N</i> ( $p = 0.02$ )	
Control vs Thin	0.08
Control vs Burn	0.89
Control vs Dual	0.15
Thin vs Burn	0.06
Thin vs Dual	<b>0.003</b>
Burn vs Dual	0.19
<i>C:N ratio</i> ( $p = 0.02$ )	
Control vs Thin	0.06
Control vs Burn	0.20
Control vs Dual	0.41
Thin vs Burn	<b>0.003</b>
Thin vs Dual	<b>0.01</b>
Burn vs Dual	0.63
<i>Bray-P</i> ( $p = 0.0005$ )	
Control vs Thin	<b>0.0003</b>
Control vs Burn	0.73
Control vs Dual	0.26
Thin vs Burn	<b>0.0001</b>
Thin vs Dual	<b>0.006</b>
Burn vs Dual	0.15
<i>pH</i> ( $p < 0.0001$ )	
Control vs Thin	<b>0.004</b>
Control vs Burn	0.37
Control vs Dual	<b>0.03</b>
Thin vs Burn	<b>0.03</b>
Thin vs Dual	<b>&lt; 0.0001</b>
Burn vs Dual	<b>0.003</b>

Treatments varied in mean soil N content in the main ANOVA ( $F_{3,24} = 3.8$ ,  $p = 0.02$ ) and mean N differed between soil depths ( $F_{1,12} = 98.67$ ,  $p < 0.01$ ) (Table 1). There was no evidence of an interaction between treatment type and depth ( $F_{3,12} = 0.91$ ,  $p = 0.46$ ). Mean soil N content was estimated to be 1.63 times higher in the Thinning treatment compared to the Dual treatment ( $p = 0.003$ ,  $DF = 24$ ,  $CI = 1.18$  to  $2.16$ ). The difference between the Thinning treatment and the Burn treatment was  $1.39\times$ , however  $p = 0.06$  (Table 2); only suggestive of a statistical difference. Mean N was estimated to be 2.1 times greater in the upper soil layer than the lower ( $p = 0.009$ ,  $DF = 12$ ,  $CI = 1.55$  to  $2.55$ ).

For plant available P there was strong evidence of a treatment effect ( $F_{3,12} = 4.97$ ,  $p = 0.02$ ) as well as a depth effect ( $F_{1,12} = 42.57$ ,  $p < 0.01$ ) (Table 1) and no evidence of an interaction between treatment type and depth ( $F_{3,12} = 1.71$ ,  $p = 0.22$ ). Mean Bray P was estimated to be  $24.38 \text{ mg/kg}$  soil greater in the Thinning treatment compared to the Control treatment ( $p = 0.01$ ,  $DF = 12$ ,  $CI = 9.68$  to  $39.08 \text{ mg/kg}$  soil),  $26.38 \text{ mg/kg}$  soil greater in the Thinning treatment compared to the Burn treatment ( $p \leq 0.01$ ,  $DF = 12$ ,  $CI = 12.82$  to  $39.94 \text{ mg/kg}$  soil), and  $17.62 \text{ mg/kg}$  soil greater in the Thinning treatment compared to the Dual treatment ( $p = 0.03$ ,  $DF = 12$ ,  $CI = 4.05$  to  $31.18$ ) (Table 1). Mean Bray P concentration was estimated to be  $14.68 \text{ mg/kg}$  soil greater in the upper soil layer than the lower ( $p = 0.06$ ,  $DF = 12$ ,  $CI = 6.66$  to  $22.72 \text{ mg/kg}$  soil) (Table 1).

For pH there was evidence of a treatment level effect ( $F_{1,12} = 5.92$ ,

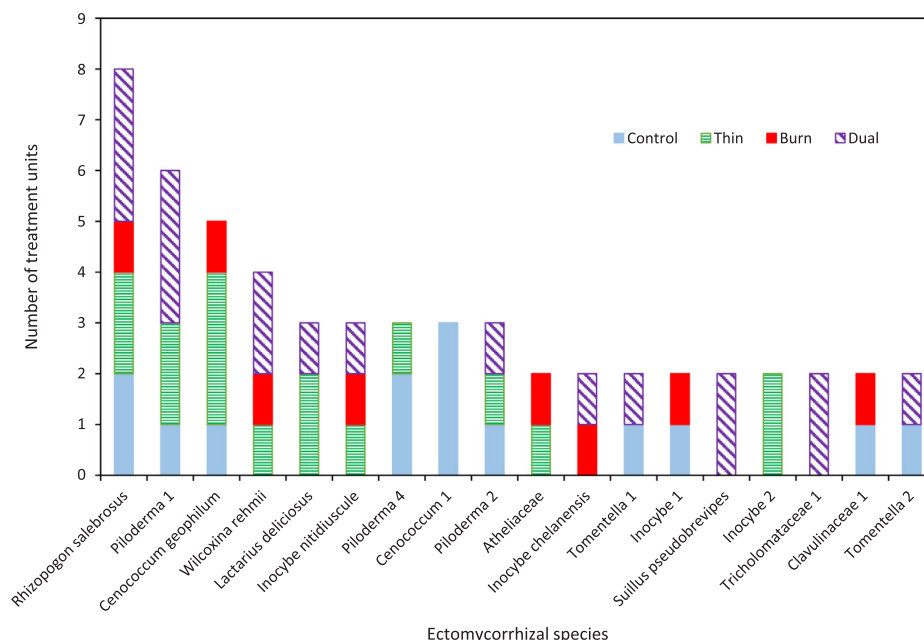


Fig. 1. Species occurrence by treatment for the 18 most frequent EMF species (species occurring in two or more treatment units).

$p = 0.01$ ) but no difference between depths ( $F_{1,12} = 0.06, p = 0.81$ ) (Tables 1 and 2), and no evidence of an interaction between treatment type and depth ( $F_{3,12} = 1.42, p = 0.28$ ). pH was estimated to be 0.45 units lower in the Thinning treatment compared to the Dual treatment ( $p = 0.01, DF = 12, CI = -0.45$  to  $-0.23$ ).

### 3.3. EMF communities

A total of 174 purified PCR samples submitted for ITS sequencing yielded 117 taxa among treatments and depths, with 49 taxa (42%) in the upper soil depth and 68 taxa (58%) in the lower depth. Of these, 60 OTUs were successfully assigned family level designation or lower. A list of 108 high quality, representative ITS sequences that were submitted to GenBank is provided in Supplementary Table 1, including the accession number for each sequence. Only 18 taxa occurred in two or more treatment units (Fig. 1), 45 (38%) taxa were detected from only 1 plot, indicating that species were widely scattered across the study site. This pattern of a few dominant and a large number of infrequent taxa was also found by Smith et al. (2005).

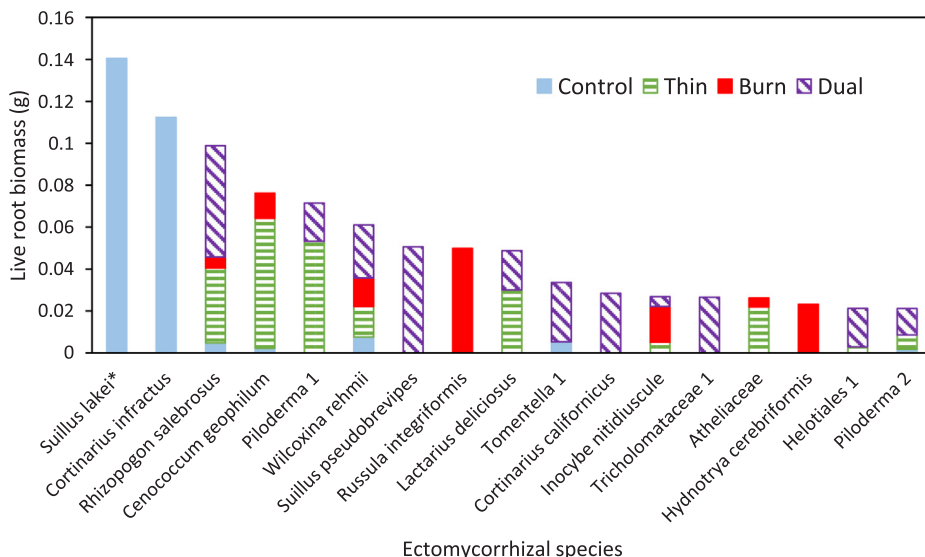
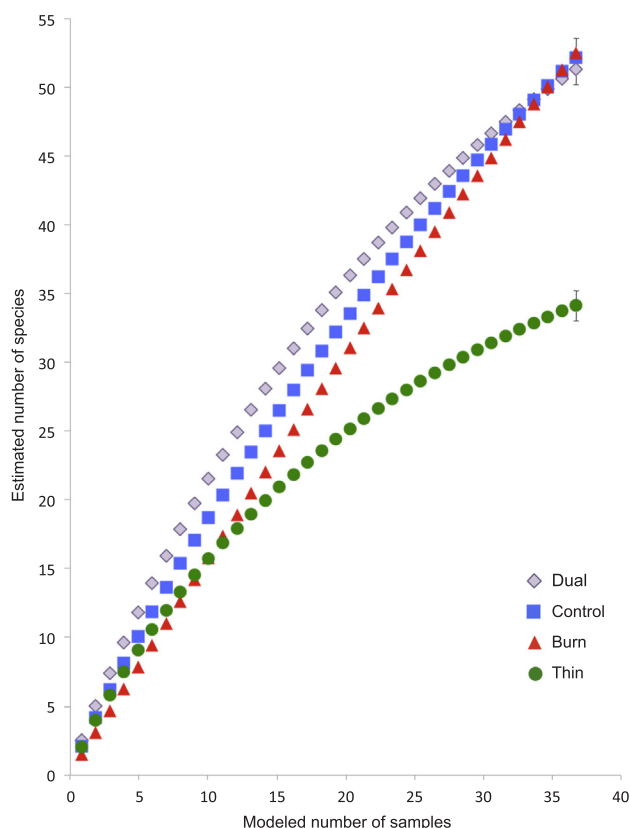


Fig. 2. Live root biomass by treatment for the 17 most abundant OTUs as assigned to closest species groups. Seventeen OTUs, each with  $\geq 0.02$  g or  $\geq 1.7\%$  of total biomass, accounted for 79% of the total biomass. Representative ITS sequences for all OTUs were deposited with GenBank. Accession numbers and submitted OTU names are presented in Supplementary Table 1. \*Known associated with *Pseudotsuga menziesii*.

Seventeen OTU species, each with 1.7% or more of the total EMF biomass, accounted for 79% of the total EMF biomass (Fig. 2). For live root biomass, there was no evidence of a treatment level effect ( $F_{3,12} = 0.66, p = 0.59$ ) or an effect of depth ( $F_{1,12} = 1.06, p = 0.32$ ) (Table 1).

For mean soil sample species richness, there was no evidence of a treatment level effect ( $F_{3,12} = 0.41, p = 0.25$ ) or an effect of depth ( $F_{1,12} = 2.53, p = 0.86$ ) (Table 1) and no evidence of an interaction between treatment type and depth ( $F_{3,12} = 1.13, p = 0.61$ ). However, species accumulation curves for each treatment showed that the density of ectomycorrhizal fungus species across the thinning treatment units (total EMF species richness at the unit landscape scale;  $n = 4$ ) was about 35% lower as compared to the other treatments when estimated for 36 modeled samples (Fig. 3).

Diversity measures for Fisher’s alpha, Simpson’s inverse (reciprocal), and Shannon’s indices are presented in Table 3. Fisher’s alpha and species richness are lowest in the Thinning treatment. Simpson’s inverse and Shannon’s indices show the Control treatment to have the lowest values. The Control treatment was strongly dominated by two species (Fig. 2) and this was reflected in the evenness values (Table 3).



**Fig. 3.** Species accumulation curves for each treatment ( $n = 4$ ) generated using the rarefaction/extrapolation sample-based estimator of EstimateS show that the density of ectomycorrhizal fungus species across the thinning treatment units is about 35% lower as compared to the other treatments when estimated for 36 modeled samples. The reference sample size is 12 and species richness curves are based on extrapolation for samples 13–36. The standard error intervals (vertical bars) are shown for the values estimated for 36 modeled samples.

**Table 3**

Diversity measures of ectomycorrhizal fungi detected on *Pinus ponderosa* roots for each treatment.<sup>1</sup>

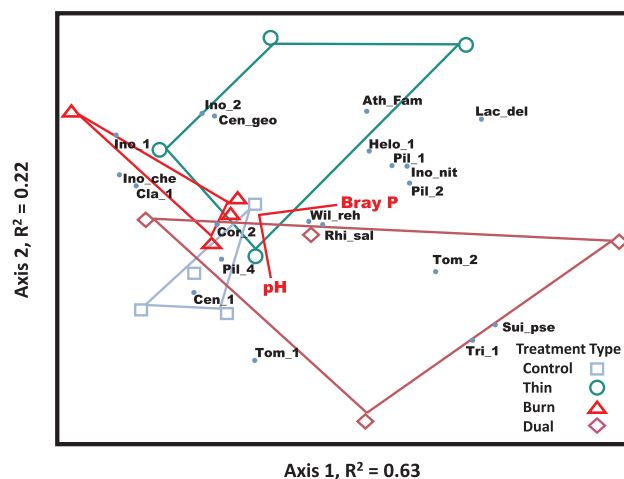
Diversity measure	Treatment			
	Control	Thin	Burn	Dual
Species richness	22	18	19	25
Fisher's alpha	3.19	2.56	3.00	3.63
Simpson's inverse	3.87	8.66	7.22	11.76
Shannon's	1.85	2.43	2.38	2.71
Evenness	0.60	0.84	0.81	0.84

<sup>1</sup> Based on data pooled across four replicates of each treatment.

The Thin, Burn, and Dual treatments are similar to each other with evenness values ranging between 0.81 and 0.84. The value for the Control treatment is much lower, 0.60, due to the high relative dominance of two taxa.

Multi-Response Permutation Process (MRPP) results yielded no evidence of a difference in EMF composition among treatments ( $T\text{-stat} = -1.66$ ,  $p = 0.06$ ); test results also had a very low effect size value ( $A = 0.04$ ). Indicator Species Analysis showed that OTU *Cenococcum 1* was correlated with the Control treatment ( $p = 0.02$ , Indicator Value = 75.0).

The NMS ordination, based on sample units in species space, provided a stable two-axis solution (Fig. 4). The axes accounted for a total of 85.4% of the variation among sample units based on the species abundance, with axes 1 and 2 accounting for 63.1% and 22.2% of



**Fig. 4.** Graphical display of Non-metric Multidimensional Scaling (NMS) ordination. NMS produced according to relative abundance of EMF taxa ( $n = 60$  taxa in 16 treatment units and six environmental variables). Color coded lines represent the region in which treatment units are located.  $R^2 =$  proportion of variance (using the Sørensen distance matrix) among treatment units explained by the axes. The phosphorus gradient was correlated with axis 1 at  $R = 0.54$  ( $p = 0.03$ ). The pH gradient was correlated with axis 2 at  $R = 0.46$ , but the significance was marginal ( $p = 0.07$ ).

variation, respectively. Sample unit points that are closer to each other share a higher proportion of species similarity. Graphically grouping the sample units by treatment in the ordination space demonstrated moderate overlap (Fig. 4). None of the treatment types were completely isolated from the others. A phosphorus gradient was correlated with axis 1 ( $R = 0.54$ ,  $p = 0.03$ ). The Burn and Dual treatments showed the least overlap on this gradient (Fig. 4). Axis 2 was correlated with pH but the significance was marginal ( $R = 0.46$ ,  $p = 0.07$ ). The Thinning and Dual treatments showed the least overlap on this gradient (Fig. 4).

## 4. Discussion

### 4.1. Project overview

The results of this study suggest that effects of fuel reducing restoration efforts on the variables measured here are largely short-term, and that recovery of soil biological, chemical, and physical attributes to levels similar to the control occurred within less than 15 years. The only treatment with significant differences from the control was the Thinning treatment, but of particular management interest is that the Thinning treatment in some cases differed from the Burn treatment and consistently differed from the Dual treatment. A 15-year recovery period is well supported as sufficient time for these soil responses to thinning and burning (Binkley et al., 1992; McKee, 1982; Hart et al., 2005b; Busse et al., 2009). In a meta-analysis of the Fire Fire-Surrogate study as a nationwide network, Boerner et al. (2009) found that regardless of treatments across multiple landscapes and environments, the impacts to soil physical and chemical attributes were both “modest in magnitude and transient in duration”. At a site similar to the Hungry Bob, also located in the Blue Mountains of eastern Oregon, Hatten et al. (2008) found no differences in soil C, N, pH or plant-available nutrients 7 years after spring or fall prescribed burning compared to the control.

It is important to note that the sampling methods for this study targeted the ectomycorrhiza community response. Our finding of similar fine root biomass in the upper and lower 5 cm cores did not support the hypothesis that greater mycorrhiza abundance would be found in the upper core. However, microbial abundance and colonization is often highest near the soil surface (Oliver et al., 2015) and decreases with depth (Anderson et al., 2014). Our methods included

exclusion of the loose litter layer (Oi) but *included* the early and late litter de-compositional layers (Oe and Oa respectively). The inclusion of organic matter in the upper soil cores may help explain why values for C, N, and P are higher and bulk density is lower in the upper soil cores than would be expected if only mineral soil had been collected.

#### 4.2. Soil chemical properties

In analysis of the long-term effects of fuels treatments within the study site, significant differences were found among the treatments for various soil chemical properties. However, while none of the treatments differed in soil biogeochemical and physical responses from the control (with the exception of greater plant available P in the Thinning treatment), treatments differed significantly in particular response variables from each other. The effects of the Thinning treatment created soil nutrient conditions that differed from those with a burning component, and differences with the Dual treatment in particular, were pronounced. This finding is likely attributable to the effects of the post-treatment woody harvesting residual debris and how it was managed. In the Thinning treatment, harvesting residuals were left on site to decompose. In the Dual treatment, harvesting residuals were incorporated into the subsequent burning. Decomposition in the Thinning treatment residuals may have resulted in elevated microbial activity; in contrast with the Dual treatment where reduced microbial activity resulted in less organic matter. Moreover, annual temperature and precipitation regimes of the eastern Blue Mountains permit for mobilization/leaching of nutrients for a relatively short period each year, which could in part explain why trends created during initial treatment may still be detectable in this study over a decade post-treatment.

Total soil C was found to be highest in the thinning treatment, which aligns with findings by Chatterjee et al. (2008) and Yanai et al. (2003) who hypothesized that long-term increased soil C following harvesting based restoration treatment was likely due to incorporation of forest residuals into the soil layer. Other mechanisms proposed include increased leaf/root turnover from the forb/shrub community (Campbell et al., 2009) and interactions of soil order and time (Nave et al., 2010).

The generality of N trends following C trends (Turk and Graham, 2009) is supported in this study. Nitrogen mobilization can increase as more labile sources of N are released by decomposition of slash in concert with labile C availability that promotes microbial activity (Grady and Hart, 2006; Monleon and Cromack, 1996). Johnson and Curtis (2001) report increases of up to 18% of soil C and N could be expected when tree harvesting residuals are left on site. Our detection of increases in N of up to 30% in the Thinning treatment is likely attributable to the incorporation of organic material found within our sampling methodology. Combustion of woody material initially leads to a pulse of N within the first few years of burning (Covington and Sackett, 1992; Wan et al., 2001; Schoch and Binkley, 1986) but this effect diminishes within a few years (Binkley and Fisher, 2012). Due to lack of further treatment since initial burning and little residual organic matter on the floor of burned units, it is probable that uptake by plants in burned units have left overall N levels lower than the Control and Thinning treatments.

Given that the Control treatment units in this study were atypical compared to other pre-treatment areas in this study, one of the considerations addressed was if there were any existing differences in soil chemical make-up at the time of treatment. An exploratory ANOVA of pre- and post-treatment soil chemistry data revealed that prior to treatment, the Control was not significantly different from the other treatments. However, similar to the current study, measurements indicated a significant difference of soil C in the Thinning treatment compared to the Burn and Dual treatments. This finding indicates there may have been some inherent difference in stand structure or nutrient deposition from the canopy that was driving some of the post-treatment trends seen.

Soil chemical processes are often linked to pH which may in part explain our findings for plant-available P. In this study, plant-available P was the only response variable where the Thinning treatment was significantly different from the Control as well as Burning and Dual treatments. Ash production from combustion of organic material is known to increase soil pH (Neary et al. 1999; Arocena and Opio, 2003; Moghaddas and Stevens, 2007) due to the higher concentrations of hydroxides in ash and by the process of calcium, magnesium, and potassium displacing hydrogen and aluminum ions in soil. In areas such as the Blue Mountains where precipitation is low, this effect could last for several years. Values for pH were significantly different between the Dual and Thinning treatments (0.45 units lower in the Thinning). In a review of the Fire Fire-Surrogate Network by Stephens et al. (2012), it was determined that even at the network scale, higher pH also occurred in the Dual treatment. Deposition of harvest residuals that were then burned provided the organic material needed to raise pH in the Dual treatment. Conversely, unburned harvest residuals left in the Thinning treatment likely contributed to acidification of the soil (Binkley and Fisher, 2012).

Bray-P can be elevated in response to fire. Temperatures exceeding 400 °C can mobilize P into orthophosphates that are available for plants utilization (Binkley et al., 2012). However, such extreme temperatures generally are not found in prescribed fires where mineral soil temperatures rarely exceed 120 °C (Cowan et al., 2016) unless induced to higher levels by practitioners (Smith et al., 2016). In a 20-year interval study of controlled burns in SW Arizona, Wright and Hart (1997) found no differences in mineral P compared to controls. This is consistent with other authors (Binkley et al., 1992; Kaye et al., 2005; Saa et al., 1993) and the study presented here.

#### 4.3. Soil physical properties

Soil bulk density did not significantly differ among treatments, even though it is known that prescribed fire can increase soil bulk density (Certini, 2005). Bulk density could also be related to treatment placement on landforms within the study area. Although the treatments were assigned to sample units by complete randomization, the outcome by chance was that units with a burning component were often along ridges where soils were shallow and rocky; Thinning units typically had a lower slope position and deep soils (Morici, 2017).

Harvesting equipment in Thinning units operated on trails covered with harvested tree limbs and tops, thus adding low-density organic material to the soil. Litter accumulation (Oi) and subsequent “duff” depth (Oe and Oa) also did not differ among treatments, providing strong evidence that productivity of these sites has recovered well since treatments. Litter accumulation may be correlated with densities of mycorrhizal roots (Malajczuk and Hingston, 1981) and the recovery of litter depth could in part reflect on our finding of no difference among treatments in mycorrhizal root biomass and mean EM species richness per soil core. EM species richness has been shown to be related to EM root-tip density (Luoma and Eberhart, 2014; Erland and Taylor, 2002). Smith et al. (2005), determined that one year post-burning and two years post-thinning at this study site, mycorrhizal root abundance, as well as duff depth, was significantly lower in the Dual treatment. Whereas now, litter depth and fine root biomass have recovered to levels that are similar to those found in the control.

#### 4.4. EMF communities

Outcomes from the forest restoration treatments studied here indicate that the ectomycorrhizal symbionts of ponderosa pine responded positively during a period of recovery without further disturbance. Several studies point to EMF reductions after disturbance in the short-term (Smith et al., 2004, 2005; Barker et al., 2013) as well as the long-term (Klopatek et al., 1990). The historic fire return interval in the region where this study was conducted is estimated to be 15–20 years



(Heyerdahl et al., 2001). It could be expected that the EMF communities that had developed prior to fire suppression would have been adapted to recovering within this time period. Other studies support this idea, showing community similarity to controls in as little as 6 years after burning in the southeastern United States (Oliver et al., 2015), or recovered communities within 12 years in severely burned boreal systems (Treseder et al., 2004). Covington et al. (1997) estimated 10–20 years before soil properties stabilize around long-term averages. Other authors suggested similar timeframes: two decades (Holden et al., 2013), twelve or more years (Fritze et al., 1993), or at a minimum a decade (Oliver et al., 2015) to fifteen years (Treseder et al., 2004) before soil microbial populations recover to pre-disturbance levels. These findings support 15 years as being an adequate period of time for various aspects of soil microbial communities to recover, as was found in this study.

Negative effects from heat disturbance on microbial communities have been shown to be transient and community recovery has been reported (Hart et al., 2005a; Overby et al., 2015). Cowan et al. (2016) suggest that patchiness of burn intensities coupled with rapid hyphal penetration into areas where EMF were extirpated due to fire could be one mechanism for recolonization of burned areas. Latent spore banks within the soil have also been shown to facilitate EMF community recovery after fire (Bruns et al., 2005). The strip-head type ignition pattern used in the burning treatments of our study produced a mosaic of patchiness, leaving areas of low to high intensity burning as well as unburned areas (Youngblood et al., 2008). Low-intensity burns can produce lethal temperatures of 60 °C (Dunn et al., 1985) to a depth of a few centimeters in the soil profile (Cowan et al., 2016; Smith et al., 2016). That scenario was likely the case in this study, yet EMF abundance recovered in the Dual treatment, indicating that even with the hottest temperature penetration and duration as postulated by Smith et al. (2005), recovery of EMF can be dramatic.

The lower rate of species accumulation detected across the thinning treatment suggests that over-all species richness for that treatment is lower than that of the other treatments (Fig. 3). This is in contrast with the results for the mean species richness per soil core (as a point measurement), which did not differ among treatments; though even at the reference sample size of 12, the trajectory of the species accumulation curve is starting to diverge from that of the other treatments (Fig. 3). The strong divergence of the thinning treatment curve indicates that the thinning treatment supports fewer less-common EMF species, which is an important insight on EMF species response when integrated across the sampled landscape.

Since the singletons are excluded from the NMDS analysis, the extrapolation is also important to capture information from the tail of the distribution that is not included in the NMS ordination. The evenness values, not surprisingly, match the patterns shown in Fig. 2. The Thin, Burn, and Dual treatments are similar to each other in evenness (0.81–0.84, Table 3). The value for the Control treatment (0.60) is much lower due to the high relative dominance of two taxa (Fig. 2).

From the diversity measures (Table 3), we see that Fisher's alpha and species richness are lowest in the Thinning treatment. This is not surprising since Fisher's alpha is dominated by the richness component of the index. Simpson's inverse and Shannon's indices show the Control treatment to have the lowest values. This is also not surprising since those two indices are sensitive to dominance of a few species and, as noted above, the Control treatment was strongly dominated by two species.

The fact the Fisher's alpha is low in the Thinning treatment (2.56 vs. 3.00–3.63) is further evidence for our interpretation that fewer rare species are found across the thinning treatment. That species richness does not differ among treatments at the individual soil core level suggests that ectomycorrhizal root tip density is similar across the treatments. Yet, despite this, a sparser complement of EMF species was recovered from roots in the thinning treatment. This finding correlates well with differences in N levels and corresponds to what is known from

the literature regarding nitrogen effects on EMF, as discussed below.

Ectomycorrhizal fungus species richness and community composition are sensitive to N levels in the soil (Kluber et al., 2011). Though fertilizer effects on EMF are varied and complex, we hypothesize that the unburned litter across the thinning treatment could influence EMF abundance and richness via a “fertilizer” effect. Luoma and Eberhart (2010) found that a one-time application of N (as urea) decreased feeder-root density by 62% and decreased ectomycorrhiza type richness by 33%. Those reductions in ectomycorrhizae closely mirrored the results of Nilsson and Wallander (2003) who found that EMF produced about 50% less mycelium in the soil after N fertilization (as ammonium sulfate) for ten years. Kårén and Nylund (1997) found a 49% reduction in EMF root biomass associated with N fertilization of 100 kg ha<sup>-1</sup> yr<sup>-1</sup> (as ammonium sulfate) for six years prior to sampling. Conversely, Jonsson et al. (2000) found that moderate N additions (as ammonium nitrate) for four years had little effect on EMF root tip density.”

The vertical partitioning of mycorrhizal fungi was one of the questions of interest in this study, and evidence for EMF to preferentially distribute within the soil profile has been demonstrated by several authors (Beiler et al., 2012; Dickie et al., 2002; Rosling et al., 2003; Scattolin et al., 2008; Tedersoo et al., 2003). Statistically the upper partition of the cores was not greater in EMF than the lower, so this finding may warrant further investigation with more intensive sampling and include a seasonal aspect. Whether or not this could be attributed to litter recovery and the lack of nutrient cycling in this water-limited system that effect on decomposition has yet to be investigated. For most soil chemistry and EMF community variables, the largest differences in responses were between the Thinning and Dual treatments. Although ash deposition has been thought to facilitate recovery of microorganisms in relation to increased pH (Knicker, 2007), it could also be that nutrient depleted soils are driving the EMF response. Trees in soils that are rich in N and P have been found to support less abundant EMF (Nilsson et al., 2005; Toljander et al., 2006), presumably because fewer carbohydrates need to be allocated to root growth for absorption. Chatterjee et al. (2008) found that long-term increases in soil C resulted in decreased microbial levels as well. Soils that are impoverished of nutrients necessary for tree growth may be driving increased belowground fine root production which in turn increases the abundance of symbiotic soil fungi.

Among the 18 most prevalent (those occurring in two or more treatment units) taxa forming ectomycorrhizae in this study, half reproduce by forming aboveground fleshy fruiting bodies (e.g. mushroom, club or cup fungi). The other form truffle-like fruiting bodies, are cryptic in various substrates, or are non-fruiting. This group of fungi includes members of the genera *Cenococcum*, *Piloderma*, *Rhizopogon*, or *Tomentella*. These fungi, despite not usually having airborne spores, are well represented in studies of both short- and long-term responses to disturbance (Visser, 1995; Dahlberg et al., 2001; Horton and Bruns, 2001; Smith et al., 2004, 2005). It is possible that colonization by these typically early seral species that can then persist across many seral stages is disproportionately important, especially if the ecosystem is subject to regular disturbance such as fire. Eleven of the 18 (61%) most prevalent taxa of the 60 found in this study were also the most abundant (by root biomass) (Figs. 1 and 2). However, some abundant species were infrequently detected including the most abundant species, *Suillus lakei*, collected from a single core and exemplifying the patchy distribution of EMF. The presence of this species indicates that Douglas-fir roots were detected in this soil core since *Suillus lakei* only associates with Douglas-fir. Dominance of a few ectomycorrhizal taxa within species-rich assemblages is common in pine forest systems (Gehring et al., 1998; Grogan et al., 2000; Stendell et al., 1999; Smith et al., 2004, 2005). Ponderosa pine appears to associate with similar EMF assemblages across a fairly broad ecological range, as many of the more prominent species reported in Trappe et al. (2012) from southern Oregon, Garcia et al. (2016) from south-central Oregon, and Smith et al.

(2004) from the southern portion of the Blue Mountains of north-eastern Oregon were also found in this study.

#### 4.5. Resiliency of fire-prone landscapes and management implications

One of the strengths of the nationwide Fire and Fire Surrogate study was the commitment to assessing the effectiveness of fuels reduction treatments at what is known as “the operational scale” (Youngblood et al., 2006). This means that treatments were implemented on a size scale that reflected typical management considerations and not at the smaller sizes more common in experimental research, often due to costs of implementation. As mentioned previously, even prescribed fires have inherent patchiness associated with their burning patterns. During treatment operations at the Hungry Bob site, fire managers were instructed to ensure that sampling areas were burned in order to capture the effect of treatment. In the short-term (one year post-burn), both burning treatments significantly reduced EMF species richness, live root biomass, and litter depth (Smith et al., 2005). The general recovery of EMF communities to levels not differing from the control is encouraging with regard to the longevity of outcomes of fuel reduction treatments.

Carbon, N, and Bray P levels were highest in the Thinning treatment and EMF community recovery was similar or greater in the Dual treatment where nutrient levels were lowest. This EMF response may have a relationship to the demand for the services EMF provide in terms of nutrient acquisition. With regard to a changing mosaic of diverse habitats, if a management objective is to maintain high diversity and abundance of ectomycorrhizal fungus species on the landscape, then incorporating a burning component to a restoration management strategy may be preferred to thinning alone.

Unfortunately, even though a relatively low-cost strategy, prescribed burning alone is a resource sink and does not provide revenue. Thinning alone does accomplish the objective of lowering stand densities and modifying stand composition to accelerate development of late seral characteristics (Youngblood 2006) but it does not remove downed wood on the forest floor and as our study has shown, does not promote a higher mycorrhizal presence compared to the Dual treatment. The Dual treatment, which removes organic material from the forest floor by combustion during prescribed burning also recoups expenses from revenue generated during the thinning process.

Burning operations conducted in the fall diminish surface layer EMF more than burning conducted during the spring (Smith et al., 2004). Since it is widely recognized that the process of restoring fire suppressed stands in the western United States will require multiple entries into stands (Hessburg et al., 2005; Spies et al., 2006; Youngblood et al., 2006; McIver et al., 2013), it is important to minimize negative impacts during preliminary entries to ensure ecosystem recovery.

Management focusing on mitigation of risks associated with high severity fire as it is relevant to individual stand conditions is needed. The use of thinning activities exclusively is useful for reducing forest crown connectivity, lowering stand densities as well as elevating crown base height while also selecting for larger trees with more desirable crown structure and increased fire resistance. Perhaps the most practical aspect of thinning is the associated economic returns that can provide revenue for further restoration techniques. However, the potential nutrient deposition from fine needles and branches associated with thinning alone promotes continued fine root development near the soil surface. Re-introduction of the ecological processes of prescribed fire removes fuel from the forest floor and can remove smaller diameter trees by heat girdling, but cannot accomplish goals of broader tree removal from overly dense stands without particularly hot fires that may damage or remove desirable trees as well. Combining the two treatment types with a preparatory thin followed by prescribed burning captures the benefits of both. The data support that using a burning treatment can be successful in terms of maintaining the mycorrhizal communities. Treatments receiving burning recovered more thoroughly than the thinning alone treatment over the same time period. The data also show

that either of the burning treatments have equivalent outcomes in regard to the recovery of the ectomycorrhizal community. Therefore we propose that the dual thinning and burning treatment overall is more effective at reaching broad management objectives and specific outcomes regarding EMF because ectomycorrhizal communities associated with the Dual treatment were not negatively impacted in the long term while above-ground plant communities were restored to a more resistant and resilient condition. If managers seek to reduce the severity of future wildfires in the shortest and most economical manner, the use of prescriptions similar to those used in the Dual treatment may be the optimal option, precluding stand specific conditions that would merit an alternative approach.

#### 5. Implications for forest management

The results of this study indicate that EMF populations are able to re-establish in areas where they had previously declined as a result of initial thinning and burning restoration activities in the Blue Mountains of eastern Oregon (Smith et al., 2005). This finding supports a growing body of evidence that EMF communities are resilient to low-severity disturbance (Hart et al., 2005a; Overby et al., 2015; Cowan et al., 2016), and expands it to include the activities of mechanical thinning and prescribed burning. However, this research has just “scratched the surface” with regard to sampling the EMF community. The more common taxa recovered well over 15 years, but more remains to be known about the less common species that form the tail of the species abundance distribution. This is well illustrated by the species accumulation curve (Fig. 3).

This research is valuable to land managers as it provides evidence that aggressive restoration treatments seemingly have little long-term effect on EMF and certain soil parameters and this new information may improve confidence in various options for future management. There is an increased need for accelerated restoration over large landscapes to reduce the risk of stand-replacing wildfire. The species richness and abundance of EMF were equally high in the Dual treatment as compared to the others, suggesting that lower soil nutrients found in association with regular fire return ecosystems may increase the importance of maintaining EMF in these forest types. Thinning alone resulted in soil conditions with higher nutrient values, while thinning followed by prescribed burning yielded lower-nutrient soil.

If managers are faced with resistance to prescribed burning, thinning alone can be an effective way of reducing stand basal area densities and promoting a discontinuous canopy. However, in order to return ecological processes to the fire-suppressed forests of the mountain west, inclusion of a burning component to restoration treatments is essential. Ultimately, objectives of land managers in the future will include creating stands of trees that are resistant to the effects of drought and higher fire-risk resulting from climate change. Reduction of competitive stress by reduction of stand basal area will be a primary approach of restoration efforts. Multiple entries to remove live trees will likely be required.

The findings of this study are limited to fire-suppressed low-elevation stands of ponderosa pine in the Blue Mountains. Further research to evaluate the outcomes presented here in other fire-suppressed forest ecosystems and across elevations would greatly widen the scope of knowledge about the effects of fuel treatments on ectomycorrhizae. The ability to broaden application of these insights would benefit land managers who are faced with an uncertain future of fire, but with a certain call to action.

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