Effects of High-Severity Wildfires on the Ectomycorrhizal Fungal Communities of Ponderosa Pine Ecosystems in Eastern Washington

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Abstract

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Ectomycorrhizal (EcM) fungi have the ability to mediate ecosystem responses to post-fire environmental changes, yet we have limited understanding of how high-severity wildfires influence the EcM community, especially in systems adapted to low-severity fires. To elucidate the effects of high-severity fires on the EcM community of ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws) ecosystems, I performed a chronosequence study of the post-fire period from 2006 to 2015, with the objective of identifying the changes to species richness and community composition. Additionally, we addressed if an eleven-year span was sufficient time for species richness to reach pre-fire levels. Microsite variability has been shown to affect the EcM community, thus, we also tested for differences in soil nutrients, total carbon (TC), phosphorus (TP), nitrogen (TN) and their respective ratios, to determine if they play a role in the EcM community assemblage. Comparison of the EcM communities between burned and neighboring unburned areas revealed distinct differences between these communities. Both species richness and community composition were significantly lower in the high-severity sites, leading to a complete turnover in species composition. The new environment became dominated by taxa that

respond positively to high-severity fires. Most of these taxa were members of the Ascomycota phylum, and include the genera *Cennococum* and *Pustularia*, which increased on average by 9-fold (adj-p<0.005) in the post-fire environment. Of importance to this community was the presence of *Wilcoxina rehmii*, a known PIPO symbiont and pyrophytic fungi. We found no relationship between species richness and increased time since fire, possibly due to the small scale of the chronosequence. However, the significant differences in community composition between the burned and unburned sites suggest that an 11-year recovery period is not sufficient for the EcM fungi communities to return to pre-fire levels. The changes to the post-fire communities were not solely dependent on fire disturbance. We found significant associations between the EcM communities and physicochemical site characteristics, such as elevation and slope, as well as TC and TC: TP. High-severity fires significantly affected the soil nutrient composition, but the alterations were shown to be ephemeral. Total carbon, TN, TC: TN and TC: TP decreased with increase time since fire. In contrast, TP and OM depth increased with increase time since fire.

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Dedication

Para mi mama, la persona más importante en mi vida. ¡Siempre has sido tú la fuerza impulsora de mis éxitos y por eso y más te doy las gracias!

For my mom, the most important person in my life. You have always been the driving force of my success and for that, I thank you.

&

To my husband, thank you for all of your love, help, and support.

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Effects of High-Severity Wildfires on the Ectomycorrhizal Fungal Communities of Ponderosa Pine Ecosystems in Central Washington

Introduction

Wildfire is an important ecological component of forested ecosystems. Thus, various tree species, such as ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws) have developed adaptations to frequent, low-severity wildfires (Heyerdahl et al., 2001; Oliver & Ryker, 1990; Cooper, 1961). However, decades of fire exclusion, altered land use patterns, and timber harvesting, combined with climate change, have caused wildfires to deviate from historical patterns (McKenzie et al., 2004; Littell et al., 2009; Naficy et al., 2010: Mallek et al., 2013). Today, wildfire extent and severity have increased all across North America (Mantgem et al., 2013; Littell et al., 2009; McKenzie et al., 2004), such that landscape structure has been severely impacted resulting in increased research of the response of the ecosystem to high-severity (HS) wildfires. Although alterations to the aboveground ecosystems have been extensively studied, little is known about the effects of altered fire severity regimes on the ectomycorrhizal (EcM) community of ponderosa pine (PIPO).

EcM fungi are a key component of the soil community in forested ecosystems due to their associations with dominant tree species, such as PIPO (Smith and Read, 2008). In some ecosystems, such as boreal forests, EcM fungi make up approximately 32% of total microbial biomass (Anderson et al., 2007). Via their obligate associations, EcM fungi attain their sole source of photosynthetically derived carbon from their host (Smith and Read, 2010). In exchange, the EcM fungi supply their host with mineral nutrients, assist with water acquisition, as well as physical and chemical protection from pathogenic fungi (Taylor and Bruns, 1999; Smith and Read, 2010), and allow for the transfer of carbon, via connections to the common mycorrhizal network (Molina and Trappe, 1982; Song et al., 2015). In addition, EcM fungi contribute to carbon storage and nutrient soil fluxes (Clemmensen et al., 2013; Klein et al., 2016). The ability of EcM fungi to minimize microsite alterations and allow their hosts to thrive in environments where resources are limited makes them invaluable for the maintenance of a healthy, thriving forest, and for restoration of disturbed ecosystems.

Research in both prescribed and wildfire affected ecosystems have shown that soil surfaces have the potential to reach temperatures between 400°C - 600°C (Neary et al., 1999; Smith et al., 2016). At these temperatures, soil chemical and physical characteristics, as well as the EcM community are drastically affected (Debano et al., 1998: Neary et al., 1999; Debano, 2000; Hart et al., 2005). High soil surface temperatures lead to the volatilization of soil nutrients, such as nitrogen and phosphorus (Debano et al., 1998), resulting in a decrease in total phosphorus (TP), total nitrogen (TN), and total carbon (TC) (Smith, 1970; Kutiel and Naveh, 1987; Baird et al., 1999; Certini, 2005; Eldiabani et al., 2008). Furthermore, the combustion of the organic layer and the increase temperatures of the mineral layer during a HS fire have the potential to surpass the lethal temperature thresholds of EcM fungi, which approximate 60 ˚C (Dunn et al., 1985; Neary et al., 1999).

Previous studies have shown that fires can have various effects on EcM fungi and that depending on the severity of the fire; the effects can be transient or long lasting. For example, Johnson et al., (1999) reported little changes to the composition and densities of EcM fungi during prescribed burns. In contrast, the impact of HS fires can be dramatic and long lasting. Research has shown that HS fires alters the community composition of EcM fungi, creating a simple community composed of a few taxa (Visser, 1995; Smith et al., 2004; Martin-Pinto et al., 2006; Buscardo et al., 2011; Glassman et al., 2016). Additionally, shift in species abundance and lower production of sporocarps have been reported following HS fires (Grogan et al., 2000; Treseder et al., 2004; Martin-Pinto et al., 2006). Although research has shown that certain EcM taxa are highly active in the post-fire environment (Baar et al., 1999; Hart et al., 2005; Claridge et al., 2009), the overall community composition requires 15-18 years to return to pre-fire conditions (Treseder et al., 2004; Kipfer et al., 2011). Given that PIPO requires an EcM association for proper growth and survival, the potential, long-lasting alteration to its symbiont could significantly alter the ecosystem for decades.

Ponderosa pine is a wide-ranging conifer found throughout the Western United States (Graham et al., 2005). Before fire exclusion, PIPO ecosystems experienced frequent, low-severity wildfires approximately every 40 years (Agee, 1993; Hann et al., 1997) that removed understory vegetation and released nutrients into the system. Unfortunately, shifting fire regimes have led to the homogenization of the flammable landscape by increasing the density of fire-intolerant species and increasing accumulation of the soil organic layer (Graham et al., 2005). Consequently, PIPO ecosystems have been predisposed to burn at HS. Given that PIPO ecosystems and most of their organisms evolved under the shared selective pressures of low-severity wildfires, the above- and belowground communities are experiencing high mortality rates. Consequently, the post-fire landscape is riddled with large, HS burn patches. High severity, per this study, consists of two components, vegetation burn severity, and soil burn severity. High vegetation burn severity is defined as areas where more than 75% of the basal area in the stand has been removed (Agee, 1993). In contrast, high soil burn severity is defined as areas where the ground cover and the soil organic layer have been completely consumed (Parsons et al., 2010). Given the ecological importance of PIPO for increasing forest resiliency, its relationship to forest fires, and its dependence of EcM fungi, PIPO has been the center of increased research. Recent studies in PIPO ecosystems have used prescribed wildfires, lab experiments, and wildfires at differing severities (Johnson et al., 1999; Cowan et al., 2016) in order to understand the response of the EcM community. To the best of my knowledge, no study has looked at PIPO ecosystems affected by HS fires over a chronological period. Thus, we aim to increase the knowledge about the EcM taxa present in the post-fire environments of PIPO ecosystems and allow forest manages to develop a proper management plan that incorporates understanding of the soil microbiome. Given the interrelationship between fire severity, soil chemical and physical alterations, and the highly complex and heterogeneous effects of the fire upon the landscape (Near et al., 1999; Certain, 2005; Hart et al., 2005), we limited this study to fire adapted ecosystem that have experience abnormally HS wildfires. Since the year 2000, Eastern Washington has experienced 18 large wildfires, which burned over 8,084 ha, with approximately 25% of the area burning at HS (MTBS). These fires have provided the opportunity to investigate the impact that HS wildfires have upon the ECM community composition of PIPO by making it possible to implement a chronosequence study of 11 years of post-fire history. In this study, we addressed the following objectives: (a) Determine the differences in soil nutrients between fire impacted forest and neighboring unburned forest; (b) Assess if the changes to the nutrient levels, in the burned forest, change with increase time since fire; (c) Determine if alpha diversity differs between the burned and neighboring unburned forest; (d) Determine if the EcM community composition differs between burned and unburned forest; (e) Determine if the post-fire community composition is composed of rare taxa; (f) Assess if species richness, in the burned forest, is altered with increased time since fire.

We hypothesized that: (a) Soil nutrients will be lower in the burned sites as opposed to the unburned sites; (b) The observed changes in soil nutrients in the burned sites will decrease with increase time since fire; (c) EcM fungal species richness and diversity (species richness, evenness, and diversity) will be lower in the burned sites as opposed to their neighboring unburned sites; (d) The EcM community composition will differ between burned and unburned sites; (e) The post-fire community will be dominated by a few rare taxa; (f) Species richness will increase with increase time since fire.

Methods

The methods for this study are presented below, beginning with site selection criteria and study site locations. Followed by the experimental design and sampling techniques. Data analyses consist of soil chemical and molecular analysis, followed by bioinformatics, and statistical analysis.

Site Selection Criteria and Description

For this study, I implemented the time-for-space substitution, in which four wildfires occurring in the span of eleven years, between 2006 and 2015, were chosen to explore the temporal changes and underlying processes affecting the EcM fungal communities of PIPO ecosystems in Eastern Washington. Selection criteria required that each sampling site (1) be located in an area with high soil burn severity; (2) have PIPO as the dominant tree species (>80% of the site); (3) exhibit no evidence of previous wildfire, prescribed fire, or mechanical treatment for a minimum of 30 years; (4) have no salvage logging; and (5) be within the defined elevation range of 1050 m and 1300 m (Table 1). The elevation range was chosen, because one of the sites—affected by the Tripod fire—had burned at high elevations only. Thus, all other sites needed to be within the same elevation range to maximize consistency and minimize confounding factors. Although maintaining the same aspect was an initial requirement, it proved difficult to locate sites with a south-facing aspect for two of the fire sites; ultimately, this criterion was removed from the site selection requirements.

The study sites are located in eastern Washington, in an area spanning from the eastern Cascades slopes and foothill (Table mountain fire), up to the northern Cascades (Carlton and Tripod Complex Fires), and to the east in the northern Rockies (North Star fire; Figure 1). During the decade spanning these wildfires, the mean average temperature, and precipitation for the eastern Cascades slopes was 7.4˚C and 88.4 cm, with a mean summer temperatures and precipitations of 14.51˚C and 9.3 cm, respectively. The mean average temperatures and precipitation for the northern Cascades was 6˚C and 59.4 cm respectively. The northern Cascades experienced mean summer temperatures and precipitation of 13.78˚C and 11.0 cm, respectively. Additionally, the northern Rockies have a mean average temperature and precipitation of 6.18˚C and 50.9 cm, with summer temperature and precipitation averaging at 14.29˚C and 12.87 cm, respectively (NOAA, 2018).

Site 1 was located on the East section of the Tripod Complex Fire, approximately 2 km from the NE corner of Methow Wildlife Area (120°5’52.279”W, 48°34’13.209”N). The unburned site was placed NW from the burned site, directly across National Forest Road 37 (120°7‘24.75”W, 48°35’58.362”N; Table 1; Table 2).

Site 2 was located on the east corner of the 2012 Table Mountain Fire, approximately 4.5 km west from Mission Ridge Ski and Board Resort (120°28’50.787”W, 47°15’29.234”N). The unburned site was located directly to the SE of the burned site, on the land of Department of Natural Resources (120˚26’10.089”W, 47°13’31.147”N; Table 1; Table 2).

Site 3 was located in the 2014 Carlton Complex Fire, 11 km SE from South Summit Sno Pak (119˚54’41.47”W, 48°18”44.624”N). The unburned site was located in the unburned island within the fire perimeter (119˚57’1.812”W, 48°13”54.708”N; Table 1; Table 2).

Site 4 was located in the 2015 North Star Fire which burned within the Colville Reservation, approximately 7 km SE from Moses Mountain (118°59’41.607”W, 48°21’31.352”N. Correspondingly, the unburned site was located 5 km N of Moses Mountain (119°6’20.255”W, 48°26’52.655”N; Table 1; Table 2).

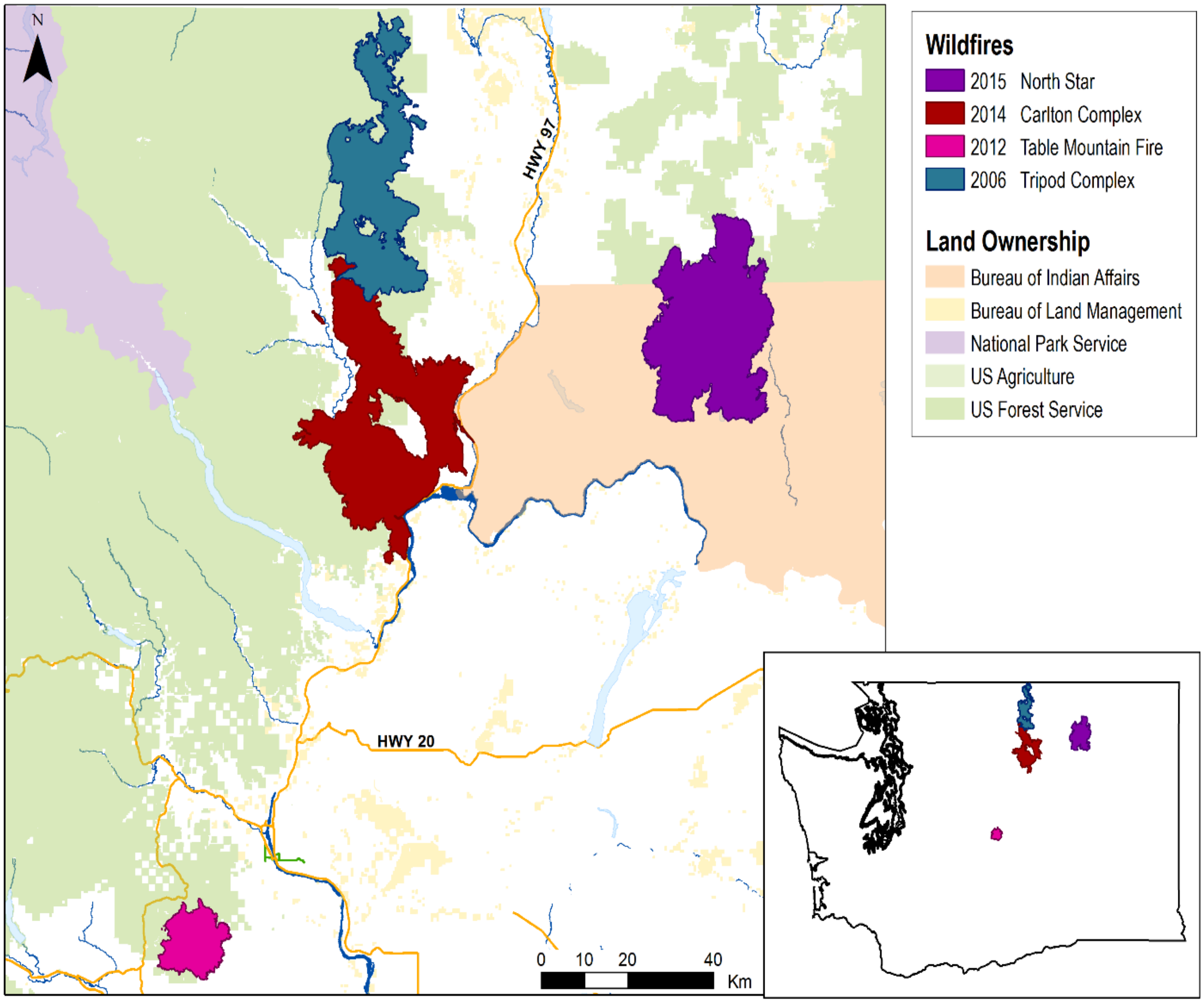

Figure 1. Study sites in central Washington depicting the four study sites (wildfires). Three sites located in Okanogan-Wenatchee National Forest: Table Mountain, Carlton Complex and Tripod Complex, and North Star fire in Colville Reservation.

Table 1. Site Description and Characteristics, per fire (site) and their respective units (3 per site).

Site	Unit	Latitude, Longitude	Aspect	Avg Elevation	Avg Slope
Tripod Burned	1	120˚05’53.620”W, 48˚34’14.544”N	SE	1200	18.84
	2	120˚05’52.279”W, 48˚34’13.209”N	SE	1184	17.14
	3	120˚05’50.768”W, 48˚34’14.375”N	SE	1181	19.62
Tripod Unburned	1	120˚07’23.873”W, 48˚35’59.928”N	S	1233	16.15
	2	120˚07’24.750”W, 48˚35’58.362”N	SW	1218	21.89
	3	120˚07’22.624”W, 48˚35’58.239”N	S	1224	13.15
Table Mountain Burned	1	120˚28’52.974”W, 47˚15’28.455”N	S	1322	9.22
	2	120˚28’50.787”W, 47˚15’29.234”N	S	1325	10.75
	3	120˚28’51.621”W, 47˚15’30.769”N	S	1334	13.35
Table Mountain Unburned	1	120˚26’ 8.108”W, 47˚13’30.940”N	S	1107	14.35
	2	120˚26’10.089”W, 47˚13’31.147”N	S	1115	13.90
	3	120˚26’12.108”W, 47˚13’31.431”N	S	1115	23.27
Carlton Burned	1	119˚54’43.769”W, 48˚18’44.140”N	S	1177	14.27
	2	119˚54’41.470”W, 48˚18’44.624”N	S	1175	12.83
	3	119˚54’42.069”W, 48˚18’42.791”N	E	1162	12.84
Carlton Unburned	1	119˚57’ 1.858”W, 48˚13’53.687”N	E	1063	4.18
	2	119˚57’ 1.812”W, 48˚13’54.708”N	SE	1161	6.18
	3	119˚57’ 3.872”W, 48˚13’56.541”N	S	1161	6.45
North Star Burned	1	118˚59’38.975”W, 48˚21’33.506”N	S	1074	12.48
	2	118˚59’41.607”W, 48˚21’31.352”N	*SW*	*1058*	*12.10*
	3	118˚59’ 40.221”W,48˚21’30.312”N	*S*	*1056*	*11.67*
North Star Unburned	1	119˚06’20.255”W, 48˚26’52.655”N	*S*	*1181*	*14.83*
	2	119˚06’22.668”W, 48˚26’54.181”N	SW	1176	11.14
	3	119˚06’23.988”W, 48˚26’51.962”N	S	1173	12.13

Site and unit descriptions, elevation, and slope measurements are based on the average of four separate measurements taken at each unit. Elevation (meters) and slope (degrees).

Table 2. Wildfire burn severity statistics for each study site (modified from MTBS datasets) to show area burned in hectares for each fire severity; unburned, low, moderate and high-severity. Location of each wildfire based on the county of the wildfire and their respective Ecoregions.

		Hectares burned					Location	
Fire Name	Ignition date	Unburn (ha)	Low (ha)	Mod (ha)	High (ha)	Total area burned (ha)	County	EPA Ecoregion
Tripod Complex	7/3/2016	8,140	15,646	17,845	28,210	69,841	Chelan-Okanogan County	North Cascades
Table Mountain	9/8/2012	6,508	9,725	4,786	5,592	26,611	Kittitas County	Eastern Cascades Slopes & Foothills
Carlton Complex	7/14/2014	15,607	49,526	36,764	13,287	115,184	Okanogan County	North Cascades
North Star	8/13/2015	10,786	37,737	25,989	13,223	87,735	Ferry County	Northern Rockies

Location and hectares burned per fire based on MTBS data. Regions based on EPA ecoregion classifications (Omernik, 2004) and counties.

Experimental Design

The experiment was designed as an ex-post-facto chronosequence study on four fire sites: Tripod Complex, Table Mountain, Carlton Complex, and North Star. At each site, three 30 m x 40 m units spaced >5m apart, were laid out systematically in order to avoid young patches of PIPO and/or rock outcrops. To sample as much of the variation within each unit and capture the diversity of the EcM community, nine plots measuring 3 m x 5 m, spaced >3 m apart, were laid out following the same cardinal direction of their respective unit (Figure 2). Units were restricted to areas were PIPO was the dominant tree species in order to reduce confounding variables. To avoid edge effects, unit boundaries were placed at least 30 m from roads, streams, and fire lines. Three coordinate readings were recorded, two on diagonally opposite ends of each unit, and one in the centroid (Table 1) with a Garmin GPS (GPS64s, Garmin International, Inc., Olathe, KS) via the waypoint function.

Soil Sampling

Soils were sampled from each plot in the summer of 2017. Within each plot, four individual soil samples were randomly collected from the mineral soil down to a depth of 17 cm using a stainless steel auger. The severity of the fires consumed the organic layer in the burned units, hence, in order to ensure that only the mineral layer was sampled in the unburned units any vegetation and organic layers were manually removed prior to sampling. At each sampling location, forest floor depths, and distance to nearest live or dead PIPO was recorded to test for environmental effects. To avoid cross contamination between plots, the auger was sprayed with 70% ethanol, air dried, and cleaned with a paper towel. One hundred and eight (108) soil samples were collected from each site, placed in individual freezer bags, and stored at -20°C, at the School of Environmental and Forest Sciences (SEFS) at the University of Washington, until soil DNA extractions were performed.

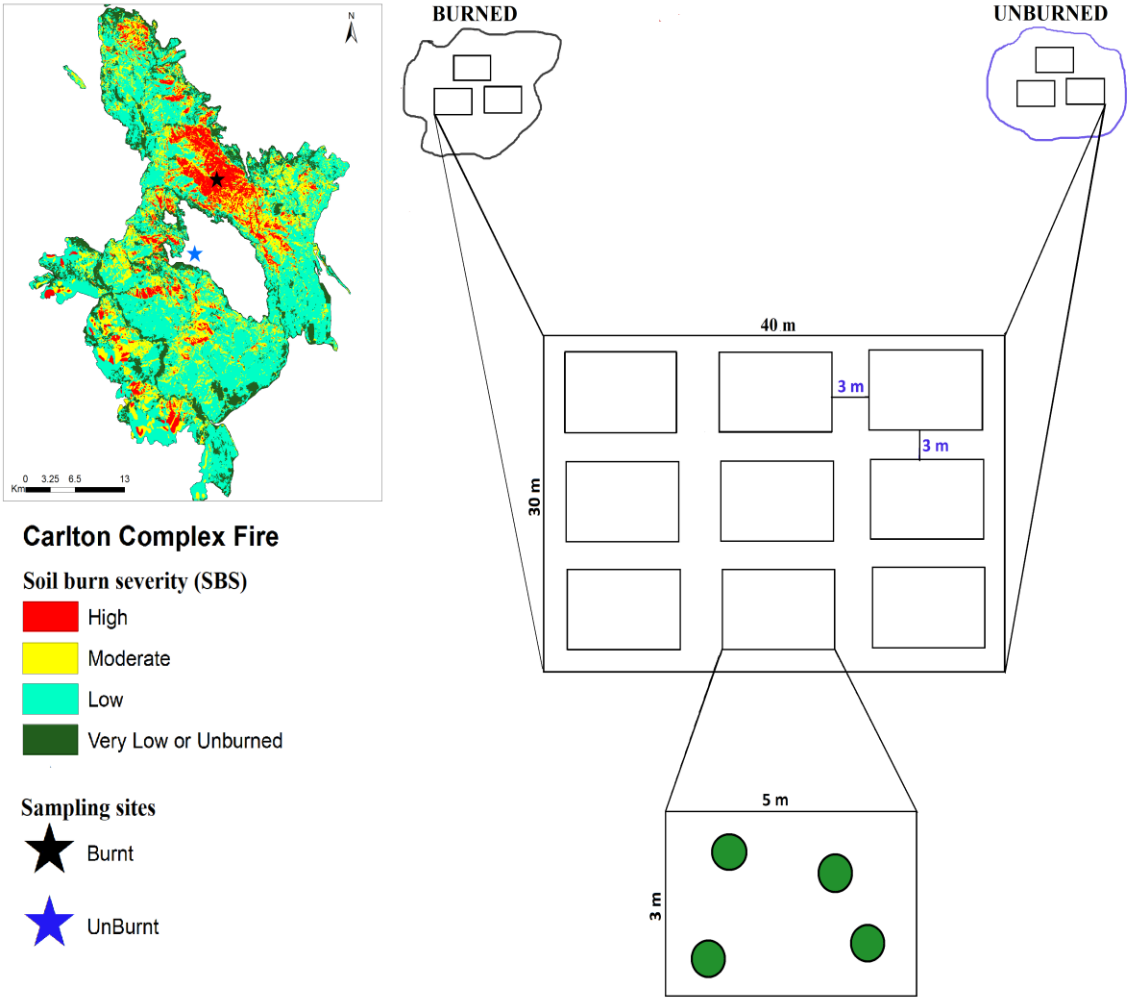

Figure 2. Experimental design and soil sampling layout for burned and unburned sites. Locations shown based on their placement within the Carlton Complex Fire (blue/black stars). Green circles represent the four random soil samples taken per plot, for a total of nine plots (5 m x 3 m) and three units (30 m x 40 m), resulting in 108 soil samples per site (burned vs. unburned).

Sample Preparation and Analysis

All soil samples were homogenized by hand and sieved through a 2 mm mesh to remove any rock and root fragments. In order to prevent degradation of DNA, all soil samples were stored at -20°C until the day of processing, with the exception of the soil used for chemical analysis, which were air dried prior to processing.

Soil Chemical Analysis

All soil chemical analysis were performed in the Soil Analytical Service Center in SEFS at the University of Washington, WA. Total carbon and TN were analyzed by dry combustion with a Perkin Elmer 2400 CHN/S Analyzer (Perken Elmer Inc., Waltham, MA, USA). Total phosphorus was analysis via the digestion method following EPA method 3050A (Edgell, 1988) using ICP 6000 (Thermo Fisher Scientific, Waltham, MA, USA).

Molecular Analysis

Qiagen DNeasy Power Soil Kits (Mo Bio Laboratories Inc. Carlsbad, CA 92010) were used to extract the DNA from each individual soil samples. To ensure that the extractions were successful, 2 ul of the extracted DNA were quantified by gel electrophoresis. The remaining DNA was used in polymerase chain reaction (PCR), and for amplification of internal transcribed spacer regions (ITS) using primers ITS-1F (White et al. 1990) and ITS2 (Gardes and Bruns, 1993). PCR reactions were carried out in 25 ul reaction mixtures with the following concentration in ul/rxn 2.5 ul of 10x HotstarTaq PCR buffer, 0.5 ul of 2 mM of deoxynucleotide triphosphates (dNTPs), 1.0 ul of 10 uM ILL-1F-primer, 0.13 ul of HotStarTaq (5 units/ul), 0.25 ul of 100x bovine serum albumin (BSA), 14.62 ul of molecular grade water, and 2 ul of the DNA template. PCR cycling parameters consisted of an initial cycle of 5 min at 95˚C for denaturation of the DNA, followed by 29 cycles consisting of: 30s at 95˚C, 30s at 51˚C, and 1 min at 72˚C of annealing and 10 min at 72˚C for extension. A negative control and a positive control were used during amplification to ensure amplification of target DNA and exclude contamination of the products. For samples that failed to amplify the concentration of DNA was increased to 5 ul (10:90 PCR product). PCR was performed in collaboration with the Bruns Lab at the University of California-Berkeley (UCB). PCR plates containing 215 samples were submitted to Vincent J. Coates Genomic Sequencing Laboratory (GCL) at UCB for library preparation, quality control (QC), and Next-Generation Sequencing (NGS). Next Generation Sequencing was performed by synthesis (SBS) technology on the MiSeq platform, with 300 paired-end-reads (PE) which equate to 150 loci per run, for two runs.

Bioinformatics

Paired-end demultiplexed sequence files, containing 4,535,423 raw sequences in 215 samples were processed using Qiime2, version 2018.6 (Caporaso et al., 2010). Two samples were returned as undetermined by the sequencing facility, hence, they were not included in the analysis. The primers and adapter sequences from the 5’ were removed by the sequencing facility. The 3’ primer and adapters, *CTTGGTCATTTAGAGGAAGTAA*, were removed using the qiime2-cut-a-dapt plugin (Martin, 2011). Quality control to filter out chimeric sequences, and low quality regions was done by parsing the sequences through DADA2 (Callahan et al., 2016), resulting in 3,632,059 merged sequences.

Taxonomic analysis was performed by first training a taxonomic classifier using UNITE reference database with clustering at ≥ 99% reference levels (Kõljalg et al 2005; UNITE, 2017). The sequences were then assigned taxonomy using the fitted classifier, classify-sklearn plugin, (Pedregosa et al., 2011) resulting in a clustered Occupational Taxonomic Unit (OTU) table clustered at 99%. Since the ITS1F-ITS2 allow for the amplification of all members of the fungal family, the OTU table was parsed through FUNGuild (Nguyen et al., 2016) in order to assign guild and remove all non-EcM samples. In order to account for uneven sampling depth, the OTU table was rarefied to remove the bottom 15% of the sequences. All analysis were performed on the rarefied OTU table, which was rarefied to 32,041 sequences, per sample. A detailed description of the bioinformatic processing steps can be found in supplementary methods S1.

Statistical Analysis

Univariate soil nutrient data were analyzed in Rstudio. Analysis of statistical assumptions yielded data, which were not normally distributed. Total carbon, TN, TC: TN and OM depth were log-transformed. Square root transformation and cube transformations were applied to TP and TC: TP, respectively. Transformed data was used to test for differences between treatments, using a one-way ANOVA. The data was further partitioned to maintain only the data belonging to the burned sites in order to test the changes to soil nutrient with increased time since fire. Non-normally distributed data were transformed using log, square, cubed transformations, and regressed using a linear regression.

EcM fungal diversity and community composition was compared using species richness, as determined using observed OTU's, Simpson's Diversity Index (D), and Simpson's evenness (Simpson, 1949). Simpson evenness measures the relative abundance of the different species making up species richness. Simpson's Diversity Index measures is a diversity measure which takes into account the number of species present as well as their relative abundance. These metrics were calculated in Qiime2, and exported to R for graphing purposes using the phyloseq package.

In addition, the difference in community composition was compared using Bray-Curtis (BC) distance measure (Bray and Curtis, 1957). A distance defined by McCune and Grace (2002) to be the best measure for community composition of abundance data. Statistical significance of alpha diversity was tested using Kruskal-Wallis (Kruskal and Wallis, 1952), and Kruskal-Wallis pairwise test. Comparisons were considered significant at $p \leq 0.05$.

To visualize community composition between treatments, non-metric multidimensional scaling (NMDS; Legendre & Legendre, 2012) using Vegan function metaMDS (Oksanen et al., 2018) was applied to the relativized abundance data. Permutational analysis of variance (PERMANOVA) using the adonis2 function in R, with 9999 permutations and Bray-Curtis (BC) distance was used to test the hypothesis that EcM communities differed between treatments. Using a negative binomial regression on Deseq2 (Love et al., 2014), and calculating the log2 fold-change of the abundance of each taxon, determination of the contribution that each taxon has on the differences presented between burned and unburned units were determined. Significance was tested using nbinom Waldtest in the Deseq2 package. Those taxa, which exhibited significant differential abundance, $p \leq 0.05$, were visualized to species and genus levels.

To test for correlation between the environmental variables and the EcM community, Pearson correlation (Pearson, 1901) was performed using Qiime2. Additionally these variables were tested using PERMANOVA with 9999 permutations and BC distance. Since some of the explanatory variables contained levels, pairwise Adonis (Martinez, 2017) was used to depict whether community composition differed among any of the variables that were proven significant by PERMANOVA. Multivariate homogeneity was tested using betadisper to ensure equal variance between sites.

To test the significance of time since fire, a negative binomial regression using the glm.nb function in the MASS package (Venables, 2002) was fit to the rarefied observed OTU table of the burned sites. This model was chosen due to the over-dispersed variance observed in the OTU table. Results were considered significant at $p < 0.05$.

All statistical analysis were performed on Rstudio (R CoreDevelopment Team, 2018, version 3.5.1) using Vegan package (version 2.5-2; Oksanen et al., 2018) and Phyloseq (version 1.24.2; McMurdie and Holmes, 2013) unless otherwise noted.

Results

Soil Nutrients between Treatments

High-severity fires significantly altered the soil nutrients in all sites. Burned sites contained lower percentages of TC (2.08 % ± 1.00), TC: TN (18.83 % ± 5.13), and TC: TP (18.76 % ± 15.54). Additionally, the mean OM depth in the burned sites was 6 times lower than the OM depth in the unburned sites (0.76 % ± 0.80 and 4.74 % ± 2.65, respectively). In contrast, TN (0.12 % 0.08) and TP (0.15 % 0.06) experienced a slight increase in the post-fire environment, in comparison to the unburned sites (Table 3). Analysis of variance showed that these differences were statistically significant ($p<0.05$) between burned and unburned units, with the exception of the ratio between TC: TN ($p=0.455$; Table 4).

Table 3 Mean and standard deviations of OM depth (cm) and soil nutrient concentrations (%) for total carbon, total nitrogen, total phosphorus and the ratios between total carbon/nitrogen and total carbon/phosphorus by treatment (burned vs. unburned) and by fire year (year since fire).

	Treatment				Fire Year							
	Burned		Unburned		2006(11)		2012(5)		2014(3)		2015(2)	
	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev	Mean	Std. Dev
TC	2.08	1	2.3	0.96	1.11	0.2	1.65	0.47	2.7	0.6	3.15	1
TN	0.12	0.08	0.11	0.05	0.06	0.01	0.24	0.03	0.13	0.03	0.15	0.04
TP	0.15	0.06	0.08	0.02	0.19	0.02	0.09	0.01	0.09	0.04	0.16	0.07
TC:TN	18.83	5.13	20.6	2.66	19.84	2.38	13.30	7.98	20.67	1.85	20.56	2.33
TC:TP	18.76	15.54	35.2	23.87	6.01	1.20	18.92	6.11	38.76	21.57	22.55	9.70
OM depth	0.76	0.8	4.74	2.64	1.08	0.89	0.94	0.43	1.02	1.25	0.27	0.49

Time since fire shown in parentheses, all values based on percent; TC=total carbon, TN= total nitrogen, TP= total phosphorus and the ratio between TC: TN and TC: TP, with the exception of OM depth, which is measured in cm.

Table 4 One-way ANOVA results for the mean comparison among soil nutrients and treatment groups (burned vs. unburned).

	Df	Sum Sq	Mean Sq	F value	Pr (>F)
Total Carbon	1	0.84	0.8432	4.565	0.0340*
Nitrogen	1	0	0	0	0.9970
Total Phosphorus	1	31.83	31.83	91.17	<2e-16*
TC:TN	1	6.879e-07	68788	5.769	0.0173*
TC:TP	1	23.32	23.319	54.76	5.29e-12*
OM depth	1	82.51	82.51	257.8	<2e-16*

Significance at p <0.05 marked with an asterisk, N=178. TC: TN= ration of total carbon and total nitrogen and TC: TP= ratio of total carbon and total phosphorus. Nutrients based on percent, OM depth measured in cm.

Changes in Soil Nutrients with Time since Fire

Out of the five soil nutrient measured, four differed significantly ($p < 0.05$) in relation to time since fire (Table 5 and Figure 3). Total carbon (t=-13.57, p<2e-16), TN (t=-8.182, p=1.84e-12), and TC: TP (t=-12.55, p<2e-16) showed a decrease in percentage with increase time since fire. In contrast, TP (t=4.036, p=0.0001) and OM depth (t=2.913, p=0.0045) increased with increase time since fire.

Table 5 Linear regression relationship between soil nutrients (%) and OM depth (cm) with increase time since fire (years).

	Estimate	Std. Error	t value	Pr(>|t|)
Total carbon*	-0.1104	0.00813	-13.57	<2e-16*
Total nitrogen*	-0.1579	0.01929	-8.182	1.84E-12*
Total phosphorus**	0.00906	0.00225	4.036	0.0001*
OM depth*	0.0405	0.0139	2.913	0.0045*
TC:TN***	-81.56	108.61	-0.751	0.4550
TC:TP*	-0.23775	0.01895	-12.55	<2e-16*

Significance at p< 0.05 displayed with asterisk. Nutrients based on %, OM depth measured in cm. *Log transformed **square root transformed ***Cubed transformed

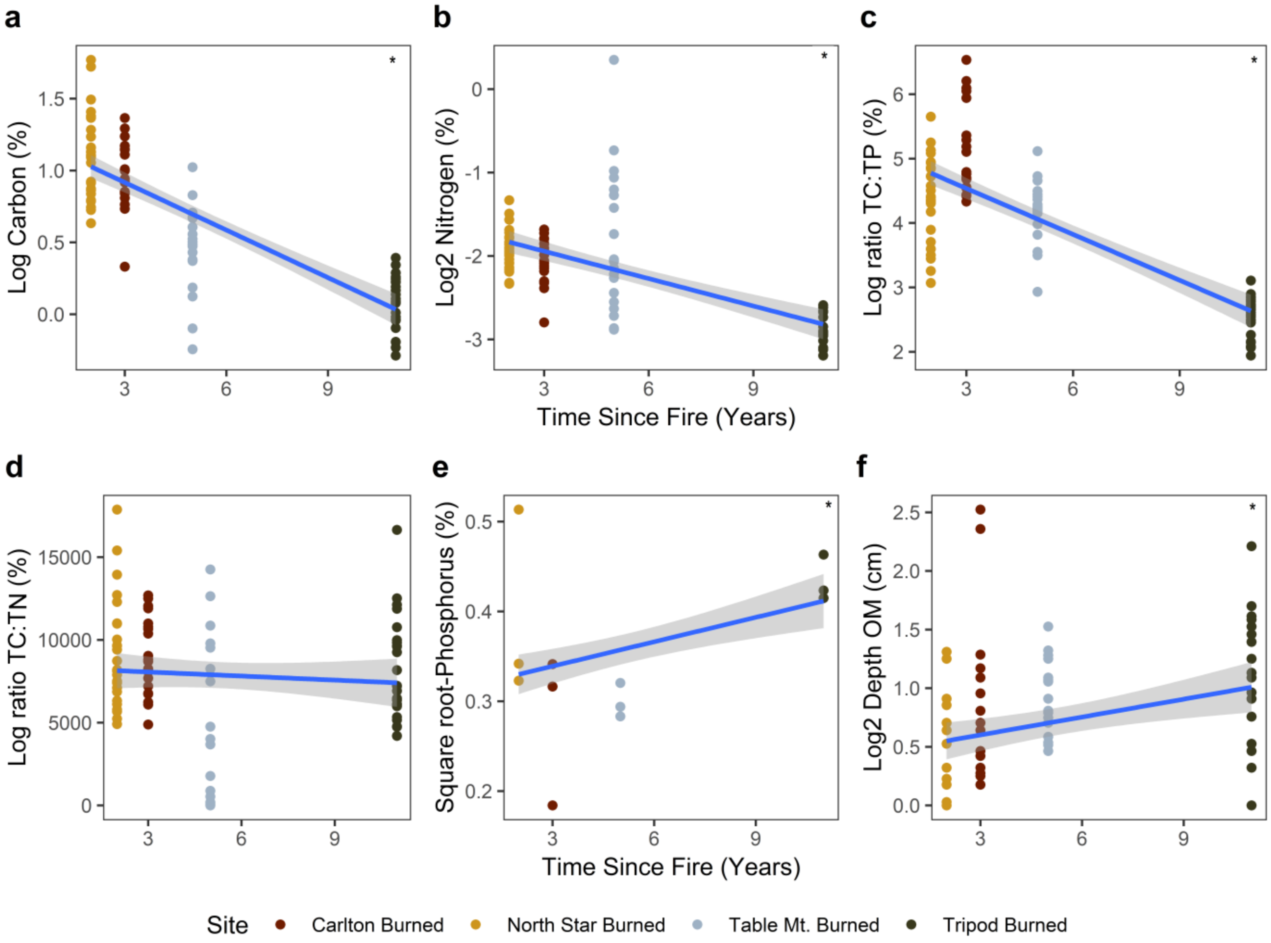

Figure 3. Linear regression between percent nutrients—total carbon (TC), total nitrogen (TN), and total phosphorus (TP), and the ratio between TC: TN and TC: TP—and time since fire. Points represent transformed data, and lines are predicted values based on the transformed data. Logistic regression equations for each nutrient are; total carbon= -0.11+1.25, total nitrogen = -0.16 -2.33, ratio of TC: TP = -0.237+5.25, and OM depth=0.05+0.44. Cubed transformed regression equation for TC: TN = -81.6 +8307.27. Square root transformed regression equation for P= 0.01+0.31, and significance at $p<0.05$ depicted with an asterisk in upper right corner of each graph, per Table 5.

Alpha Diversity

After rarefying the data to 32,041 sequences, per sample, a total of 165 OTU's were identified across the study area, with 100 identified to the burned sites and 130 to the unburned sites (Table 9). The significance of the low number of OTU's in the burned sites was expressed in species richness and Simpson Diversity for the burned sites, when comparing treatment/burn effects ($p<0.0000$, Figure 4a; Table 6). Species evenness was higher in the burned sites in comparison to the unburned sites ($H=78.2693$, $p<0.0000$). As expected, comparison between sites followed the same trend observed in when comparing alpha diversity per treatment (Figure 4b; Table 6). The Kruskal-Wallis post-hoc test on site diversity metrics did not reveal significant differences ($p>0.05$) for any of the three metrics recorded: species richness, diversity, or evenness. However, for species richness, the mean rank between all sites in comparison to the Tripod burned sites were shown to be similar ($p>0.05$; Table A1), but for species evenness, the mean rank between all sites was significantly different from Tripod unburned ($p<0.05$), suggesting high variability within the sites.

Alpha Rarefaction Curves

Although we sufficiently sampled the burned and unburned units to depict the changes in species richness, we failed to sufficiently sample to test the difference among sites (Figure 5). More specifically, we failed to sufficiently sample the Carlton Complex sites, thus resulting in improper comparison between the other sites and Carlton Complex.

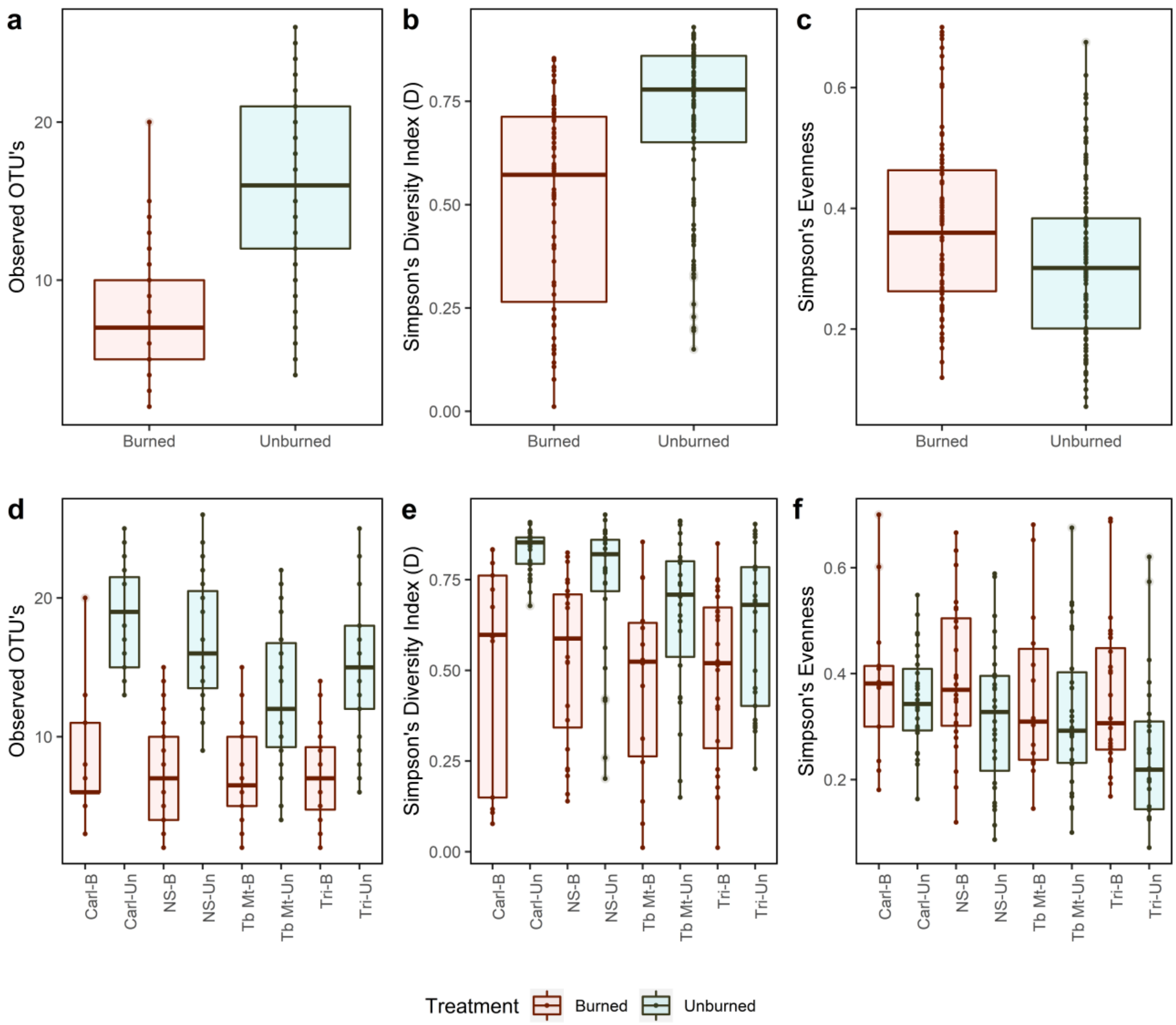

Figure 4. Alpha diversity metrics per treatment (a) and per site (b), colors based on treatment for both (a) and (b), based on rarefied OTU table to 32,041 sequences per sample. Simpson's richness, Simpson's evenness, and observed OTU's are shown for burned (red) and unburned (blue) sites. All metrics were significant at $p < 0.05$. Sites names are as follows: Carl B/U=Carlton burned/Unburned, NS-B/U= North Star Burned/Unburned, Tb.Mt. B/U= Table Mountain Burned/Unburned, Tri B/U= Tripod Burned/Unburned.

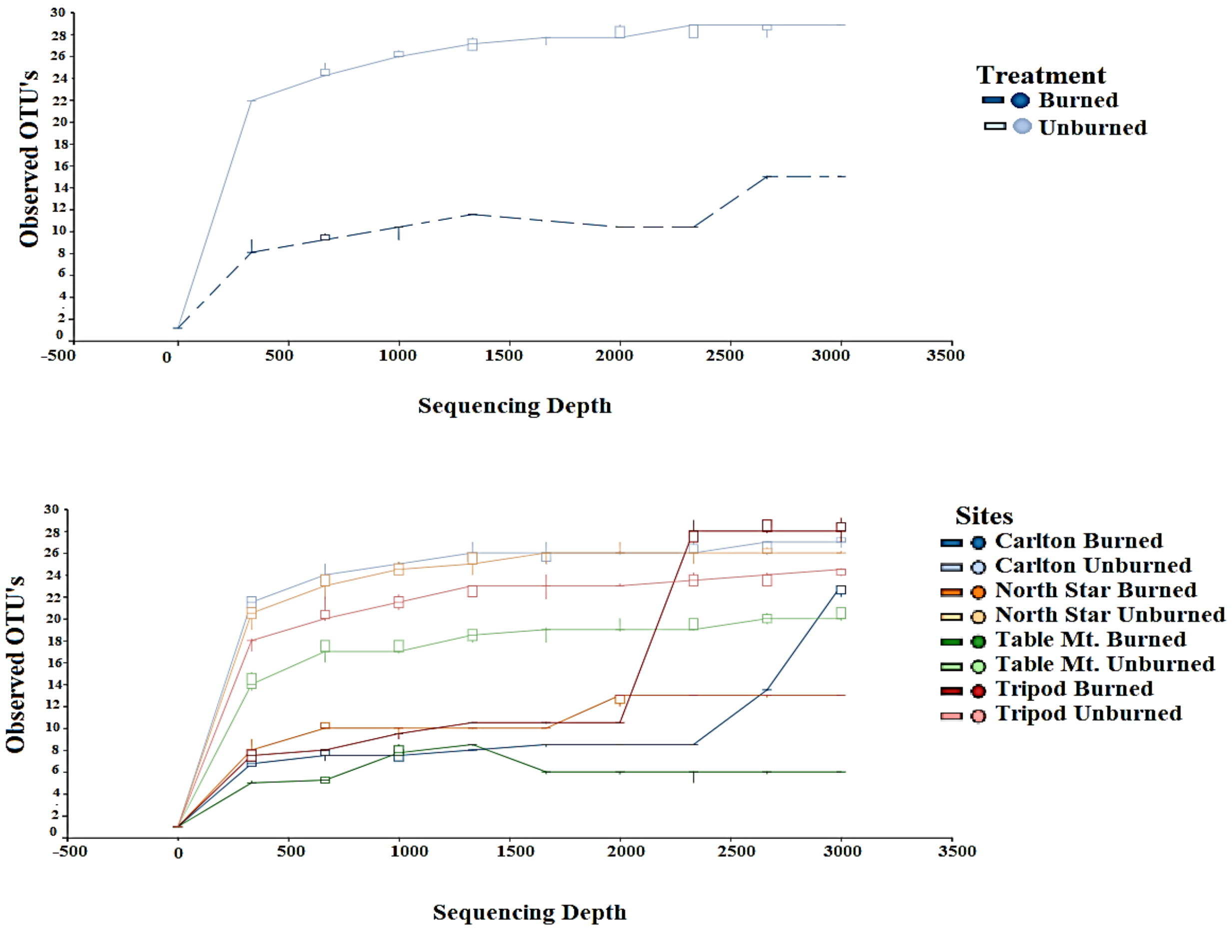

Figure 5. Rarefaction curves describing observed OTU's (species richness). Rarefactions curves indicate the number of observed Operational Taxonomic Units (OTU's) at a rarefaction depth of 3,000 sequences (median sequence in all samples of the dataset). Sequences are based on ITS1F and ITS2 for soil samples per treatment (burned vs unburned) top graph and per sites (bottom graph): Carlton Complex, North Star, Table Mountain, and Tripod.

Table 6. Diversity metrics, species richness (observed OTU's), Simpson's evenness, and Simpson's Diversity per treatment (burned and unburned) and between sites (Tripod Complex, Table Mountain, Carlton Complex, and North Star Fires.

	Treatment		**Site**	
Diversity metric	H	p-value	H	p-value
Species Diversity	35.2529	0.0000*	56.728	0.0000*
Species Evenness	7.5813	0.0059*	21.9377	0.0026*
Species Richness	78.2693	0.0000*	91.922	0.0000*

Kruskal-wallis (H), Significance at p<0.05 displayed with an asterisk.

Community Composition

Burn effect was a significant driver of EcM community composition (p=0.0001; Table 7). These results were corroborated via analysis of homogeneity (F=0.999, p=0.3184). Samples from the burned sites differed in species composition from those in the neighboring unburned sites, as observed in the NMDS ordination (Figure 6). Additionally, the lack in species overlap between burned and unburned sites, suggest that an eleven-year time span, was not sufficient for the EcM community to resemble the pre-fire community.

Table 7 Results of Adonis showing effects of different physicochemical site variables, treatment, and year of fire using Bray-Curtis dissimilarity matrix on the ectomycorrhizal soil community.

	Df	Sum of Squares	R^2	F	Pr(>F)
Fire year	1	3.041	0.03828	7.6912	0.001*
Treatment	1	1.917	0.02414	4.8497	0.001*
Elevation	1	1.706	0.02147	4.3146	0.001*
Slope	1	1.479	0.01862	3.7412	0.001*
Total carbon	1	1.467	0.01847	3.7111	0.001*
OM depth	1	0.875	0.01101	2.2131	0.001*
TC:TN	1	0.876	0.01103	2.2161	0.001*
TC:TP	1	0.87	0.01096	2.2015	0.001*
Residual	170	67.213	0.84603		
Total	178	79.445	1		

Significance at p<0.05 displayed with an asterisk. TC: TN = ration total carbon: total nitrogen and TC: TP=total carbon: total phosphorus.

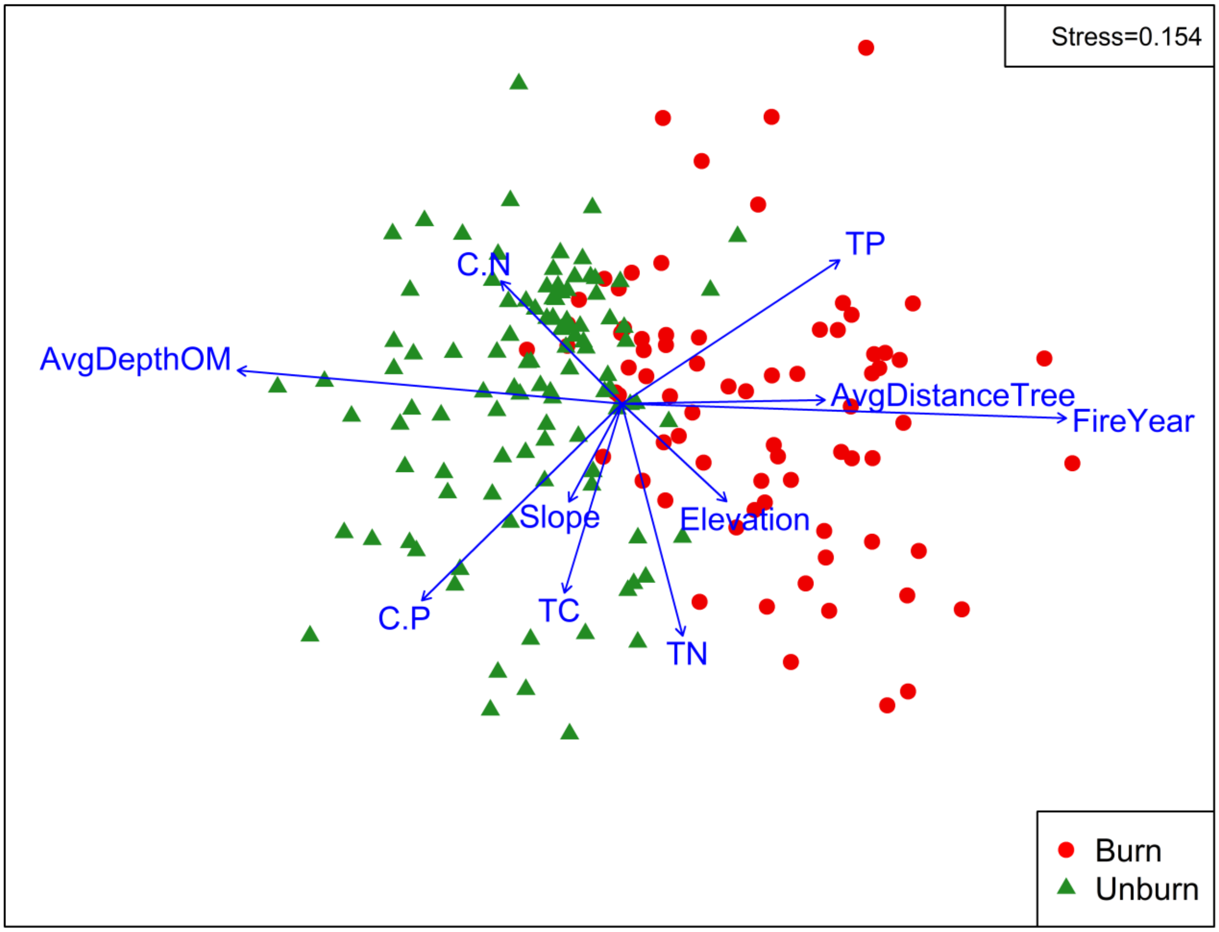

Figure 6. NMDS plot of ectomycorrhizal community assemblages per treatment, burned (circles), and unburned (triangles). Ordination of ectomycorrhizal spore pools on 3-dimensions, based on Bray-Curtis dissimilarity matrix, stress level 0.154. Points closer together in the ordination space have more similar ectomycorrhizal assemblages (significance at $p<0.05$). Statistical analyses per treatment were performed on ADONIS2 (Table 7). Environmental vectors whose significance was $p<0.05$ are displayed in relation to the EcM fungal community assemblage. Statistical test per envfit (Appendix 5).

Environment Drivers of Species Richness and Community Composition

Environmental variables, per Pearson correlation, were shown to contribute to the differences among alpha diversity. Species evenness positively correlated with fire year ($p=0.0066$, $n=179$). In contrast: fire year, time since fire, average distance to tree, elevation, slope, and TP negatively affected both, species diversity and richness (Table 8). Organic matter depth and the ratio between TC: TN were the only variables that positively affected species diversity ($p=0.000$ and $p=0.0061$, respectively) and species richness ($p=0.000$, $p=0.0033$, respectively). Additionally, TC imparted a positive effect on species richness ($p=0.0181$, $n=179$).

Permutation analysis showed that there were other components influencing the EcM fungi community composition (Table 7). In fact, treatment only accounted for 2.4% of the variance (Table 7). The full model showed that various physical site characteristics such as elevation ($F_{1, 177}=4.315$, $p=0.001$), slope ($F_{1, 177}=3.741$, $p=0.001$), and OM depth ($F_{1, 177}=2.213$, $p=0.001$), influenced the EcM communities. Additionally, the alterations to TC ($F_{1, 177}=3.711$, $p=0.001$), TC: TN ($F_{1, 177}=2.216$, $p=0.001$), and TN: TP ($F_{1, 177}=2.202$, $p=0.001$) led to differences in community assemblages between the burned and unburned sites.

Table 8. Pearson’s correlation matrix between physicochemical site variables between; Tripod Complex, Table Mountain, Carlton Complex and North Star Fires and their respective neighboring unburned sites, in response to three alpha diversity metrics (Simpson's evenness, Simpson's Diversity, and species richness (observed OTU’s).

		Species Evenness		Species Diversity		Species Richness	
EV variable	N	Test Stat	p-value	Test Stat	p-value0	Test Stat	p-value
Fire year	179	0.2023	0.0066*	-0.4190	0.0000*	-0.6498	0.0000*
Time since fires	179	0.1081	0.1499	-0.3574	0.0000*	-0.4972	0.0000*
OM depth	179	-0.1149	0.1256	0.3469	0.0000*	0.5541	0.0000*
Avg. dist. tree	179	0.1381	0.0652	-0.1898	0.0190*	-0.3527	0.0000*
Elevation	179	-0.1436	0.0552	-0.2697	0.0003*	-0.1898	0.0109*
Slope	179	-0.0286	0.704	-0.2315	0.0018*	-0.2743	0.0002*
Total carbon	179	0.0127	0.866	0.0446	0.5535	0.8105	0.0181*
Total phosphorus	179	0.1226	0.102	-0.2103	0.0047*	-0.3288	0.0000*
TC:TN	180	-0.0446	0.5524	0.2038	0.0061*	0.2176	0.0033*
TC:TP	181	-0.0566	0.4505	0.0939	0.2101	0.1011	0.1770
Total nitrogen	179	0.0176	0.8151	-0.0429	0.5687	-0.0693	0.3568

Significance at $p<0.05$ displayed with an asterisk, TC: TN= total carbon: total nitrogen and TC: TP = total carbon: total phosphorus.

Structural Composition

Comparison of the top ten most abundant taxa showed a difference in dominance across taxonomic classes based on burned effects. Across the burned sites, Ascomycota was the dominant phyla (48%; Figure 7). The most frequent genus in the burned sites were *Wilcoxina* (21%), *Pustularia* (20.5%), and *Lyophyllum* (14%; Figure 8a). In contrast, the unburned sites were dominated the phylum Basidiomycota (79%; Figure 7), with the genus *Wilcoxina* (47%), *Russula* (14%) and *Cortinarius* (11%) being the most frequent (Figure 8a). It is worth noting that species composition also differed between burned and unburned sites, although the most abundant species in the burned sites were a group of unidentified species (40%; Figure 8b).

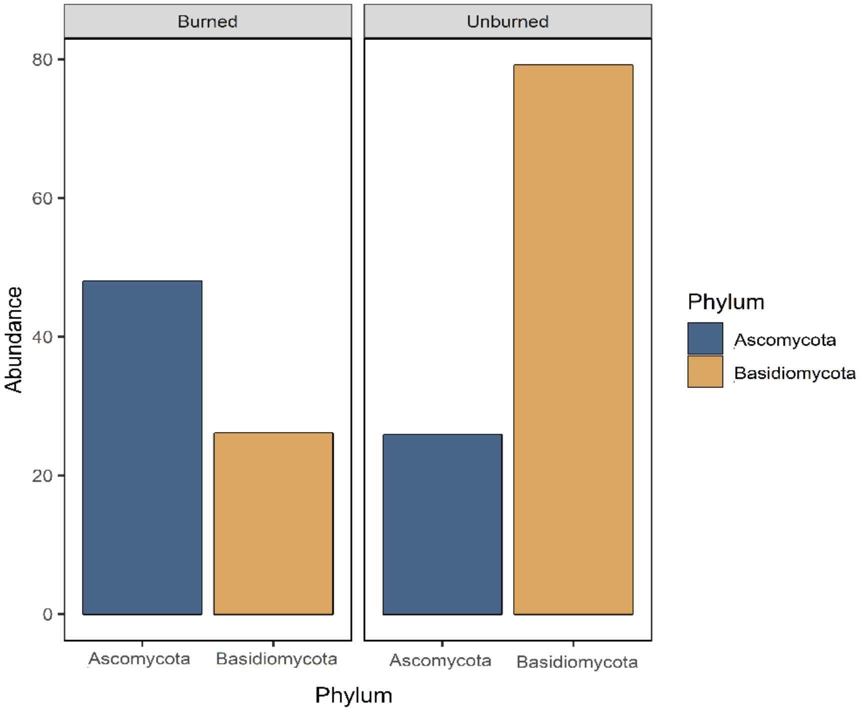

Figure 7. Percent relative abundance at the Phylum taxonomic rank, per treatment, burned (left) vs. unburned (right). Percent relative abundance based on data rarefied to 32, 042 sequences and the cumulative relative abundance of each phyla per site: burned and paired unburned sites: Tripod Complex fire, Table Mt. Fire, Carlton Complex Fire and North Star Fire.

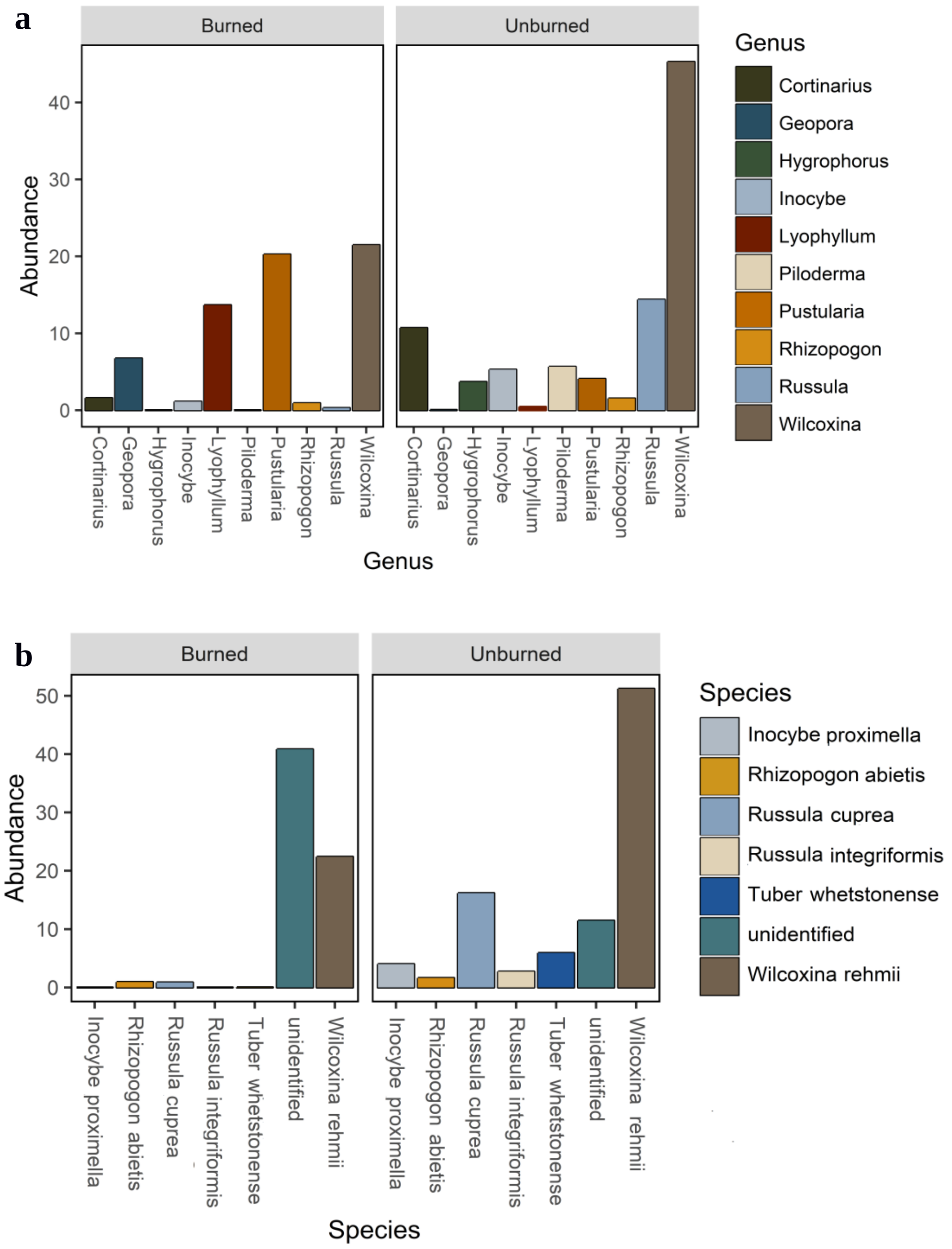

Figure 8. Relative abundance of the top ten most abundant taxa (OTU's), per taxonomic rank. a) Percent relative abundance of the top ten most abundant genus per treatment, burned (left) vs. unburned (right). Colored based on genus which is also represented on the x-axis. b) Percent relative abundance of the top ten most abundant species per treatment, burned (left) vs. unburned (right). Colors follow graph (a), where the species is colored based on the genus it belongs. Percent relative abundance based on data rarefied to 32, 042 sequences. Sites include HS wildfire site and paired unburned sites: Tripod Complex fire, Table Mt. Fire, Carlton Complex Fire and North Star Fire.

Table 9. List of genus and species observed across experimental treatments (burned vs. unburned) and the frequency of the sequence recovered from soil samples. Sequences based on ITS1F and ITS2. Species richness (total observed OTU's) is shown in the bottom row.

		Frequency				Frequency	
Genus	**Species**	**B**	**U**	**Genus**	**Species**	**B**	**U**
Cenococcum	geophilum	78	157	Tricholoma	moseri	0	125
Cenococcum	unidentified	309	3	Tricholoma	triste	4	229
Unidentified	unidentified	102	9	Unidentified	Unidentified	7	20
Hymenoscyphus	caudatus	2	0	Amphinema	byssoides	7	3
Hymenoscyphus	tetracladius	3	0	Amphinema	unidentified	9	435
Meliniomyces	bicolor	364	51	Piloderma	Unidentified	0	49
Meliniomyces	unidentified	43	60	Piloderma	bicolor	0	2
Rhizoscyphus	unidentified	0	2	Piloderma	byssinum	0	1
Unidentified	Unidentified	4	0	Piloderma	lanatum	6	100
unidentified	unidentified	100	0	Piloderma	olivaceum	0	180
Peziza	ampliata	0	1	Piloderma	unidentified	24	1248
Peziza	lobulata	8	0	Tylospora	unidentified	15	224
Peziza	polaripapulata	10	0	Rhizopogon	Unidentified	0	44
Peziza	proteana	5	0	Rhizopogon	abietis	112	340
Peziza	udicola	28	0	Rhizopogon	ellenae	0	6
unidentified	unidentified	5	1	Rhizopogon	hawkerae	0	47
Unidentified	Unidentified	1236	33	Rhizopogon	salebrosus	88	124
Genabea	cerebriformis	0	16	Rhizopogon	vesiculosus	1	0
Geopora	cooperi	24	8	Rhizopogon	villosulus	2	16
Geopora	tolucana	3	4	Scleroderma	bovista	6	0
Geopora	unidentified	867	126	Scleroderma	unidentified	16	0
Gilkeya	Unidentified	0	68	Suillus	grevillei	2	0
Gilkeya	unidentified	0	11	Suillus	pseudobrevipes	20	213
Pulvinula	convexella	20	0	Suillus	quiescens	3	9
Pulvinula	unidentified	5	0	Sistotrema	confluens	0	149
Pustularia	unidentified	1887	51	Sistotrema	unidentified	91	226
Wilcoxina	rehmii	3393	3490	Clavulina	coralloides	2	3
unidentified	unidentified	88	30	Clavulina	rugosa	3	0
Tuber	whetstonense	1	512	Clavulina	unidentified	26	0
Amanita	Unidentified	0	6	Membranomyces	unidentified	1	5
Amanita	pantherina	0	4	unidentified	unidentified	3	26
Cortinarius	Unidentified	543	1177	Hydnum	unidentified	2	7
Cortinarius	caninus	4	0	unidentified	unidentified	3	0
Cortinarius	clandestinus	0	8	Hysterangium	crassirhachis	0	48
Cortinarius	croceus	1	43	Leucogaster	rubescens	5	0
Cortinarius	helobius	10	6	Unidentified	Unidentified	0	17
Cortinarius	myrtilliphilus	0	1	Gymnomyces	Unidentified	0	13
Entoloma	Unidentified	12	1	Lactarius	Unidentified	0	17
Entoloma	atrosericeum	32	140	Lactarius	splendens	0	70
Entoloma	conicosericeum	0	10	Russula	Unidentified	12	465
Entoloma	flocculosum	35	1	Russula	acrifolia	37	405
Entoloma	undatum	21	0	Russula	adusta	0	5
Gliophorus	psittacinus	0	4	Russula	badia	0	167
Hygrophorus	agathosmus	1	0	Russula	brevipes	36	141
Hygrophorus	chrysodon	0	429	Russula	cuprea	24	790

Hygrophorus	gliocyclus	0	278
Hygrophorus	hypothejus	0	4
Hebeloma	Unidentified	0	1
Hebeloma	mesophaeum	1	2
Hebeloma	unidentified	2	6
Hymenogaster	subalpinus	0	15
Inocybe	Unidentified	43	639
Inocybe	abjecta	0	57
Inocybe	bufonia	0	92
Inocybe	chelanensis	0	263
Inocybe	egenula	32	268
Inocybe	exilis	2	11
Inocybe	flocculosa	0	1
Inocybe	fuscescentipes	6	10
Inocybe	giacomi	19	393
Inocybe	inodora	50	0
Inocybe	maculipes	0	4
Inocybe	mixtilis	0	109
Inocybe	nemorosa	0	10
Inocybe	nitidiuscula	36	243
Inocybe	petiginosa	101	4
Inocybe	proximella	18	612
Inocybe	pruinosa	154	26
Inocybe	purpureobadia	19	1
Inocybe	rimosa	0	2
Inocybe	herbaceae	96	125
Inocybe	stellatospora	3	0
Inocybe	xanthomelas	4	0
Inocybe	unidentified	177	123
Mallocybe	unidentified	32	7
unidentified	unidentified	9	0
Lyophyllum	Unidentified	739	6
Lyophyllum	shimeji	0	9
Lyophyllum	unidentified	1544	4
Myochromella	inolens	11	0
Tricholoma	bonii	0	5
Tricholoma	equestre	0	16
Russula	integriformis	0	478
Russula	puellaris	2	23
Russula	queletii	2	85
Russula	recondita	0	54
Russula	sanguinea	0	235
Russula	turci	5	56
Russula	violaceoincarnata	0	19
Russula	xerampelina	37	237
Russula	unidentified	1	72
Helvellosebacina	concrescens	5	37
Helvellosebacina	unidentified	0	312
Sebacina	Unidentified	0	6
Sebacina	unidentified	15	155
unidentified	unidentified	5	5
Hydnellum	caeruleum	25	0
Hydnellum	gracilipes	3	0
Sarcodon	quercinofibulatus	0	1
Unidentified	Unidentified	13	372
Pseudotomentella	Unidentified	0	9
Pseudotomentella	griseopergamacea	0	1
Pseudotomentella	unidentified	16	50
Thelephora	Unidentified	12	70
Thelephora	atra	22	12
Thelephora	terrestris	0	1
Thelephora	unidentified	88	327
Tomentella	Unidentified	11	57
Tomentella	badia	7	81
Tomentella	bryophila	0	5
Tomentella	coerulea	0	9
Tomentella	ellisii	56	0
Tomentella	fuscocinerea	0	112
Tomentella	galzinii	0	3
Tomentella	stuposa	0	71
Tomentella	subclavigera	0	24
Tomentella	sublilacina	12	31
Tomentella	terrestris	0	6
Tomentella	unidentified	0	1
Total (Species richness)		**13,246**	**18,795**

B=Burned sites, U= Unburned sites

Differential Abundance

OTU’s were identified as significantly differentially abundant via DESEQ. At the genus level, 26 individual OTU’s were significantly differentially abundant (adjusted p<0.005) between treatments (burned and unburned) (Figure 9; Table A3). Six OTU’s were shown to present a positive respond to fire; three basidiomycetes in the *Lyophyllum* genera, which increased in average, by 9-fold, and three ascomycetes, *Cenoccocum, Pustularia,* and an unidentified genus, with an average 5-fold increase. Additionally various OTU’s responded negatively, including but not limited to *Thelephora, Rhizopogon, Cortinarius, Tuber, Hygrophorus,* and *Helvellosebacina* (average 5-fold decrease). In response to fire, only one *Basidiomycete* in the *Tuber* genera decreased by 9-fold. Differential analysis at the species level showed a significant decrease in various species, many belonging to the previous genera, but the most abundant being a group of unidentified species. (Figure 10; A3)

Comparison of the differentially abundant taxa shows that with increase in time since fire, all fires in comparison to the Tripod fire only differed by 1-2 taxa (OTU’s; P-adjusted <0.0005), most which were unidentified at the species level (Appendix 4).

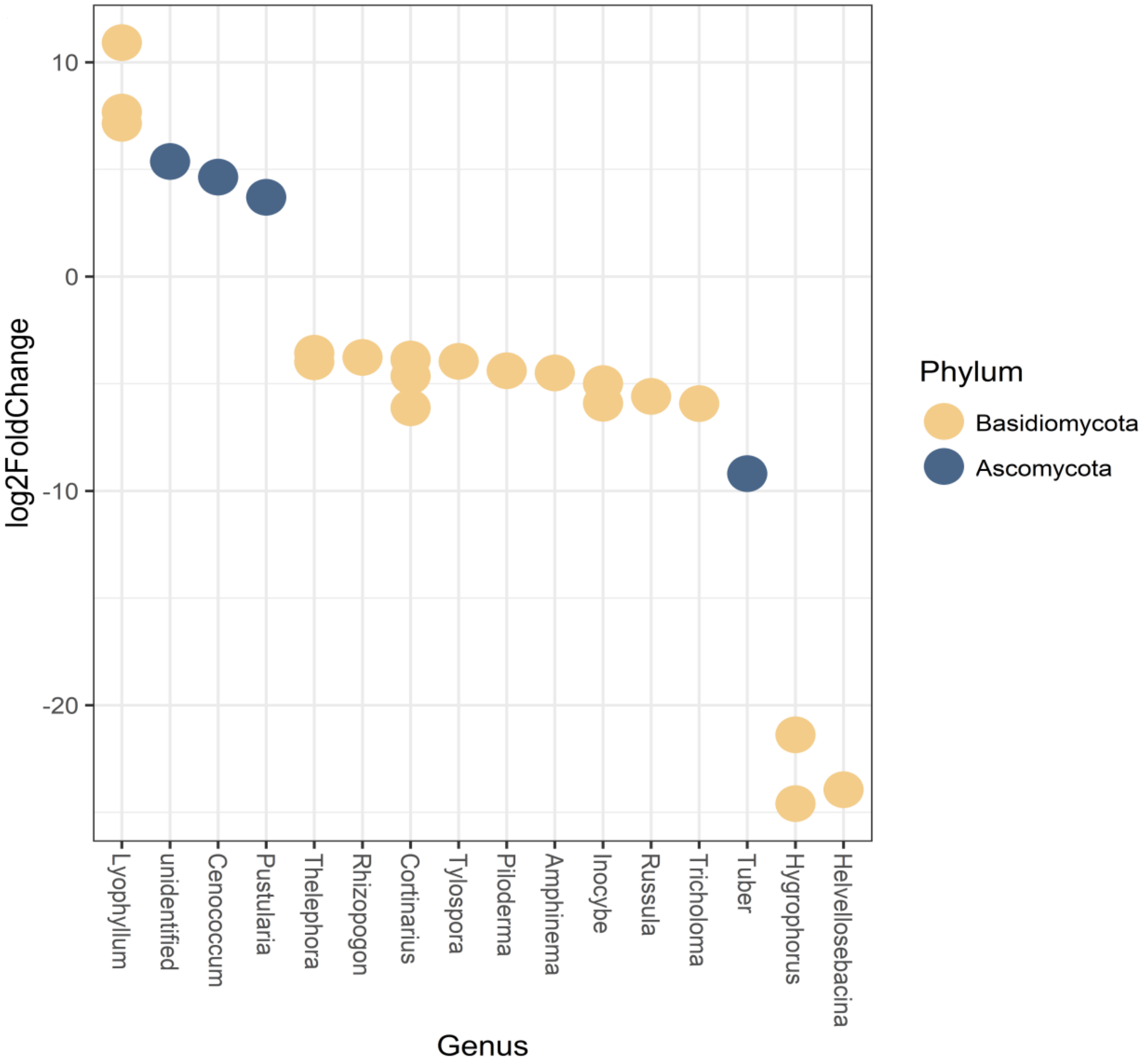

Figure 9. Differential representation of OTU's between burned and unburned sites. Differential abundance of OTUs in soil samples from burned an unburned sites, were assessed by fitting a local regression model with a negative binomial distribution to the raw OTU data. Testing for differential abundance based on nwald, likelihood ratio implemented in DESEQ package with phyloseq. Dots represent the taxa, colored by phylum and labeled by genus. Positive values indicate a positive response to fire. Negative values represent a negative response to fire and higher representation in the unburned sites. Only the probabilities with a differential abundance at p.adj <0.05 and a log2fold change above zero are included (Statistical test in appendix 3).

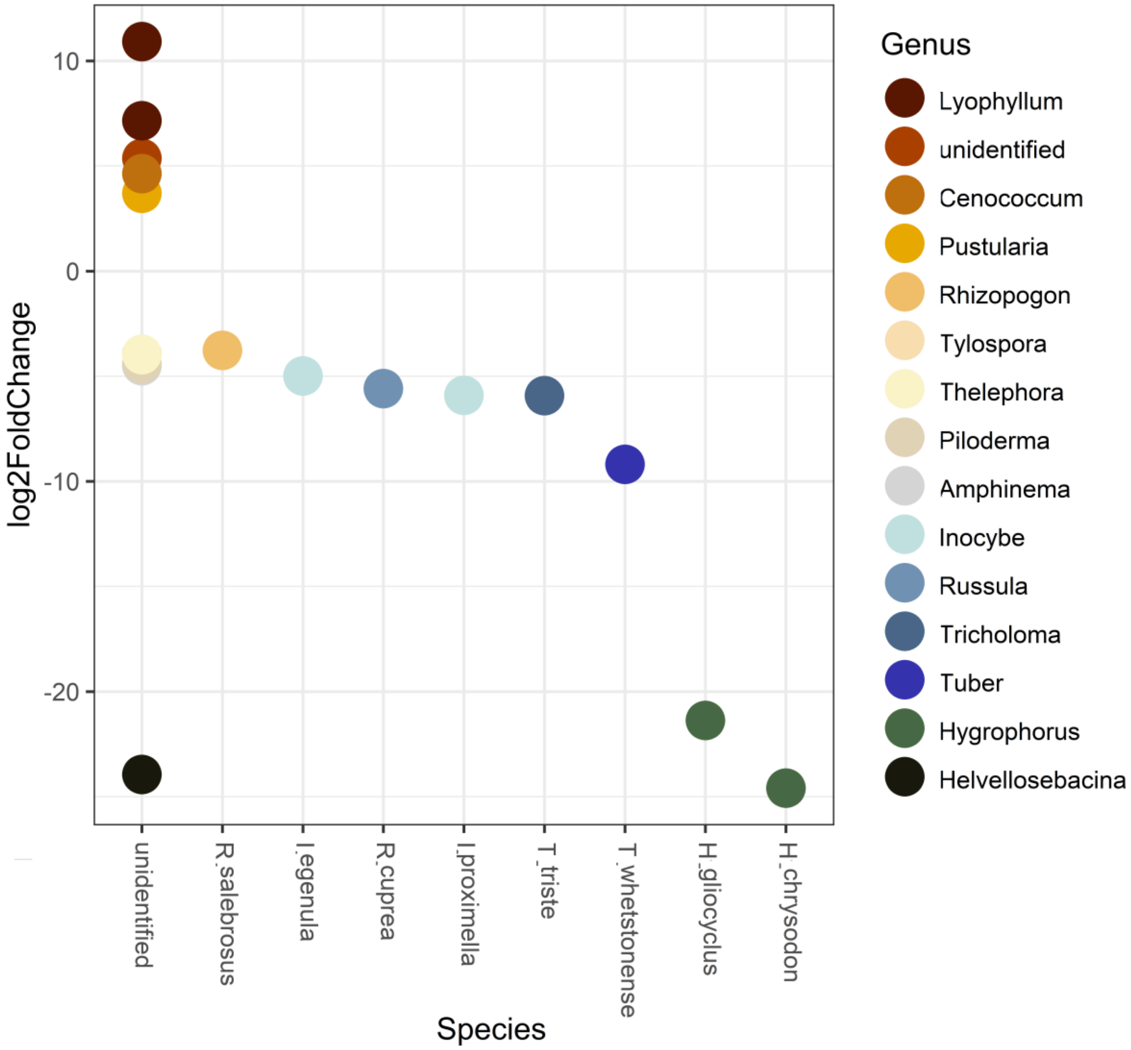

Figure 10. Differential representation of OTU's between burned and unburned sites. Differential abundance of OTUs in soil samples from burned an unburned sites, were assessed by fitting a local regression model with a negative binomial distribution to the raw OTU data. Testing for differential abundance based on nwald, likelihood ratio implemented in DESEQ package with phyloseq. Dots represent the taxa, colored by genus and labeled by species. Positive values indicate a positive response to fire. Negative values represent a negative response to fire and higher representation in the unburned sites. Only the probabilities with a differential abundance at p.adj <0.05 and a log2fold change above zero are included (Statistical test in Appendix 3).

Changes of Species Richness with Time since Fire

Although there was a decreasing trend in species richness with increased time since fire, the negative binomial distribution showed it was not statistically significant ($p=0.2883$, $z = -1.063$; Table 10; Figure 11).

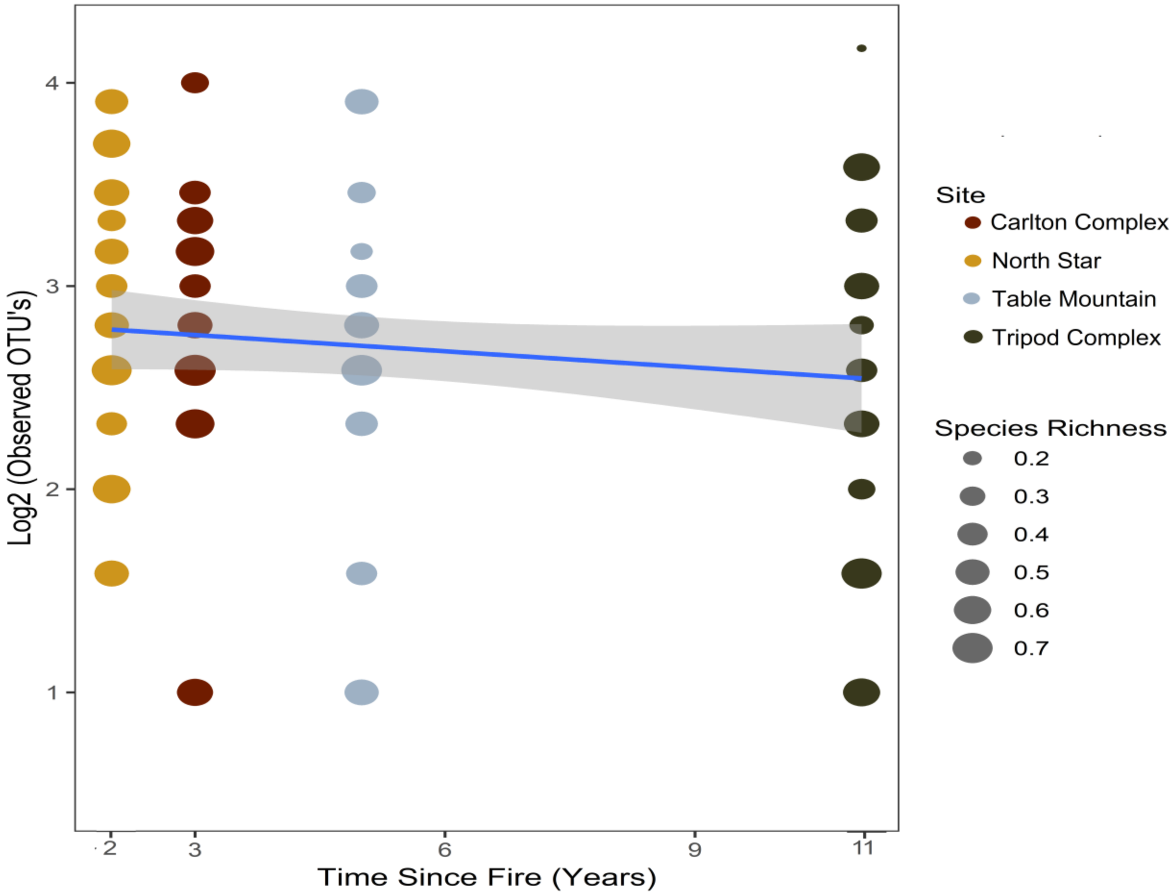

Figure 11. Negative Binomial Regression of species richness with time since fire. Relationship of species richness based log2 transformed OTU data (dots). Dashed line represents the predicted values based on the log2 transformed data of the changes between species richness and time since fire (years) in the burned sites. Regression equation = $-0.014x+2.05$, $p<2e-16$, thetha =17.03.

Table 10. Negative binomial regression comparing the response of species richness (observed OUT's) to increase time since fire. Results are based on comparison between the newest fire (2015, North Star Fire = base level).

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	2.04867	0.08169	25.079	**<2e-16 *****
Time Since Fire (year)	-0.01374	0.01293	-1.063	0.288

Significance at p <0.05 shown with asterisk

Discussion

Nutrients

The complete consumption of the organic layer, in the burned sites, showed a clear relationship with both the changes in the EcM community and soil environment. Previous research has shown that during fire the nutrients which were held by the vegetation, litter, and soil are released by the combustion of the organic layer via volatilization (Evans and Allen, 1971; Neary et al., 1999), resulting in significant decreases in TC and TN directly post-fire (Fernandez et al., 1997; Knicker, 2007). This same relationship was present in the case of TC. Total nitrogen did not react as expected; in fact, there was no change between the burned and unburned sites. The response of TN corroborates previous research from (Alban, 1977; Richter et al., 1982; Knoepp and Swank, 1993; Palviainen et al., 2017). The amount of available nitrogen within the soil is relatively small in comparison to the TN pool. Consequently, although fire induced changes to nitrogen are high, the change to TN are comparatively small accounting for approximately 5% of the cumulative nitrogen lost in a system (Wright and Bailey, 1982; Gillon and Rapp, 1989; Debano et al., 1998). We did not measure available nitrogen in these systems, but it is suggested that future research incorporates changes to both forms of nitrogen in order to better elucidate its relationship to the EcM community. The study sites expand a large portion of eastern Washington, thus, there is a large variation between sites, which could disguise the minimal changes to the TN pool in the post fire environment. Additionally, we only sampled the mineral soils, which tend to be insulated from heat, by the organic layer. Therefore, it is possible that the mineral layer did not reach extreme temperature to cause volatilization of the nutrients. In addition, heat-induced changes only occur in the upper 0-2 cm layers of the mineral soil (Wan et al., 2001; Debano and Conrad, 1978). Consequently, because we homogenized the samples, it is possible that the minute changes to TN were obscured thus resulting in either no significant changes between the burned and unburned sites (Table 3).

Total phosphorus behaved in the same manner as TC in the post-fire environment. In the post-fire sites, the percent TP in the sites doubled in comparison to the unburned sites, supporting previous research from (Kutiel and Naveh, 1987; Debano, 1989; Serrasolsas and Khanna, 1995). The reported TP in the post-fire environments is associated with ash deposition, organic phosphorus mineralization, and the solubility of phosphorus via changes to soil pH (Kutiel and Naveh, 1987; Garcia-Olivia and Jaramillo, 2011). In this study, we did not measure changes to soil pH, therefore, whether the increased TP is associated with alterations to the changes in the soil pH is unknown, but we can associate a portion of the increase in TP to the large amounts of ash deposits in the burned sites (Appendix 6).

Time since fire imposed significant changes to the soil nutrients in the post-fire environment. The ephemeral increases in TC and TN in 2015 were shown to decrease in average by 0.56 and 0.6 percent, respectively, by 2006 (Table 3). Previous research have reported similar results of small increments of TC and TN in the post fire environment with increased time since fire (Debano et al., 1979; Mackenzie et al., 2004; Cowan et al. 2016; Ficken and Wright, 2017). Successional changes in the post fire environment, especially succession of woody shrubs and N-fixing vegetation, result in reduced nutrient availability due to plant uptake (Grogran et al., 2000; Deluca et al., 2002; Mackenzie et al., 2004). Additionally, we see a slight increase in TP with increase time since fire (0.18%), but this relationship with time since fire, differed depending on the years post fire. For example, we see a decrease in TP up to five years post fire, but eleven years post fire, we see an increase in TP. Although the results were significant, p=0.004, we did not attain the TP in every soil sample. Thus, it is possible that the results are a fabrication of the analysis procedure. Additionally, it is possible that four sites in a chronosequence is not ample time to depict the full results of the effects of time since fire on TP. Previous research have depicted fluctuating results similar to ours, but the results only spanned one year (Ranalli, 2004) and these changes were attribute to adsorption by calcium carbonate or the precipitation of phosphorus to insoluble calcium compounds. We did not measure there variables, thus, we recommend that our results be taken lightly as they might not depict the overall effects changes to TP with increasing time since fire.

Species Richness and Community Composition

The community composition of EcM fungi was shown to be significantly altered by HS wildfires. The decrease in species richness, and diversity led to a shift in the post-fire communities, which were dominated by a few pyrophytic fungi. This shift coincides with previous studies (Visser, 1995; Fujimura et al., 2005; Smith et al., 2004; Glassman et al., 2016). However, the decline in species richness in post-fire fungal communities is in contrast to previous research

(Reazin et al., 2016). Species evenness was higher in the burned sites, than unburned sites. The increase in species evenness is attributed to the dominance of a few taxa suggesting a possible decrease in site functional diversity (Maestre et al., 2011; Hillebran et al., 2008), thus having long lasting effects on the restoration of the ecosystem. However, the precise role of EcM fungi in ecosystem processes, in post-fire environments, is not fully understood. In the case of Ascomycetes, a phylum dominated by saprotrophs, the EcM lineage has evolved multiple times suggesting the possibility of saprotrophic capabilities (Claridge et al. 2009), which can give Ascomycetes an advantage in these highly disturbed environments. In fact, previous isotopic research has shown that the genus Morchella possess saprotrophic capabilities (Hobbie et al., 2016). Hence, it is possible that the prolific fruiting of Ascomycetes in these environments is due their potential ability to assimilate C and N from the same resource pool as the various saprotrophic fungi present in the post-fire environments (Hobbie et al., 2016). The reduction of Basidiomycetes in response to fire, and the dominance of Ascomycetes could be a response to the removal of the organic layer and the increase nutrient availability observed in the burned sites, an idea that is supported by previous research (Saikkonen et al., 1999; Tederso et al., 2006; Hobbie and Horton, 2007).

The results of this study do not support the hypothesis that the EcM fungal community will be dominated by a few rare taxa. Although nine of the ten top species in the post-fire environment were rare in the unburned environment, composing less than <10% of the total abundance, *W.rehmii* was a dominant taxa in both pre- and post-fire environments (Figure 9a, 9b). These results differ from those previously reported (Visser, 1995; Horton et al., 1998; Smith et al., 2004; Fujimura et al., 2005; Reazin et al., 2016). *W. rehmii* is a common Ascomycete in PIPO stands (Fujimura et al., 2005) which has previously been shown to survive artificial heating (Peay et al., 2009).Hence, its dominance in the post-fire ecosystem is attributed to its large abundance in the pre-fire environment and to its resistant stage with the soil spore pool. It is also possible that *W.rehmii* is an efficient competitor and can effectively attain the limited nutrients in the disturbed ecosystem. Thus, allowing it to be prolific in these environments. Further research is needed to determined functionality and uptake capabilities of *W.rehmii* in order to assess if and how *W.rehmii* can dominate these HS burned systems.

Overall, HS wildfire imposed a significant alteration to the structural community composition, shifting dominance of Basidiomycetes in the neighboring unburned sites to Ascomycetes in the burned sites. Post-fire communities were dominated by the genera *Wilcoxina*, *Pustularia,* and *Lyophyllum* and the species *W. rehmii*. Although previous studies and in lab test have suggested that *Rhizopogon* species (Horton et al., 1998; Smith et al., 2004; Izzo et al., 2006; Kabir et al., 2009) were highly abundant post fire and even survived after artificial heating in temperatures of 65˚C, this study did not support those results. In fact, although *Rhizopogon* species were present in the pre-fire environment they were rare post-fire. The research in question was performed in low-mixed severity fires, thus the lower fire severity could have allowed *Rhizopogon* species to survive, but HS fires appear to have the opposite effects. It is also possible that *Rhizopogon* survived heating, but was unable to survive the altered environment, thus decreasing in the post-fire sites. This study suggests that *Wilcoxina*, *Pustularia* and *Lyophyllum* respond positively to fire, but soil sampling did not take place directly after the fire event. Consequently, it is difficult to classify the dominant species present in this study as pyrophilic fungi, but there is evidence that fungi belonging to the pezizales clade can produce large amounts of resistant propagules, which emerge after complete drying, and mixing of the soils (Baar et al., 1999; Taylor and Bruns, 1999). Suggesting both resistant to disturbance and a rapid colonizing ability in environments where competition is scarce (Dickie and Reich, 2005).

Species Richness Response to Time since Fire

Although there was a decreasing trend in species richness with time since fire, statistical analysis showed that the trend was not statistically significant. This result differs from those previously observed in other studies (Kipfer et al., 2011; Oliver et al., 2015). This study was constrained to study the effects of the changes to the EcM community in PIPO ecosystems affected by HS fires. Therefore, site selection was limited to four sites spanning eleven years. Consequently, the limited time span used for this chronosequence could have minimized the changes to species richness with increase time since fire. All analysis were performed on rarefied data, resulting in the loss of samples from both the Carlton Complex fire (year 3) and Table Mountain fire (year 5), 6 and 8 sample, respectively. This could have masked some of the effects of the fire on species richness since we are removing samples from the intermediate years. Additionally, there has been mixed opinions about whether or not microbiome data should be rarefied. Some research report no changes after rarefying the data (Glassman et al., 2016), while other suggest that not rarefying could lead to false positives (McMurdie et al., 2014). For this study, all analysis were performed on the rarefied data with the exception of the differential analysis, which requires the full dataset. Therefore, it is unknown whether the results would have differed if the raw data was utilized. Future research should carefully consider when and why to rarefy, or they can perform double analysis as done by Glassman et al., (2016) to better understand if rarefying alters their overall results.

Environmental Drivers

The fire-related response of the EcM community was a significant change in dominance resulting in a simplified community. However, it was evident that fire alone was not the only acting agent in this environment. The EcM communities were linked to physicochemical alteration at the microsites scale (Table 7; Table 8). In this study, elevation, and slope account for 4.0 % of the variance in the EcM community composition (Table 7). Although we attempted to control for site variability, site elevation differed in average by 269 m, between the lowest elevation (1056 m) and the highest elevation (1325 m), thus differences in climatic conditions, which we did not measure could have driven the difference in community composition associated with elevation. Further research is required to understand the changes to the EcM community in response to elevation and their respective climatic variables. Additionally, the differences between TC and TC: TN accounted for 2.34% of the variance in the community composition. Organic matter depth was shown to be correlated to species richness and alpha diversity, explaining 1.10% of the variance of the EcM community assemblage. Although the combination of these metrics explained less than 10% of the community variance, we note that other studies have reported that the EcM communities are driven various physicochemical site characteristics (Gehring et al., 1998; Vasutova et al., 2016; Nickel et al., 2017). Of importance is the organic layer. The organic layer has been shown to contain the largest diversity of EcM fungi (Pierrer et al., 2008; Schenk et al., 2002). Consequently, HS fires which leads to the complete combustion of the organic layer (Neary et al., 1999; Hart et al., 2005) resulting in significant losses to fungal biomass (Koster et al., 2014) which is what we saw in this study (Table 8).

Assumptions and Limitations

Working with chronological studies requires that all sites be as homogeneous as possible. Although we attempted to ensure that we had similar characteristics between sites, we could not control for some factors, including; aspect, elevation, and stand age, resulting in significant differences in the EcM community, which could be, attributed site variation. In fact, aspect was shown to be a significant factor affecting community composition (Appendix 3). We believe that these differences in community composition are associated with differences in solar radiation and soil moisture, which can lead to indirect mortality of the EcM fungi (Hart et al., 2005). Additionally, we assume that the unburned sites are representative of the burned sites, before the fire, but we do not know the OM depth of the burned sites, pre-fire. Given that a vast majority of the EcM community is associate with the organic layer (Pierrer et al., 2008; Schenk et al., 2002) we could be over or underestimating the changes to the EcM community. Unfortunately, it is difficult to pinpoint future time of ignition of a wildfire, thus, at this time this is the only way of assessing the changes to the EcM community.

Conclusion

Results from this study supported three out of the six hypothesis. Nutrients were in fact lower in the burned sites and were altered with increase time since fire. Similarly, the EcM community composition differed between the burned units. Although two metrics of alpha diversity decreased post-fire, species evenness did not, thus we reject our hypothesis. Additionally, the post-fire ectomycorrhizal community was not dominated by rare taxa and species richness did not increase with increase time since fire. We believe this was due to the short time span in this chronosequence and the high site variability, which confounded the results.

In forested ecosystem, maintaining an EcM association is dire for growth and proper resource acquisition. In this study, we see a turnover in the EcM community composition. This decrease in species richness and diversity could have significant impact in the post-fire restoration of the system due to the possible lack of the appropriate EcM species needed for PIPO seedling establishment and growth. We did find that one of the predominant PIPO symbionts, *W. rehmii*, dominated the post-fire environment. Therefore, it is possible that this symbiont can effectively colonize the new seedlings, potentially aiding in post-fire forest restoration. Unfortunately, whether *W.rehmii* can associate with PIPO seedlings is yet to be determined. This area requires further research, especially in the fire-adapted communities, where increase mortality rates of both host and symbiont are prevalent under high-severity fires.

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Management of High-Severity Burned Ponderosa Pine Forest to Maintain the Ectomycorrhizal Community

The preceding chapter will report some of the possible pre/post-fire management techniques that forest managers can implement to help sustain the ectomycorrhizal (EcM) communities and ensure restoration, thereof, in high-severity (HS) burned areas.

Conclusions

Over the eleven-year span that covered this study (2006-2015), the EcM community of ponderosa pine in eastern Washington was drastically affected. High-severity (HS) wildfires resulted in the complete turnover of the EcM community due the decrease of both, species richness (number of different species) and diversity (the number and abundance of each species). Community composition changed post-fire, but eleven years was not sufficient enough for it to converge to pre-fire levels, although, *Wilcoxina rehmii*, a known pyrophilic fungi and well known symbiont of PIPO, dominated both pre- and post-fire environments.

Recommendations

Minimize the risk of HS wildfires

High severit wildfires results in long-lasting impact to the EcM community (Treseder et al., 2004; Kipfer et al., 2011). Given that these types of fires are detrimental to the above- and belowground organism, the first line of defense would include implementing management practices to minimize the propensity of HS wildfires. Fuel reduction treatments, such as thinning and prescribed fires can help eliminate ladder fuels and the understory vegetation (Pollet and Omi, 2002; Agee and Skinner, 2005) thus reducing wildfire severity. Although these management techniques have been shown to alter the EcM community composition, the effects are transient (Grogan et al., 2000; Barker et al., 2013; Cowan et al., 2016), especially when the burns occur in the spring (Anna, 2009). Additionally, reducing the risk of HS fire in PIPO forest can help maintain the EcM refugia (Cowan et al., 2016) by (1) Maintaining large trees which serve as the host the EcM community; 2) Controlling burn severity which minimizes the impact on the EcM community, and; 3) Reducing HS burn patches which increases spore dispersal from unburned/low severity burned forest.

Replanting

Restoration of the post-fire environments can results in increased mortality of the newly planted seedlings, leading to high economic losses to forest managers including the United States Forest Services (USFS), the Department of Natural Resources (DNR) and private owners (timber companies and reservations). Although, seedling inoculation is a current technique for planting conifers, it is important to understand that the disturbed environments do not mimic the natural environment, for some conifer species. Consequently, inoculation of the seedlings with EcM fungi does not always results in positive effects. Therefore, to increase both seedling productivity and the EcM fungi community, it is advantageous to inoculate the seedling with EcM species that can provide benefits to the seedlings in the specific environments the seedling will experience (Garbaye, 1984). Unfortunately, we have yet understand the functionality of the EcM species in these environments, so it is difficult to ascertain what benefit, if any, they may confer to the seedlings. Per this study, inoculation of PIPO seedling with *W. rehmii* might be beneficial, as it was present in both pre-and post-fire environments. The dominance of *W. rehmii* in both systems may be indicative of a competitive fungus, that can thrive in disturbed environments, and that can attain resources in a limited environment. Thus, *W. rehmii* could be the percent species to inoculate PIPO seedling for planting in eastern Washington.

Post-fire seeding and soil erosion

In current years, managers have actively planted HS burned sites with grasses to decrease soil erosion (Peppin et al., 2010; Robichaud and Ashmun, 2012). Ectomycorrhizae have the ability to aggregate soil via their mycelium (Borchers and perry, 1992; Bogeat-Triboulot, et al., 2004; Demenois et al., 2018), which can effectively aid in post-fire erosion. Given that HS-wildfires reduce the EcM soil inoculum, some potential strategies that managers can implement include the manual dispersion of spores into the affected sites. This can be done via animal vectors, such as squirrels from non-burned forest (Allen et al., 1992), but this would require extensive catching and release work which can potentially be expensive. I recommend that soil, from the nearby-unburned forest be used. This healthy soil can serve as inoculum in the burned forest, especially in the center of these HS burn-patches where spore wind dispersal is not feasible. Although, the movement of soil has not been tested in burned environments, the movement of soil to serve as inoculum has been documented in various exotic plantations, and the results have always been positive (Marx, 1991; Pringle et al., 2009). Therefore, manually moving small amounts of soil into the center of the burn patches has the potential to help minimize soil erosion, increase the EcM community in the center of the burn patch and increase the inoculum potential to aid in post-fire seedling restoration.

Summary

Wildfires are predicted to increase in both area burned and area burned at HS (Littell et al., 2010; Peterson and Littell, 2014). Thus, it is important to understand the effects of HS fires on the soil microbiome, especially on EcM fungi, due to their obligate association with the dominant conifers in temperate forest. Conifers in the family Pinaceae, are obligate EcM host who require an EcM association for proper seedling establishment and survival (Smith and Read, 2008). In HS affected sites, it is recommended that managers maintain a healthy EcM community, which has the potential to aid in seedling regeneration, seedling resiliency to disturbed environments and soil erosion. This can be done by (1) minimizing HS fires via fuel treatments; (2) by moving small amount of soil into the HS burn patches; (3) inoculating seedling with EcM species that are native to the location, that have been known to associate with the specific conifer in question and which can thrive in the altered environment.

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Appendices

Appendix 1: Kruskal-Wallis Pairwise Comparison Analysis for Diversity Metrics

Table A1. Kruskal-Wallis pairwise analysis for comparison of alpha diversity metrics: species richness, Simpson’s Evenness and Simpson’s Diversity (D), between all burned sites: Tripod Complex, Table Mountain, Carlton Complex, and North Star fire and their corresponding neighboring unpaired sites.

			Species Richness			Species Evenness			Species Diversity		
Group 1	Group 2	N	H	p.value	p.adj	H	p.value	p.adj	H	p.value	p.adj
CarlB (n=13)	CarlU	27	21.20	0.000	0.000*	0.42	0.516	0.651	15.09	0.000	0.001*
	NSB	23	0.09	0.766	0.858	0.12	0.730	0.757	0.033	0.856	0.856
	NSU	27	18.83	0.000	0.000*	5.27	0.022	0.087	6.20	0.014	0.035*
	TMB	14	0.05	0.825	0.889	1.85	0.174	0.349	0.85	0.357	0.434
	TMU	26	6.23	0.013	0.019	2.88	0.090	0.251	1.35	0.245	0.354
	TriB	24	0.01	0.936	0.936	0.91	0.340	0.567	0.68	0.408	0.457
	TriU	25	12.70	0.000	0.001*	10.14	0.001	0.013*	0.48	0.489	0.526
CarlU (n=27)	NSB	23	33.13	0.000	0.000*	0.16	0.690	0.743	27.52	0.000	0.000*
	NSU	27	0.32	0.573	0.727	4.13	0.042	0.131	3.33	0.068	0.119
	TMB	14	25.16	0.000	0.000*	0.47	0.492	0.651	21.33	0.000	0.000*
	TMU	26	13.25	0.000	0.001*	2.08	0.150	0.322	13.56	0.000	0.001*
	TriB	24	32.37	0.000	0.000*	0.21	0.651	0.743	31.63	0.000	0.000*
	TriU	25	6.56	0.010	0.016*	11.67	0.001	0.014*	18.13	0.000	0.000*
NSB (n=23)	NSU	27	29.06	0.000	0.000*	4.38	0.036	0.127	11.28	0.001	0.003*
	TMB	14	0.27	0.604	0.727	0.66	0.416	0.612	1.27	0.260	0.359
	TMU	26	9.32	0.002	0.004*	1.54	0.214	0.400	2.97	0.085	0.136
	TriB	24	0.24	0.623	0.727	0.84	0.360	0.567	1.04	0.307	0.391
	TriU	25	18.61	0.000	0.000*	10.04	0.002	0.014*	1.22	0.270	0.359
NSU (n=27)	TMB	14	22.57	0.000	0.000*	0.82	0.364	0.567	11.25	0.001	0.003*
	TMU	26	10.28	0.001	0.003*	0.43	0.510	0.651	2.92	0.088	0.136
	TriB	24	29.75	0.000	0.000*	2.28	0.131	0.306	14.97	0.000	0.001*
	TriU	25	4.50	0.034	0.048*	2.45	0.117	0.299	4.63	0.031	0.068
TMB (n=14)	TMU	26	8.58	0.003	0.006*	0.08	0.777	0.777	5.02	0.025	0.059
	TriB	24	0.03	0.867	0.899	0.18	0.672	0.743	0.07	0.785	0.814
	TriU	25	15.82	0.000	0.000*	5.35	0.021	0.087	3.40	0.065	0.119
TMU (n=26)	TriB	24	11.43	0.001	0.001*	0.39	0.534	0.652	6.67	0.001	0.028*
	TriU	25	1.90	0.168	0.224	5.55	0.019	0.087	0.78	0.376	0.439
TriB (n=24)	TriU	25	19.55	0.000	0.000*	8.88	0.0029	0.020*	3.69	0.055	0.110*

Significance at $p<0.05$ displayes with asterisk. H=Kruskal-Wallis H, CarlB/U (Carlton burned/Unburned), NSB/NSU (North Star Burned/Unburned). TMB/TMU (Table Mt. Burned/Unburned), TriU/TriB (Tripod Burned/Unburned).

Appendix 2: Pairwise Adonis: Community Composition per Site

Table A2. Pairwise adonis for comparison of community composition between burned and unburned sites.

Pairs		F.Model	R^2	p.value	p.adjusted
CarlU	Carl B	4.658965	0.1092142	0.001	0.028*
	NSU	4.841023	0.08516777	0.001	0.028*
	NSB	8.839593	0.15551824	0.001	0.028*
	TMU	5.849936	0.10290137	0.001	0.028*
	TMB	9.933986	0.2030079	0.001	0.028*
	TriU	5.246553	0.09496616	0.001	0.028*
	TriB	4.996178	0.09252837	0.001	0.028*
CarlB	NSU	3.699238	0.08871235	0.001	0.028*
	NSB	4.56235	0.118311	0.001	0.028*
	TMU	3.431296	0.08486733	0.001	0.028*
	TMB	5.2979	0.17486031	0.001	0.028*
	TriU	4.414748	0.10923607	0.001	0.028*
	TriB	3.006573	0.07910666	0.002	0.056
NSU	NSB	7.297956	0.13197514	0.001	0.028*
	TNU	3.860371	0.0703672	0.001	0.028*
	TMB	7.176031	0.15540596	0.001	0.028*
	TriU	6.994918	0.1227288	0.001	0.028*
	TriB	4.802494	0.08926154	0.001	0.028*
NSB	TMU	6.846119	0.12714229	0.001	0.028*
	TMB	7.420847	0.17493397	0.001	0.028*
	TriU	8.775093	0.16020225	0.001	0.028*
	TriB	5.922105	0.11629733	0.001	0.028*
TMU	TMB	6.374336	0.14364916	0.001	0.028*
	TriU	7.163891	0.12755332	0.001	0.028*
	TriB	4.095249	0.0786108	0.001	0.028*
TMB	TriU	12.194322	0.24788068	0.001	0.028*
	TriB	7.480665	0.17204579	0.001	0.028*
TriU	TriB	4.032278	0.07901427	0.001	0.028*

Significance at $p<0.05$, displayed with an asterisk
B=burn, U=Unburn
NS= NorthStar, Tri=Tripod, TM=TableMountain, Carl=Carlton

Appendix 3: Pairwise Adonis: Community Composition per Aspect

Table A3. Pairwise Adonis for comparison between community composition and aspect differences between the sites. Information on specific site aspects found on table 1.

Pairs	F model	R^2	p.value	p.adj
E vs S	2.317575	0.01942	0.001	0.006*
E vs SE	1.702615	0.03725	0.037	0.222
E vs SW	2.247265	0.05584	0.006	0.036*
S vs SE	3.290648	0.02346	0.001	0.006*
S vs SW	2.472143	0.01852	0.001	0.006*
SE vs SW	2.431849	0.04024	0.002	0.012*

Significance at $p < 0.05$, displayed with an asterisk.

Appendix 4: Differential Abundance: Results per Treatments (Burned vs Unburned)

Table A4. Differentially abundant OTU"s by treatment, burned and unburned. Only probabilities with a differential abundance p-adj <0.05 and a log2fold change >0 are included. Reference condition burned (rarefied to 32,041 sequences).

OTU-ID	base Mean	log2 Fold Chng	STE	stat	pvalue	padj	P	G	Species
ea2cd32946d49e67 ea25d401619076d4	34.7	10.9	1.00	10.96	5.9E-28	5.0E-26	B	Lyophyllum	Un-ID
7d1bf1385d2cf3c8 b74b6ab41e0c6c44	9.9	-24.6	2.68	-9.18	4.3E-20	1.9E-18	B	Hygrophorus	*Hygrophorus chrysodon*
5875aa5022959e7b 40ba95e461bbc202	6.2	-23.9	2.89	-8.28	1.3E-16	3.6E-15	B	Helvellose-bacina	Un-ID
4cc3dda56d1ab848 607d6ad2c15faa0c	3.2	-21.4	2.78	-7.69	1.5E-14	3.2E-13	B	Hygrophorus	*Hygrophorus gliocyclus*
972cc1ab6160ba 905b7fae2f5c59f605	51.9	3.7	0.53	6.96	3.4E-12	5.9E-11	A	Pustularia	Un-ID
0b67a6ffea51b302 d3dff343e9aaa35f	91.9	-5.6	0.97	-5.79	7.2E-09	1.0E-07	B	Russula	*Russula cuprea*
6caf4eb525a14906 705e57d451e34a38	37.9	-4.6	0.84	-5.49	4.0E-08	5.0E-07	B	Cortinarius	NA
b1ee378959636010 53eda3be0f2d18a8	2.7	5.4	1.03	5.22	1.8E-07	1.9E-06	A	Un-ID	Un-ID
8a0b59b3e5aaaeb7 7e85341603defa06	5.9	-4.9	1.05	-4.64	3.5E-06	3.4E-05	B	NA	NA
9da8088d6fb8cf1b f8703a2b12ab1866	8.2	-5.9	1.32	-4.48	7.5E-06	6.5E-05	B	Tricholoma	*Tricholoma triste*
50a549c1275ffe81 80aed5b638dee4bb	7.2	7.2	1.66	4.30	1.7E-05	1.3E-04	B	Lyophyllum	Un-ID
2bf7871e99fb6c90 9309c32555c1e6e0	12.2	-6.1	1.43	-4.28	1.9E-05	1.4E-04	B	Cortinarius	NA
9e8ff65e9725f4fb b2a629f617955d32	28.0	-5.0	1.18	-4.22	2.4E-05	1.6E-04	B	Inocybe	*Inocybe Egenula*
ea02a4092f702445 fdae41ff833506f5	4.9	-4.5	1.12	-4.01	6.2E-05	3.8E-04	B	Amphinema	Un-ID
e33da878b524158d 66a2357d503a33fe	5.6	-5.9	1.55	-3.80	1.4E-04	8.2E-04	B	Inocybe	*Inocybe proximella*
9bcdce1a31460eb7e f90c77e4178fd35	4.3	4.6	1.24	3.73	1.9E-04	1.0E-03	A	Cenococcum	Un-ID
4d45230268c420d 8771351cb64dc471e	4.2	-3.8	1.06	-3.54	4.0E-04	2.0E-03	B	Rhizopogon	*Rhizoogon salebrosus*
181cc5e49a40e19 ce7380e4342a51fde	13.4	-9.3	2.73	-3.37	7.5E-04	3.6E-03	A	Tuber	*Tuber whetstonense*
523e2915edb76f05 798c7c9b8980ffa1	4.3	-4.0	1.29	-3.06	2.2E-03	9.6E-03	B	Tylospora	Un-ID

431d6de8d0b5b991687ccf4d93ee6737	11.1	-4.4	1.43	-3.07	2.1E-03	9.6E-03	B	Piloderma	Un-ID
e171e29df2030cda4856f901bf71a5ea	11.6	5.9	1.94	3.02	2.6E-03	1.0E-02	A	NA	NA
7e0e01af71cdca77e037bf6db134bed3	3.2	-4.0	1.32	-3.02	2.5E-03	1.0E-02	B	Thelephora	Un-ID
a41a34ccaefaddd6a84d55074db1a111	3.0	-3.9	1.32	-2.91	3.7E-03	1.4E-02	B	Cortinarius	NA
218b005c9e82c24bfd04b9abb25b8879	4.9	-3.6	1.23	-2.89	3.8E-03	1.4E-02	B	Thelephora	NA
8e3ecdc3bf01b0a23ab073b3cc047f7b	9.3	7.7	2.73	2.81	4.9E-03	1.7E-02	B	Lyophyllum	NA
340fcc24a214cc01e124e6bcf6ffbcdb	8.0	-3.9	1.53	-2.54	1.1E-02	3.7E-02	B	Cortinarius	NA

Significance at p.adj <0.05 (all) OUT-ID is based on the OUT ID's assign by QIIME2, using UNITE
P=Phylum, G=Genus, B=Basidiomycota, A=Ascomycota

Appendix 5: Differential Abundance per Site

Table A 5 *Differentially abundant OTU''s since time of fire. Only probabilities with a differential abundant p-adj <0.05 and a log2fold change >0 are included. Reference condition = 2 year since time of fire (2015 North Star Fire).*

TSF (2 yrs.)	OUT ID	base Mean	log2Fold Change	STE	padj	P	C	F	G
2 vs. 3 yrs.	5b96fd357e0561c7829ab9412c4057e4	36.704	26.546	3.261	2.96E-14	A	Pezizomycetes	Pyronemataceae	Pustularia
	972cc1ab6160ba905b7fae2f5c59f605	71.349	5.322	1.014	8.63E-06	A	Pezizomycetes	Pyronemataceae	Pustularia
2 vs. 5 yrs.	50a549c1275ffe8180aed5b638dee4bb	24.856	26.041	2.259	2.194E-28	B	Agaricomycetes	Lyophyllaceae	Lyophyllum
	5b96fd357e0561c7829ab9412c4057e4	36.704	26.072	3.310	2.554E-13	A	Pezizomycetes	Pyronemataceae	Pustularia
	df0ded37677cac9557bec2d40775c2cc	148.934	7.115	1.382	1.491E-05	A	Pezizomycetes	Pyronemataceae	Wilcoxina
2 vs.11 yrs.	ea2cd32946d49e67ea25d401619076d4	62.475	26.745	1.427	5.545E-76	B	Agaricomycetes	Lyophyllaceae	Lyophyllum
	50a549c1275ffe8180aed5b638dee4bb	24.856	25.227	2.089	1.588E-31	B	Agaricomycetes	Lyophyllaceae	Lyophyllum
	5b96fd357e0561c7829ab9412c4057e4	36.704	26.725	3.065	1.574E-16	A	Pezizomycetes	Pyronemataceae	Pustularia
	e171e29df2030cda4856f901bf71a5ea	71.631	10.261	2.258	0.0002503	A	Pezizomycetes	Pyronemataceae	NA
	972cc1ab6160ba905b7fae2f5c59f605	71.349	4.030	0.948	0.0008076	A	Pezizomycetes	Pyronemataceae	Pustularia

Significance at p<0.05, shown with asterisk. OTU-ID is based on the OTU IS's assigned by QIIME2, using UNITE database
P=Phylum, G-Genus, B=Basidiomycota, A=Ascomycota, STE=standard error, P=phylum, C=class, F=family, G=genus,
TSF=Time since fire

Appendix 6 Results from Envfit Table of Environmental Variables in Response to Community Composition

Table A6

Envfit permutation analysis of environmental variables, 9999 permutations on Bray-Curtis distance for community composition.

EV variable	**NMDS1**	**NMDS2**	**R^2**	**Pr(>r)**
Fire Year	0.99621	0.08697	0.5533	0.0001*
OM Depth	-0.9911	-0.1333	0.4141	0.0001*
Total Carbon: Total Phosphorus	-0.7906	0.61238	0.1877	0.0001*
Total Phosphorus	0.8861	-0.4635	0.1749	0.0001*
Average Distance to Tree	0.99623	0.08673	0.1105	0.0002*
Total Carbon	-0.3284	0.94453	0.0824	0.0008*
Total Carbon : Total Nitrogen	-0.654	-0.7565	0.0737	0.0025*
Elevation	0.71376	0.70039	0.0552	0.0061*

Significance at $p<0.05$, displayed with an asterisk

Appendix 7 Negative Binomial Regression of the Response of Observed OTU’s with Increase Time since Fire

Table A7. Negative binomial regression for comparison of observed OTU's in response to increase change in time. Results based in comparison to 2015 (2 yrs.), showing comparison among the fires based on 2015 North Star fire.

	Estimate	Std. Error	z value	Pr (>|z|)
(Intercept)	2.03688	0.08364	24.352	<2e-16*
Time since fire (3)	-0.0354	0.12914	-0.274	0.784
Time since fire (5)	-0.08348	0.13242	-0.63	0.528
Time since fire (11)	-0.13179	0.12364	-1.066	0.286

Significance at p<0.05 shown with asterisk, Years shown in parentheses

Appendix 8 Study Site Photos of Burned and Unburned Sites.

2015 North Star Fire

2014 Carlton Fire

2012 Table Mountain Fire

2006 Tripod Complex Fire

Appendix 9 Picture of the Soils of Burned Sites

Carlton Complex Burned Site

Supplementary Methods

S1. Bioinformatic Analysis

Paired-end demultiplexed sequence files, containing 4,535,423 raw sequences in 213 samples were processed using Qiime2, version 2018.6 (Caporaso et al., 2010).The primers and adapter sequences from the 5’ were removed by the sequencing facility. The 3’ primer and adapters, *CTTGGTCATTTAGAGGAAGTAA,* were removed using the qiime2-cut-a-dapt plugin (Martin, 2011). Quality control to filter out chimeric sequences, and low quality regions was done by parsing the sequences through DADA2 and truncating the 3’ end to 150 bp (Callahan et al., 2016), resulting in 3,632,059 merged sequences.

Taxonomic analysis was performed by first training a taxonomic classifier using UNITE reference database with clustering at $\geq 99\%$ reference levels (Kõljalg et al 2005; UNITE, 2017). The sequences were then assigned taxonomy using the fitted classifier, classify-sklearn plugin, (Pedregosa et al., 2011) resulting in a clustered OTU table at 99% similarity, resulting in a table with 8,319 OTU’s and a mean of 15, 709 sequences per sample.

Assigning taxonomic guild

Since the ITS1F-ITS2 allow for the amplification of all members of the fungal family, the OTU table was parsed through FUNGuild (Nguyen et al., 2016) in order to assign guild and remove all non-EcM samples by parsing the table through FUNGuild.

Ectomycorrhizal bioinformatics

The FUNGuild table was used in QIIME2 to extract those OTU's belonging to the EcM guild. This resulted in a total of 852 OTU's. This table was additionally rarefied to remove the bottom 15% of the sequences in order to account for uneven sequencing depth, but because the burned sites were in average very low in comparison to the burned units, the sequencing depth had to be maintained on the lower spectrum in order minimize discarding too many burned samples. Hence, the table was rarefied to a depth of 179, retaining a total of 32, 041 (3.06%) of the sequences in 179 (84.04%)