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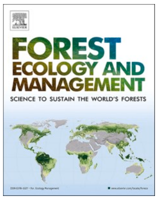

High-severity wildfire reduces richness and alters composition of ectomycorrhizal fungi in low-severity adapted ponderosa pine forests

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ABSTRACT

Ponderosa pine (*Pinus ponderosa*) forests are increasingly experiencing high-severity, stand-replacing fires. Whereas alterations to aboveground ecosystems have been extensively studied, little is known about soil fungal responses in fire-adapted ecosystems. We implement a chronosequence of four different fires that varied in time since fire, 2 years (2015) to 11 years (2006) and contained stands of high severity burned *P. ponderosa* in eastern Washington and compared their soil fungal communities to adjacent unburned plots. Using Illumina Miseq (ITS1), we examined changes in soil nutrients, drivers of species richness for ectomycorrhizal (plant symbionts) and saprobic (decomposers) fungi, community shifts, and post-fire fungal succession in burned and unburned plots. Ectomycorrhizal richness was 43.4% and saprobic richness 12.2% lower in the burned plots, leading to long-term alterations to the fungal communities that did not return to unburned levels, even after 11 years. Differences in the post-fire fungal community were driven by pyrophilous, "fire-loving" fungi, including the ectomycorrhizal Ascomycete genera *Pustularia* and *Wilcoxina,* and the saprobic Basidiomycete genus *Geminibasidium*. Ectomycorrhizal and saprobic fungi were intimately linked to the soil environment: depth of the organic matter, total carbon, total nitrogen, and their interaction with fire predicted ectomycorrhizal richness. Whereas total carbon, time since fire, treatment, and the interaction between time since fire and treatment predicted saprobic richness. We conclude that high-severity wildfires lead to lower ectomycorrhizal richness and significantly altered ectomycorrhizal and saprobic communities in fire-adapted ecosystems, selecting resilient and fire-adapted species, such as *W. rehmii* and *Geminibasidium* sp., thus initiating post-fire succession.

1. Introduction

Wildfires are an important ecological process shaping the dry Ponderosa pine (*Pinus ponderosa* Dougl. ex. Laws) forest of the Western United States. Historically, before European settlement (Fryer, 2018), ponderosa pine forest experienced a low severity fire regime of frequent surface fires (<30 yrs.) that removed the understory vegetation (Agee, 1993) and maintained a forest dominated by large, fire-resistant trees (Agee, 1993; Cooper, 1961; Odion et al., 2014). However, decades of fire exclusion, management decisions, and altered land-use patterns, together with climate change, have caused wildfires to deviate from historical patterns (Littell et al., 2009; Mallek et al., 2013; McKenzie et al., 2004). Today, wildfire extent and severity have increased across North America (McKenzie et al., 2004; Littell et al., 2009; van Mantgem et al., 2013) and the globe (Nolan et al., 2020), including in the fire-adapted *P. Ponderosa* ecosystems. Ponderosa systems are increasingly experiencing high-severity, stand-replacing fires (Westerling, 2006), that result in the removal of 75% of the basal area (Agee, 1993), and high soil burn severity that consumes all the pre-fire ground vegetation and surface organic matter (Parson et al., 2010). Consequently, the post-fire ponderosa landscape is riddled with large high severity burned patches that are larger than historically documented (Sherriff et al., 2014) and where post-fire natural regeneration is limited or lacking (Chambers et al., 2016; Haffey et al., 2018). Whereas alterations to aboveground ecosystems have been extensively studied (Bataineh et al., 2006; Chambers et al., 2016; Donato et al., 2009; Francos et al., 2016; Korb et al., 2019; Owen et al., 2017), relatively little is known about soil fungal responses in fire-adapted ecosystems, despite recent efforts.

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In temperate forests, most dominant tree species are obligate ectomycorrhizal (EcM) hosts, including *P. ponderosa* (Smith and Read, 2009). In exchange for photosynthetically derived carbon, EcM fungi provide their hosts with nutrients and water (Smith and Read 2009). Moreover, saprobic fungi co-inhabit the rhizosphere with EcM fungi; playing key roles in decomposition (Smith & Read, 2009), soil moisture retention (Egger and Paden, 1986), and are vital for the formation, stabilization, and disintegration of soil aggregates (Lehmann and Rillig, 2015; Ritz and Young, 2004; Tisdall et al., 2012). Wildfire can directly alter the soil microbial community through selective heat-induced mortality (DeBano et al., 1998), resulting in negative to neutral effects (Taudière et al., 2017). In addition, wildfire leads to indirect long-term effects via host mortality (Buscardo et al., 2011; Pec et al., 2017; Sun et al., 2015), shifts in plant dominance (Ning et al., 2021), and litter input and structure (Bhatnagar et al., 2018; Ficken and Wright, 2017). Consequently, fire can result in the selective mortality of different fungal groups (EcM vs. saprobes) while selecting for pyrophilous “fire-loving” fungi, capable of surviving wildfires and thriving in the post-fire altered environment (Carlson et al., 2017; Glassman et al., 2016; Kipfer et al., 2011). However, how the post-fire soil nutrients relate to the changes in the EcM and saprobic communities, and whether the symbiotic organisms that evolved under the shared selective pressures of low-severity wildfires are undergoing the long-lasting, high mortality rates experienced by *P. ponderosa* in these altered fire regimes, remains unknown.

The extent to which fires affect soil fungal communities has been studied under various conditions, including prescribed fires (Smith et al., 2004; Stendell et al., 1999) and varying fire severities, including high-severity wildfires (Dahlberg, 2001; Reazin et al., 2016; Smith et al., 2004). Current studies on high-severity wildfires in forests adapted to low-severity fire regimes have shown contradictory results. For example, some studies have shown an overall decrease in total fungal (Reazin et al., 2016), EcM (Cowan et al., 2016; Day et al., 2019; Owen et al., 2019), and saprobic species richness (Day et al., 2019) and alterations in the EcM community composition (Glassman et al., 2016; Owen et al., 2019; Smith et al., 2017). In contrast, other studies have indicated no change in saprobic communities (Owen et al., 2019; Smith et al., 2017). Consequently, the effects of high-severity wildfires on fungi and specific fungal groups are difficult to generalize. Moreover, most studies on changing fire-regimes on soil fungal communities are based on manipulated experiments (Cowan et al., 2016; Reazin et al., 2016; Smith et al., 2017) or a onetime sampling event of a wildfire (Day et al., 2019; Glassman et al., 2016). Thus, we lack information on the long-lasting impact of wildfire on the soil fungal communities and their post-fire successional dynamics.

Chronosequences studies, where space is substituted for time, have allowed for monitoring long-term effects on fungi. However, the limited number of studies have focused on EcM succession (Alem et al., 2020; Cairney and Bastias, 2007; Kipfer et al., 2011; McMullan-Fisher et al., 2011; Treseder et al., 2004; Twieg et al., 2007; Visser, 1995; Yang et al., 2020) or arbuscular fungi (McMullan-Fisher et al., 2011; Treseder et al., 2004), and only one study focused on ecosystem experiencing a shift in fire regimes (Kipfer et al., 2011). These studies established that EcM changes are highly drastic the first post-fire decade (Twieg et al., 2007; Visser, 1995) and reached levels comparable to unburned communities after 15–18 years (Kipfer et al., 2011; Treseder et al., 2004). Moreover, it has been established that EcM fungi subsequently replace saprobes with increased time since fire (Sun et al., 2015; Treseder et al., 2004), but fungal succession is also affected by soil geochemical changes. Yet, studies linking long-term soil changes to fungal succession, including saprobic communities, are limited (Alem et al., 2020). It is recognized that wildfires create a pulse of nutrients, a crucial resource for many fungal species to survive in a disturbed environment (Chang and Turner, 2019) and that changes in soil pH (Day et al., 2019; Glassman et al., 2017b; Whitman et al., 2019) and soil organic matter (Cairney and Bastias, 2007; Dooley and Treseder, 2012) can affect fungal communities. Additionally, the increased size of the area burned at high-severity limits fungal dispersal (Owen et al., 2019; Sherriff et al., 2014). As a result, limited fungal dispersal can hinder post-fire vegetation regeneration, negatively affecting the post-fire fungal communities, resulting in a positive feedback loop that might require manual intervention to overcome. Given the limited studies in altered fire regimes and the lack of studies linking changes in the soil nutrients to changes in the soil fungal communities, the long-term effects and successional dynamics of post-fire fungi require attention.

Between 1973 and 2019, eastern Washington experienced 20 large wildfires (>25,000 ha), and 19 of these occurred from 2003 to 2019 (WA DNR, 2019). These fires burned over 890,000 ha, and about 25% of the area burned experienced high vegetation and soil burn severity (MTBS.gov). These fires provided the opportunity to implement a chronosequence study of 11 years of post-fire history, based on four different fires that burned between 2006 and 2015 and contained stands of high severity burned *P. ponderosa* and their adjacent unburned stands. Thus, resulting in a series of sites (wildfires) that varied primarily in the time since fire, 2 years (2015) to 11 years (2006), and have similar soil types (loamy soils), vegetation, and climatic conditions. We addressed the following hypotheses: (1) high-severity wildfire will cause long-term changes (>11 yrs) to the soil nutrients; (2) high-severity burned areas will have lower EcM and saprobic species richness and a different community composition compared to unburned areas; (3) EcM and saprobic fungal communities in high-severity burned areas will be dominated by pyrophilous Ascomycetes; (4) long-term changes (>11 years) in soil fungal richness and community composition will be associated with the post-fire changes in soil nutrients; (5) An 11-year post-fire period is not enough time for EcM fungal species richness to converge to comparable unburned sites, but it will be ample time for saprobic species richness to converge to comparable unburned levels.

2. Materials and methods

2.1. Study sites

We selected sites from four high-severity wildfires in eastern Washington, USA, that had burned over 17,000 ha in the Okanagan-Wenatchee National Forest and the Colville National Forest (Fig. 1). Soil burn severity was determined using BAER soil burn severity maps (MTBS.com) and ground-truthing the selected areas for plots that we determined to be burned at high-severity based on USDA guidelines (Parson et al., 2010). Ground-truthing measurements of high soil burn severity included 1) > 70% tree mortality (Agee, 2007), per our sites, we selected sites with 100% tree mortality, and 2) the presence of at least 2 or more of the soil burn severity factors, including; complete removal of the soil organic layer, ash color, ash depth and charred stump holes (Parson et al., 2010). At each fire, burned sites were paired with an adjacent unburned sites to isolate the effects of the wildfire (Johnson and Miyanishi, 2008). Unburned sites were located between 2 and 11 km from the burned sites to avoid any potential alterations caused during firefighting (fire lines, retardants, etc.). Sites were selected to meet the following five criteria: (1) they were located in an area of high soil burn severity; (2) *P. ponderosa* was the dominant tree species (>80% of the basal area on the site (live/dead trees); (3) the sites showed no evidence of previous wildfire, prescribed fire, mechanical treatment, or salvage logging for a minimum of 30 years (U.S. Forest Service records confirmed previous management treatments); (4) to ensure that we remained outside of the mixed forest line, sites were selected to be within the lower end of the natural elevation range of *P. ponderosa* (1000–1800 m; Table S1); (5) contain similar understory vegetation. Another initial criterion was to maintain the same aspect between burned and adjacent unburned sites, but for two of the fire sites with a south-facing aspect, it proved difficult to locate control sites. Therefore, the same-aspect criterion was removed from the site selection requirements, resulting in four high-severity burned sites within the fire perimeter and four adjacent unburned sites (Fig. 1a, Table S1). The

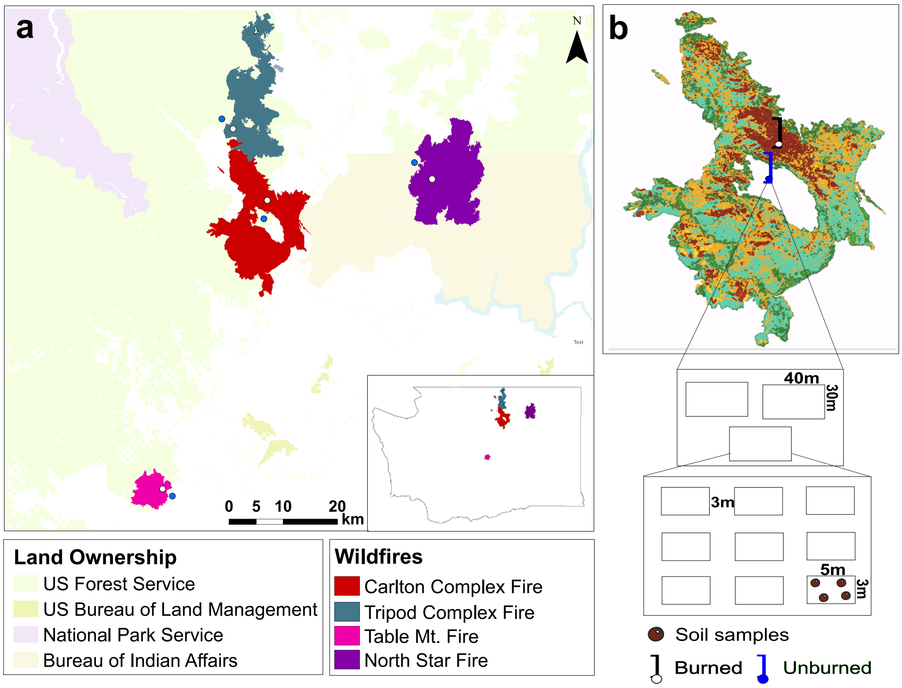

Fig. 1. (a) Study region of four wildfires, Tripod Complex fire (2006); Table Mountain fire (2012); and Carlton Complex fire (2014), and the North Star fire (2015) and respective unburned areas in Eastern Washington. Location of sites are denoted with a dot – burned (white) and unburned (blue). b) Example of the experimental design and soil sampling layout based on their placement within the Carlton Complex Fire (black pine: burned; blue pine: unburned). At each site, three (30 m × 40 m) plots were laid out systematically, with nine, 5 m × 3 m subplots. Four soil samples were collected from each subplot. A total of 216 soil samples were collected: 4 fires, 2 treatments (burned and unburned) × 3 plots × 9 subplots. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

chronosequence we studied covered an 11-year post-fire period, including the following four fires: the 2006 Tripod Complex Fire, the 2012 Table Mountain Fire, the 2014 Carlton Complex Fire, and the 2016 North Star Fire (Fig. 1a). Sites were dominated by *P. ponderosa* but also harbored western larch (*Larix occidentalis*) or Douglas-fir (*Pseudotsuga menziesii)*. The understory was dominated by *Ceanothus* spp., Balsamroot (*Balsamorhiza sagittata*), bunch grass (*Festuca idahoensis*) and bluebunch wheatgrass *(Pseudoroegneria spicata)*. In addition, fireweed *(Chamaenerion angustifolium*) was a dominant vegetation type in the burned sites. Soil types were consistent within the loamy texture and ranged from ashy sandy loam to stony loam (Table S1). Mean summer temperature and precipitation data for these sites can be found in Supplementary data 2, Table S2.

2.2. *Soil sampling*

Sampling of paired burned and unburned sites took place in summer 2017, two years after the most recent fire (North Star) and 11 years after the oldest fire (Tripod Complex). Plots were selected for similar elevation, slope, understory vegetation, and dominance and density (basal area) of *P. ponderosa*. To estimate the fungal composition of each site, three rectangular 30-m × 40-m plots, 5-m apart, were systematically laid out to avoid young patches of *P. ponderosa* as they contain an EcM community that differs from the mature stands (Deacon and Donaldson, 1983; Twieg et al., 2007; Visser, 1995). We recorded coordinate readings from each plot with a Garmin GPS device and measured organic matter depth and the distance to nearest *P. ponderosa,* where distance corresponds to live trees in the unburned plots and dead trees in burned plots. On average, the distance ranged from 1.4 m to 28 m. Edge effects were minimized by placing all plots at least 30-m from roads, streams, fire lines, and nearest unburned plot or residual trees. Spatial microbial turnover has been shown to occur within 2–3-m (Bahram et al., 2013; Lilleskov et al., 2004; Pickles et al., 2012); thus, to account for spatial autocorrelation, we placed nine, 3-m × 5-m subplots, 3-m apart, following the same cardinal direction of their respective plots (Fig. 1b). In addition, we tested for autocorrelation using Mantel test (Table S3) and controlled for autocorrelation within our analysis. Four soil samples were randomly collected from the subplots with an auger that was sterilized with 70% EtOH between samples. Samples were taken from the mineral soil up to 17 cm and were pooled for analysis (4 fires × 3 plots × 9 subplots × 2 treatments (burned, unburned), Fig. 1b), for a total of 216 soil samples. To ensure homogeneity in sampling in the unburned plots, we manually removed the soil organic layer before sampling the mineral layer, allowing for a direct comparison of the soil fungal communities between the burned and unburned plots. Soil samples were placed in Ziploc freezer bags and stored on ice for 6–24 h before transferring to the lab. All soil samples were sieved through a 2-mm sieve to remove roots and rocks and homogenize before analysis. A subsample from each sample was frozen at −20 °C for DNA extraction, and the rest of the sample was air-dried for soil nutrient analysis.

2.3. *Soil nutrient analysis*

Air-dried soil samples were analyzed for total carbon and total nitrogen by dry combustion with a PerkinElmer 2400 CHN/S Analyzer (PerkinElmer Inc., Waltham, Massachusetts, USA). For each plot, three randomly selected subplots were chosen for total phosphorus analysis following EPA Method 3050A for acid digestion of sediments, sludges, and soils (Edgell, 1989).

2.4. *DNA extraction, amplification, and sequencing*

DNeasy Power Soil Kits (Qiagen Sciences Inc., Germantown, MD, USA) were used to extract DNA from 0.25 g from each frozen soil sample according to the manufacturer's protocol. DNA extractions were PCR-amplified via single PCR reactions, using the primer pair ITS1F/ITS2 (White et al. 1990) to amplify the ITS1 region of the Internal Transcribed Spacer (ITS), the universal barcode for fungi (Schoch et al., 2012). The 5′ end of the ITS1F primer was modified to include the forward Illumina Nextera adapter and two basepairs "linker" sequences (Smith and Peay, 2014). PCR reactions were carried out in 25-ul reaction mixtures, consisting of 2.5 ul of 10x HotstarTaq PCR buffer, 0.5 ul of 2 mM of deoxynucleotide triphosphates (dNTPs), 1.0 ul of 10 uM Illumin-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 30 s at 95 °C, 30 s at 51 °C, 1 min at 72 °C of annealing, and 10 min at 72 °C for extension. For each PCR reaction we used a negative control, a sample without DNA, to exclude contamination of the products, and a positive control composed of a mock community, to ensure the amplification of target DNA. For samples that failed to amplify, DNA concentrations were increased to 5 ul of diluted DNA (10:90). We visualized PCR products with gel electrophoresis before submitting to Vincent J. Coates (VJC) Genomic Sequencing Laboratory at the University of California-Berkeley. The VJC laboratory cleaned the PCR product with AMPURE (Beckman Coulter Inc., Brea, CA, USA), quantified fluorescently with the Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, MD, USA), and pooled at equimolar concentration. Quality check was performed with the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and sequenced with Illumina MiSeq PE 2 × 300. Additional information can be found under Supplementary data 3.

2.5. Bioinformatics

Paired-end demultiplexed Illumina sequence files, containing 4535423 raw sequences in 213 samples were processed using QIIME2, version 2019.10 pipelines (Bolyen et al., 2018; Caporaso et al., 2011). The 5′ primers and adapter sequences were removed by the sequencing facility. The 3′ primer and adapters were removed using the Quiime2 CutAdapt plugin (Martin, 2011). DADA2 was used to filter out and remove chimeric sequences and low-quality regions and produce Amplicon Sequence Variants (ASVs) by truncating the 3′ end to 150 bp (Callahan et al., 2016). The taxonomic analysis was performed by first training a taxonomic classifier using the UNITE reference database (Kõljalg et al., 2005). Taxonomy was assigned using the fitted classifier with the classify-sklearn plugin in Qiime2 (Pedregosa et al., 2011). As a result of single nucleotide differences, taxa are assigned different ASVs, resulting in multiple ASV IDs for the same taxonomic group (Callahan et al., 2017). Hence, we created a high-resolution ASV table with 8585 unique ASVs and a mean sample frequency of 17,165 sequences per sample for total fungi. The selection of specific fungal guilds was performed by parsing the raw ASV table through FUNGuild (Nguyen et al., 2016). Acceptance of FUNGuild assignment for analysis was based on confidence level rankings of "possible," "probable," and "highly probable." When a taxon was classified as "possible" and represented multiple guilds, it was only used for analysis when an online Google Scholar search accounted for at least three citations in which the taxon in question was classified as EcM or saprobic.

2.6. Statistical analysis

All statistical analyses were conducted in R version 3.6.3 (R Core Team, 2020), and significance was accepted at $p < 0.05$. All Figures were made in ggplot2 (Wickham, 2016).

To account for uneven sequencing depth, total fungal tables were rarefied to 6229 sequences/samples. The EcM-only table and saprobe-only table were rarefied 70 and 2200 sequences/sample, respectively, before statistical analysis. Alpha diversity was assessed using observed ASVs as a measure of species richness. Beta diversity was based on Bray-Curtis dissimilarity matrices. In addition, we tested the homogeneity of group dispersion to verify treatment effect versus site effects using betadisper in the Vegan package. Data were tested for normality with the Shapiro-Wilk test and heteroscedasticity by graphical inference via the residuals. To improve the data's normality, total fungal species richness was log-transformed. Moreover, a square-root transformation was applied to the EcM and saprobic species richness to test for both the effects of treatment (burned vs. unburned) and time since fire. The effect of time since fire was tested only on the subset of data representing the burned sites.

To test the effect of high-severity fire on the soil nutrients, including total carbon, total nitrogen, total phosphorus, total carbon: total nitrogen ratios, total carbon: total phosphorus ratios, and depth of the organic matter, we performed a generalized linear regression (GLM) with site as random effect. To determine the effect of time since fire on soil nutrients, we used a generalized polynomial regression (quadratic and cubic polynomials) on the untransformed data, with soil nutrients as the fixed factor and site as the random effect. Additionally, we used a locally weighted smoothing method (Loess) (Cleveland et al., 1990) to capture the non-linear general pattern of the soil nutrient data.

To test the effect of high-severity wildfire on total fungal, EcM, and saprobic species richness, independently, we first tested the level of nestedness required for analysis. The nestedness level was tested by running a null model with no predictors and different nestedness (site, plot, site:plot, and site:plot:subplot). The null models showed that the linear regression for testing the effect of treatment on the total fungal richness was better (lower AIC) when the plot was included as the random effect. The regression for EcM and saprobic species richness included the plot nested within site a random effect. Note that overall results were similar whether we ran the model using site, plot, and plot nested within sites as random effects. Result comparisons can be found in the Supplementary data 1 (Table S1). We compared community composition (beta diversity) across burned and unburned sites and between fire years using Bray-Curtis dissimilarity on square-root-transformed relative abundance data calculated using Vegan's avgdist function (Oksanen et al., 2018). Results were visualized using Non-Metric Multidimensional Scaling (NMDS). To identify the most influential soil nutrient and site variables affecting the composition of EcM and saprobic fungi, we constructed a multivariate PERMANOVA model (Anderson, 2017) with post-hoc-pairwiseAdonis (Martinez, 2019). Moreover, we used the envfit function (vegan package) to understand the influences of both the soil nutrients and the site measurements on community composition. All permutation analyses were restricted to 9999 permutations with site as a random effect.

To test which EcM and saprobic taxa drove the observed differences between burned and unburned plots, we fit a GLM based on the negative binomial distribution using the DESeq function of the DESeq2 package in R (Love et al., 2014). DESeq2 allows us to identify the taxa (ASVs) that differ or respond positively to treatment (burn vs. unburn), allowing us to see which taxa are enriched in the burned sites. We used phyloseq version 1.24.2 (McMurdie and Holmes 2013) to visualize the relative abundance of the taxa driving the compositional differences between treatments and time since fire. Graphs were based on taxa composing above 2% of the total sequence abundance.

Linear mixed-effects models (LMER) in the lme4 package were used to model the relationships between EcM and saprobic alpha diversity (total ASVs) with soil nutrients and site variables. We developed 20 models, including all soil nutrients, site variables, and their respective interactions with treatment (burn vs. unburned). We also tested the null model with no predictors and different nestedness (site, plot, site: plot, and site: plot: subplot) and the full model containing all predictors (Anderson, 2008). By testing the null model with different levels of nestedness, we were able to show that including plot as the random effect for both the EcM and saprobic data accounted for the spatially nested sampling. The models were restricted to maximum likelihood estimation, and model selection was made by backward elimination using Akaike Information Criterion (AIC) (Burnham and Anderson, 2004). The significance of the best-fit model was assessed using ANOVA ($p < 0.05$), and both marginal and conditional R^2 values were calculated using the package MuMIn (Barton, 2020).

To assess the effect of time since fire on saprobic species richness, we used a GLM based on the Poisson distribution with the plot as a random effect. If the data were over-dispersed and the conditional variance was higher than the conditional mean (i.e., EcM species richness and total fungi) (Bliss, 1953; Ross and Preece, 1985), we performed a negative binomial regression, with plot as a random effect for both EcM and total fungi, using the MASS package (Venables et al., 2012). Levels of nestedness were tested using the null model with no predictors. A comparison of the different levels of nestedness can be found in the Supplementary data 1 (Table S2). Although models using site as the random effect for the EcM data resulted in very similar AIC values as when we used plot as the random effect, we decided to proceed with our analysis using plot as the random effect for parsimony with previous analysis. Marginal and conditional R^2 for the models were calculated using the“r.squaredGLMM” function in MuMIn version 1.43.17 (Barton, 2020).

3. Results

3.1. 1 High-severity fire had long-term effects on soil nutrient composition

We found that areas burned at high severity had significantly different soil nutrients and a lower depth of organic matter than the unburned sites (Fig. S1). In contrast to our first hypothesis, we found that these changes were not long-lasting (>11 years; Fig. S2). Overall, in the burned sites, total phosphorous was 69% higher ($p < 0.01$; Fig. S1a, Tables S4, S5), and total nitrogen was 87% higher than the unburned sites ($p < 0.01$; Fig. S1b, Tables S4, S5). In contrast, the ratio of total carbon to total nitrogen was 10% lower ($p < 0.01$, Fig. S1c, Tables S4, S5), the ratio of total carbon to total phosphorus was 37% lower ($p < 0.01$; Fig. S1d, Tables S4, S5), and the depth of organic matter was 82% lower in the burned sites than the unburned sites ($p < 0.01$; Fig. S1e, Tables S4, S5). Although total carbon was lower in the burned sites, 3% (Fig. S1f, Tables S4, S5), there was no statistical difference in total carbon concentrations between burned and unburned sites ($p > 0.05$, Fig. S1f, Tables S4, S5).

Time since fire (age of the fire) also significantly affected the soil nutrients (Fig. 2, Tables S4, S6). Overall, at 11 years post-fire, total phosphorous was 16% higher (Fig. 2a), and the depth of organic matter was 312% higher than at 2 years post-fire (Fig. 2b). In contrast, the ratio of total carbon to total nitrogen was 3% lower (Fig. 2c), the ratio of total carbon to total phosphorous was 73% lower (Fig. 2d), total carbon, 65% (Fig. 2e), and total nitrogen 63% lower at 11 years when compared to 2 years post-fire (Fig. 2f, Tables S4, S6). However, when the soil nutrients were compared to the unburned sites and time since fire (Fig. S2), only the depth of organic matter experienced significant long-lasting effects that did not reach levels comparable to unburned sites after 11 years post-fire (Fig. S2b). In contrast, the ratio of total carbon to total nitrogen (Fig. S2c) and total nitrogen experienced severe declines in the burned sites, but reached levels comparable to unburned sites by 11 years (Fig. S2c, f).

3.2. High-severity wildfire reduced species richness and altered community composition

After rarefying the data to 6229 sequences/sample for total fungi, 8464 ASVs were identified across the study area, with 866 ectomycorrhizal fungal ASVs and 2850 saprobic fungal ASVs. We found no significant change in total fungal species richness between burned and unburned sites ($p > 0.05$; Fig. S3a, Tables S7/8). Although total fungal species richness varied with time since fire and was lower on the older fires (11 years), results were not significant ($p > 0.05$, Fig. S3b, Table S9). In support of our hypothesis, we found that sites burned at high severity had lower EcM species richness than the unburned sites. A total of 132 unique EcM ASVs were assigned to the burned sites, and 287 to the unburned sites, with 89 ASVs shared between treatments. On average, EcM species richness was 43.4% lower in the burned sites ($p = 0.01$; Fig. 3a; Tables S7/8). In contrast, a total of 893 unique saprobic ASVs were identified across the burned sites, and 973 ASVs in the

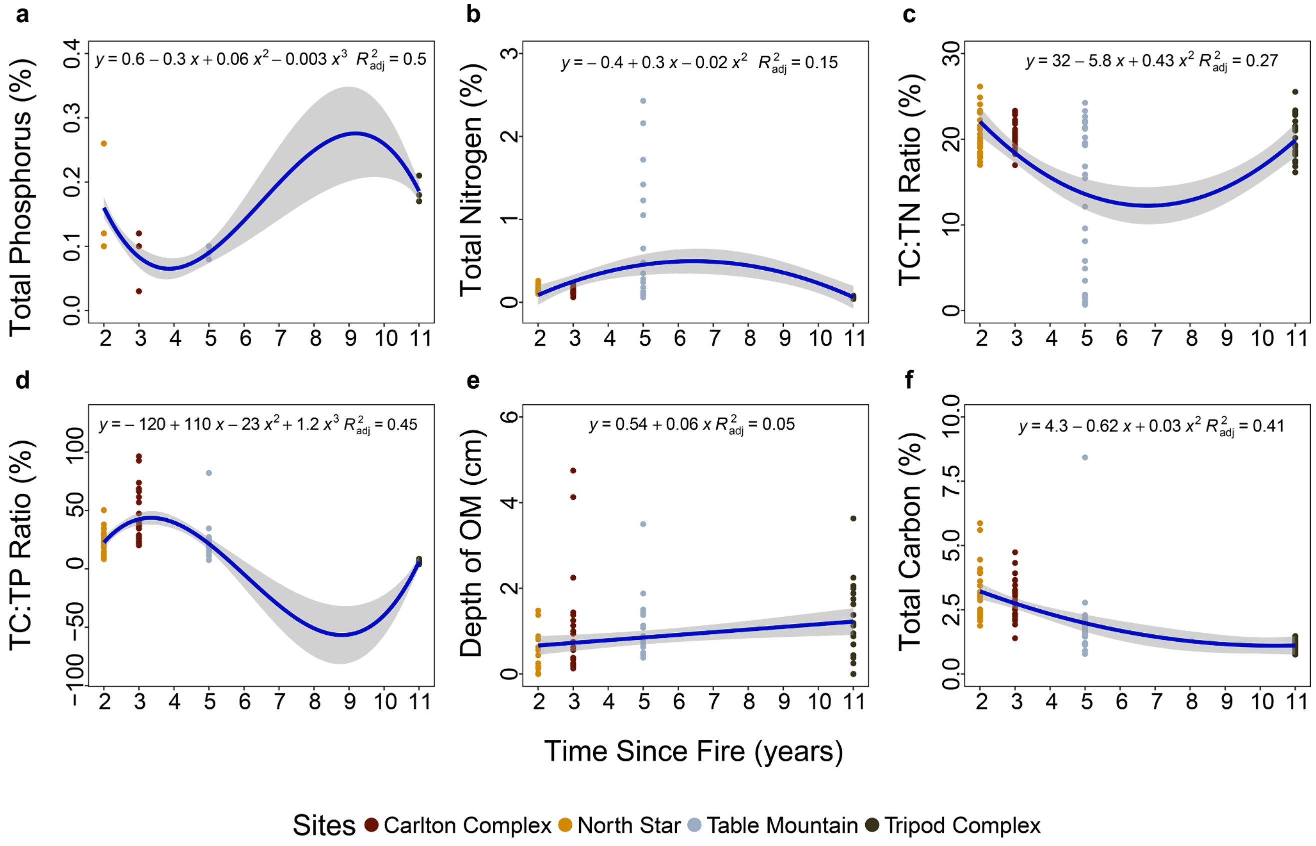

Fig. 2. Generalized linear regression (GLM) between time since fire and percentage of nutrients — total carbon, total nitrogen, and total phosphorus, and the ratio between total carbon (TC) to total nitrogen (TN) and total carbon to total phosphorus (TP) and the depth of organic matter (OM). Points represent the raw data, and lines are loess smooth regressions based on a polynomial generalized linear regression.

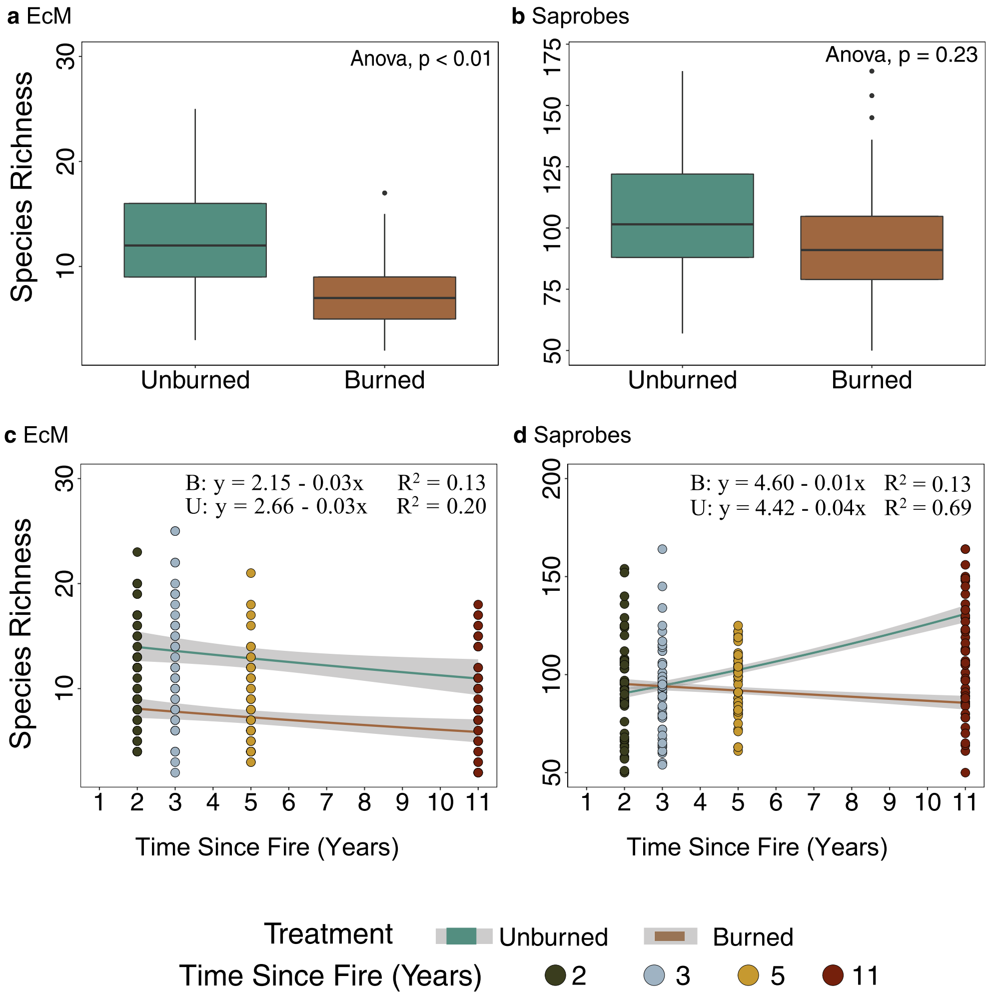

Fig. 3. Observed ASV richness, species richness, between treatment (top panels) and with time since fire (bottom panels) for all four fires— Tripod Complex fire (2006, 11-years); Table Mountain fire (2012, 5 years); and Carlton Complex fire (2014, 3 years) and the North Star fire (2015, 2 years). Points represent untransformed data and gray: 95% confidence intervals. Significance—significance $p < 0.05$, is based on a negative binomial regression for EcM fungi and Poisson regression for saprobic fungi, where B = burned and U = unburned.

unburned sites, with a total of 731 ASVs shared between treatments. Overall saprobic species richness was 12.2% lower in the burned sites, but results were not significantly different than the unburned sites ($p > 0.05$; Fig. 3b; Tables S7/8). Nonetheless, species richness for both EcM ($p = 0.01$, Fig. 3c) and saprobic fungi ($p < 0.01$, Fig. 3d) were affected by the age of the fire, where species richness was lower in the older fires (11 years; Table S7). Post-hoc analysis indicates that the changes with time since fire for both EcM and saprobic fungi were significant only when comparing species richness between the 2 year burned sites and the older sites, 11 years ($p < 0.03$, Fig. 3c/d; Table S9). In addition, total fungal community composition ($p < 0.01$, Fig. S4), EcM ($p < 0.01$, Fig. 4a), and saprobic fungal community composition ($p < 0.01$, Fig. 4b) differed between burned and unburned sites. Analysis of homogeneity further suggests a significant site effect ($p < 0.01$), where communities from each site display similar dispersal.

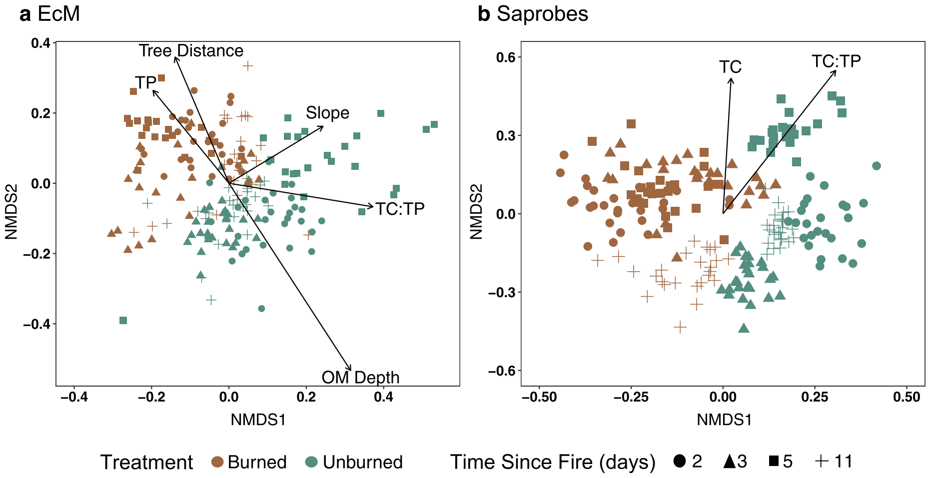

Fig. 4. NMDS plot of a) ectomycorrhizal (EcM) and b) saprophytic community assemblages per treatment (brown: burned; blue-green: unburned) on three-dimensions. Based on the Bray-Curtis dissimilarity matrix, the stress level of 0.1411 for EcM and 0.1246for saprobes. Points closer together in the ordination space have more similar community assemblages. Only significant ($p < 0.05$) soil nutrients—total carbon (TC), and total phosphorus (TP), and the TC: TP ratios and site variables —distance to the tree (*P.ponderosa*), slope, depth of organic matter (OM). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. A few taxa showed dominance in the post-fire environment

Per our third hypothesis, Basidiomycetes were lower in the burned sites, including the genera *Russula*, which was 92% lower, *Piloderma* (96%), *Hygrophorus* (100%), and *Inocybe* (43%) ($p < 0.01$; Fig. 5a, Table S10), leading to the dominance of pyrophilous Ascomycetes (Fig. 5a, Table S10). In particular, genera that were cryptic or rare in the unburned sites ($<2\%$ relative abundance) dominated the burned environment, including the genera *Pustularia,* which made up 15% of the relative abundance and *Chromelosporium* (16%; $p < 0.01$; Fig. 5a, Table S10). Interestingly, the genera *Wilcoxina*, specifically *W. rehmii,* dominated both the burned and unburned sites. On average, *W. rehmii* composed 19% of the relative abundance in the unburned sites and 24% in the burned sites (Table S10). Overall, *W. rehmii* was 29% higher in the burned sites (Fig. 5a; Table S10). However, the change in relative abundance of *W. rehmii* was not statistically significant ($p > 0.05$; Table S10), suggesting the potential resistance of *Wilcoxina* to high-severity wildfire and the post-fire altered environment. These results were corroborated by Deseq analysis, which revealed the enrichment of 9 taxa (Fig. 6a, Table S11; Fig. S5) that responded positively to the burned sites. These fire responders included taxa belonging to established pyrophilous Ascomycetes in the family Pezizaceae, genera *Chromelosporium,* and *Peziza,* and the family Pyronemataceae, genera *Wilcoxina,* and *Pustularia* ($p < 0.05$; Fig. 6a, Table S11; Fig. S5). The unburned environment was enriched by Basidiomycetes ($p < 0.05$; Fig. 6a, Table S11; Fig. S5).

In contrast to the EcM fungal community, the saprobic fungi which dominated the unburned sites also dominated the burned community, including the genera *Archaeorhizomyces* which made up *15%*, *Geminibasidium* (16%), and *Penicillium* (11%) of the relative abundance in the burned sites (Fig. 5b, Table S10). We observed the appearance of cryptic taxa in the burned sites, including two Ascomycetes in the genera *Rasamsonia* and *Calyptrozyma* (Fig. 5b, Table S10), each making up 3% of the mean relative abundance. Additionally, a Basidiomycete, *Basidioascus,* was 4897% higher in the burned sites than in the unburned sites, making up, on average, 4% of the relative abundance ($p < 0.01$; Fig. 5b, Table S10). Deseq supported these results and showed the enrichment of 41 genera that responded positively to the burned sites compared to the unburned sites (Fig. 6b, Table S11; Fig. S5), including taxa in the genera *Tephrocybe, Ochrocladosporium, Geminibasidium, Rasamsonia Pyronema,* and *Basidioascus* ($p < 0.01$; Fig. 6b, Table S11; Fig. S5).

3.4. Soil environmental drivers of EcM and saprobic fungal communities

Both EcM and saprobic fungal communities differed significantly between burned and unburned sites and with time since fire (age of the fire) (Fig. 4). PERMANOVA analysis indicated that time since fire imposed the largest effect on community composition, accounting for 6% of the variation in the EcM and 11% in the saprobic community composition ($p < 0.01$, Fig. 4, Table S12). Additionally, treatment (burned vs. unburned) accounted for 5% of the EcM and 7% of the saprobic community variation ($p < 0.01$; Fig. 4; Table S12), and aspect accounted for 3% of EcM and 6% of the saprobic community variation ($p < 0.01$; Fig. 4; Table S12). Interestingly, the interaction between time since fire and treatment accounted for 3% of both the EcM and saprobic community composition ($p < 0.01$; Fig. 4a; Table S12).

In support of hypothesis 4, long-term changes (>11 years) in EcM and saprobic community composition were correlated with post-fire changes in soil nutrients and site variables (Fig. 4). The depth of organic matter had the most significant effect on the composition of the EcM community, accounting for 38% of the EcM variation ($p < 0.01$, Fig. 4a, Table S13). In addition, the distance to nearest tree accounted for 15% ($p < 0.01$), total phosphorus for 11% ($p < 0.01$) and slope for 9% ($p = 0.04$) of the EcM community variation (Fig. 4a; Table S13). Interestingly, the ratio between total carbon and total phosphorus was associated with both the EcM and saprobic community, accounting for 14% of the EcM variation ($p < 0.01$, Fig. 4a, Table S13) and 39% of the saprobic variation ($p = 0.03$, Fig. 4b, Table S13). In addition, total carbon explained 27% of the variation in the saprobic community ($p = 0.02$; Fig. 4b; Table S13).

Ectomycorrhizal and saprobic species richness followed a similar pattern to those observed in the community composition (Table 1). Treatment, total nitrogen, and the interaction between treatment and total carbon and the depth of organic matter negativelyaffected EcM species richness ($p < 0.01$; Table 1). In contrast, the depth of organic matter ($p = 0.06$), total carbon, and the interaction between treatment and total nitrogen ($p < 0.01$; Table 1) imposed a positive effect on EcM species richness. Saprobic species richness was significantly correlated to treatment ($p = 0.1$), time since fire ($p < 0.01$), and total carbon ($p = 0.03$). Interestingly all variables imposed a positive effect on saprobic richness, except for the interaction between treatment and time since fire ($p < 0.01$; Table 1), which negatively affected species richness.

3.5. Species richness declined with increased time since fire

Consistent with our last hypothesis, we found that an 11-year post-fire period is not enough time for EcM species richness to converge to comparable unburned sites (Fig. 3c). EcM richness was significantly lower in the older sites (11 years post-fire; $p < 0.01$, pseudo $R^2 = 0.13$; Fig. 3c). However, the effect of time since fire on EcM fungi was group-dependent (Fig. 7a, Tables S14, S15). In the burned sites, there was a significant and gradual decrease of pyrophilous taxa when the older sites (11 years) were compared to the newer sites (2 years). *Pustularia* and *Chromelosporium* were both 95% lower in the older sites ($p < 0.01$). Although *Wilcoxina* was 81% lower at the intermittent burned sites (five

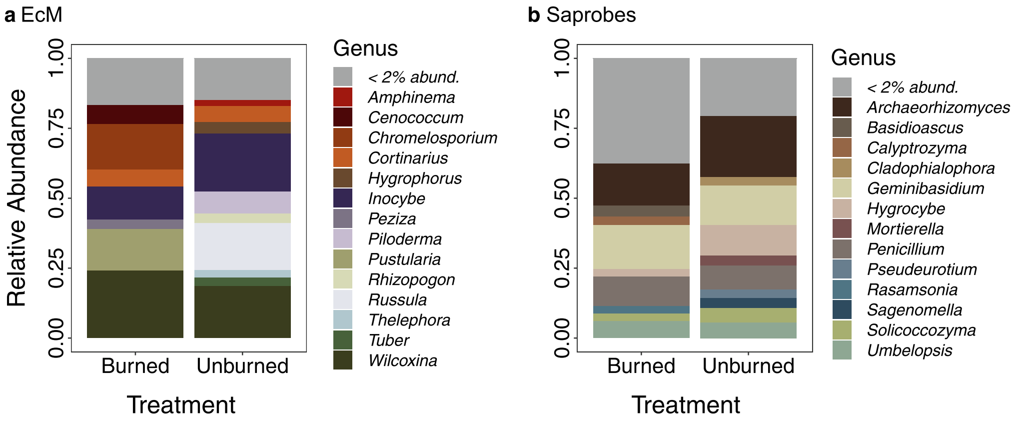

Fig. 5. Relative abundance at the genus level between burned and unburned sites for a) ectomycorrhizal (EcM) fungi and b) saprobic fungi.

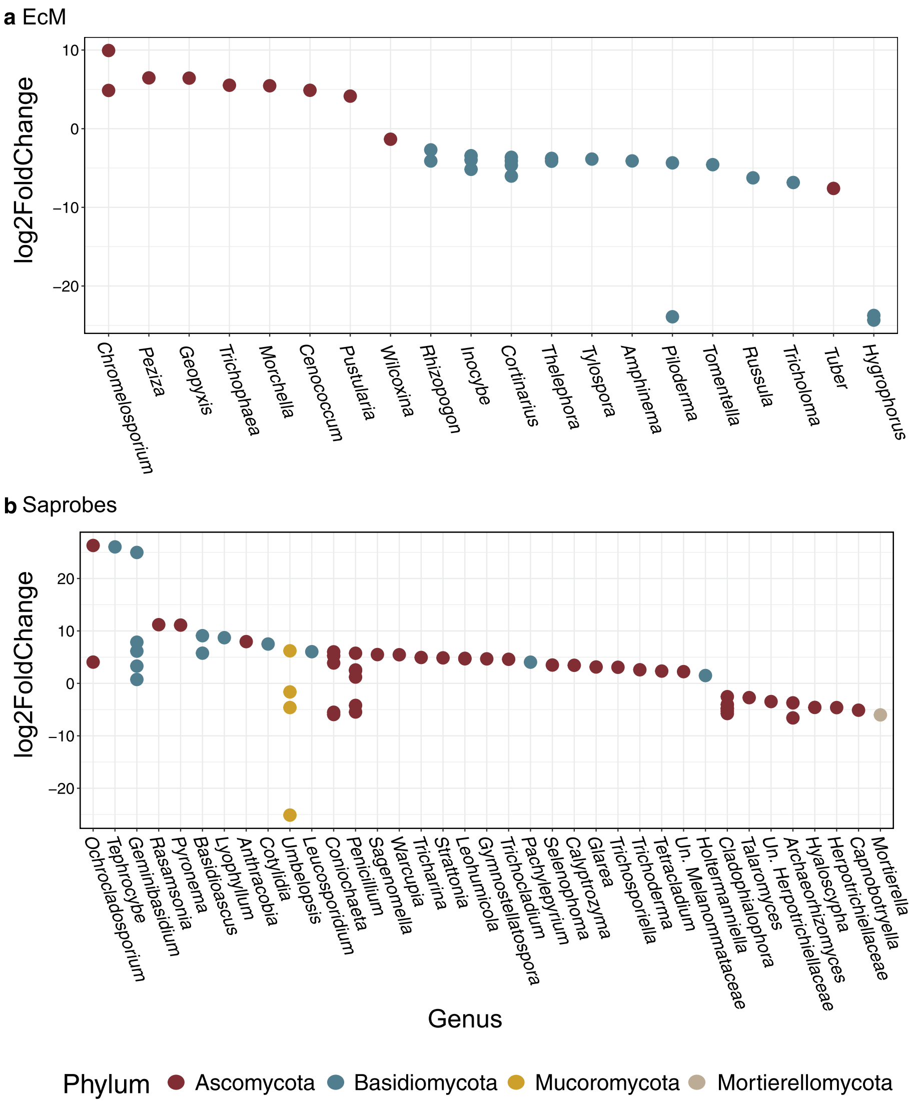

Fig. 6. Differential representation of significantly abundant ASV's between burned and unburned sites based on a negative binomial regression at the genus level. Dots represent the taxa, colored by phylum, and labeled by genus. Multiple points per genus represent different species of the given genus. Positive values indicate a positive response to fire. Negative values represent a negative response to fire and a higher representation in the unburned sites. Only the probabilities with a differential abundance at p.adj < 0.05 (EcM) and p.adj < 0.001 (Saprobes) and a log2fold change above zero are included (Table S11). Significance based on nwald, likelihood ratio.

years), relative abundance at 11 years was 159% higher than the 2 year post-fire sites (Table S14). Overall, at 11 years post-fire, *Wilcoxina* abundance was only 27% lower in the burned sites than the unburned sites ($p < 01$; Fig. 7a; Table S14). Interestingly the EcM fungal genera *Cortinarius* which composed 3% of the relative abundance at post-fire year 2, increased with time since fire (Fig. 7a); on average, it was 208% higher at year 11. However, these results were not statistically significant ($p > 0.05$; Fig. 7a, Tables S14, S15).

In contrast with our hypothesis, we found that saprobic species richness did not converge to levels comparable to the unburned sites (Fig. 3d). We found that saprobic species richness follows a similar trajectory as EcM fungi, decreasing with increased time since fire. Changes in time since fire significantly affected specific members of the saprobic fungal community (Fig. 7b, Tables S14, S15), including *Basidioascus* which was 98% lower ($p = 0.01$), *Pyronema* (98%; $p < 0.01$), *Rasamsonia* (100%; $p < 0.01$), *Coniochaeta* (59%, $p = 0.04$), and *Penicillium* (43%; $p < 0.01$) in the older sites (11 years) (Fig. 7b, Tables S14, S15). Interestingly, *Rasamsonia* and the well-known pyrophilous fungi, *Pyronema*, were two of the most abundant taxa in the burned environment at years 2 and 3, but by year 5, they had almost completely disappeared, making up <2% of the relative abundance (Fig. 7b, Tables S14, S15).

4. Discussion

4.1. Areas burned at high-severity had lower EcM species richness and an altered EcM and saprobic community composition

In this study, we sequenced fungal DNA gene amplicons from an 11-year chronosequence of high-severity burn *P. Ponderosa* stands to analyze the response of fungi during shifts in fire regimes, from historically low-severity fires to high-severity wildfires. We found that areas impacted by high-severity wildfires had lower species richness and altered community composition than unburned sites, similar to (Cowan et al., 2016; Dove and Hart, 2017; Glassman et al., 2016; Kipfer et al., 2011; Olsson and Johnson, 2010). Moreover, the differences in EcM species richness persisted for over eleven years, consistent with studies from (Kipfer et al., 2011; Kurth et al., 2013; Pérez-Valera et al., 2018; Treseder et al., 2004) who showed that fungal communities require 15–18 years to return to unburned conditions. Furthermore, we found that differences in species richness were larger for EcM than saprobic fungi, as previously shown by (Yang et al., 2020). Although we found no significant difference in saprobic species richness between the burned and unburned sites, we did show that the older burned sites contained lower species richness than the newly burned sites (11 years vs. 2 years). These results are in contrast with (Kurth et al., 2013), who found that saprobic communities recovered after nine years. However, this study

Table 1

Table of the mixed linear regression results from stepwise AIC for ectomycorrhizal and saprobic fungi with soil nutrients— total carbon (TC), total nitrogen (TN), and total phosphorus (TP) and TC: TN ratios and the depth of soil organic matter (OM) and treatment (burned vs. unburned) and time since fire (TSF) (in years). The base level for treatment is designated as burned. The final model is depicted by an asterisk as selected by AIC.

Ectomycorrhizal						
		Mod 13*			**Mod 20**	
Predictors	*Estimates*	*Statistic*	*p-value*	*Estimates*	*Statistic*	*p-value*
(Intercept)	14.53	11.39	**<0.01**	16.07	11.35	**<0.01**
Total carbon: total phosphorus	−1.12	9.59	0.09			
Treatment [Burned]	−5.48	−3.12	**<0.01**	−6.36	−3.97	**<0.01**
Time Since Fire	−0.26	−2.66	0.01	−0.27	−2.81	**<0.01**
Depth organic matter	0.31	1.91	**0.06**	0.32	1.98	0.05
Total carbon	4.18	2.76	**<0.01**	3.05	2.22	**0.03**
Total phosphorus	−10.57	−1.17	0.26			
Total nitrogen	−103.46	−3.67	**<0.01**	−99.46	−3.54	**<0.01**
Trt [Burned] * Total nitrogen	104.49	3.71	**<0.01**	100.76	3.59	**<0.01**
Trt [Burned] * Total carbon	−4.14	−2.82	**<0.01**	−3.48	−2.45	**0.02**
Trt [Burned] * Depth organic matter	−1.5	−2.73	**<0.01**	−1.67		
Random Effects						
σ^2		15			15.1	
τ_{00}		0.00_{Plot}			0.00_{Plot}	
Mar R^2 / Cond. R^2		0.451/ NA			0.44 / NA	
Saprobes						
		Mod 13*			**Mod 20**	
Predictors	***Estimates***	***Statistic***	***p-value***	***Estimates***	***Statistic***	***p-value***
(Intercept)	57.05	5.67	**<0.01**	63.26	5.94	**<0.01**
Treatment [Burned]	15.46	1.78	**0.09**	18.45	1.99	**0.06**
Time Since Fire	4.51	4.77	**<0.01**	4.17	4	**<0.01**
Depth organic matter	1.02	1.12	0.26	0.93	1	0.32
Total carbon: Total nitrogen	−3.98	−1.15	0.26			
Elevation	2.85	1.13	0.28	4.03	1.43	0.17
Total carbon	5.91	2.28	**0.03**			
Total phosphorus	21.32	0.33	0.75	106.7	1.76	**0.09**
Slope	0.41	0.83	0.42	0.54	0.98	0.34
Trt [Burned] * Time since fire	−5.5	−4.53	**<0.01**	−6.55	−5.25	**<0.01**
Total nitrogen						
Total carbon: Total phosphorus				2.59	1.21	0.23
Random Effects						
σ^2		395.7			395.39	
τ_{00}		21.73_{Plot}			41.17_{Plot}	
Mar. R^2 / Cond. R^2		0.33 / 0.36			0.31 / 0.37	

examined the impact of stand-replacing wildfires on wood-inhabiting saprobes, thus missing the potential alterations to the soil saprobic community. Additionally, in our study, we observed a slight decrease in EcM fungal richness with time since fire, consistent with previous research (Bellgard et al., 1994; Bentivenga and Hetrick, 2011). The decrease in species richness in the older fires suggests that the lack of post-fire seedling regeneration and the large, high severity burn patches that are isolated from surviving trees (Owen et al., 2019) have a high control over the recovery of EcM fungi. Given that *P. ponderosa* is adapted to low-severity, high-frequency wildfires (Agee, 1993) suggest that their soil organisms have evolved under the same selective pressures and may lack the evolutionary adaptations to thrive after high-severity wildfires. Studies of this hypothesis are lacking, and future research should explore whether soil fungal communities are adapted to their host's fire regimes and to what extent they can adapt to the changes in the fire regimes.

4.2. Ascomycetes dominated the EcM burned communities

Similar to previous studies (Bruns et al., 2019; Cairney and Bastias, 2007; Fujimura et al., 2005; Reazin et al., 2016; Smith et al., 2004; Visser, 1995; Yang et al., 2020), we observed a shift in dominance in the burned sites, from Basidiomycete- to Ascomycete-dominated. The emergence and dominance of *Chromelosporium* and *Pustularia*, both rare in the unburned sites, supports previous findings that cryptic and rare taxa tend to dominate the burned environment (Bruns et al., 2019; Fujimura et al., 2005; Horton and Bruns, 1998; Hughes et al., 2020; Smith et al., 2004; Visser, 1995). The most dominant species in both the unburned and burned environments was *W. rehmii*, a well-known symbiont of *P. ponderosa* (Fujimura et al., 2005; Glassman et al., 2015; Smith et al., 2005) that is likely to be resilient to high-severity fires. *Wilcoxina rehmii* is a common Ascomycete in Ponderosa stands (Fujimura et al., 2005) that can survive artificial heating (Peay et al., 2009) and low-severity prescribed fires (Smith et al., 2005). In the absence of competition, it is possible that *W. rehmii* can rapidly colonize the disturbed soil where competition pressures have been reduced. It is also possible that *W. rehmii* is an efficient competitor: in the presence of pines, *W. rehmii* may act as an ectendomycorrhiza and penetrate the host's roots intracellularly (Egger, 1996; Yu et al., 2001). Ectendomycorrhizal associations have been suggested to allow fungi to utilize various carbon sources, including carbon from the dead host (Raudabaugh et al., 2020; Yu et al., 2001). Therefore, in an environment where close to 100% of the hosts have died, the ability to penetrate the host cells may increase the survival of *W. rehmii*, regardless of the lack of photosynthetically derived carbon in the soil matrix. Various pyrophilous taxa, such as *Geopyxis delectans* and some taxa in the genera *Morchella,* and *Peziza* have been identified as endophytes with some vascular plants (Baynes et al., 2012; Raudabaugh et al., 2020). The ability to colonize their host in a non-mycorrhizal manner could allow these genera to survive fire events through small-scale refugia (Baynes et al., 2012; Raudabaugh et al., 2020), allowing them to rapidly dominate the burned environment where competition levels have been reduced (Moore, 1962; Seaver, 1909). Furthermore, *Pustularia*, one of the most common EcM fungi in the two year burned sites, and *Wilcoxina* both have contact and short-distance exploration types (Agerer, 2001). These types have a broad environmental range (Rosinger et al., 2018) and a preference for the

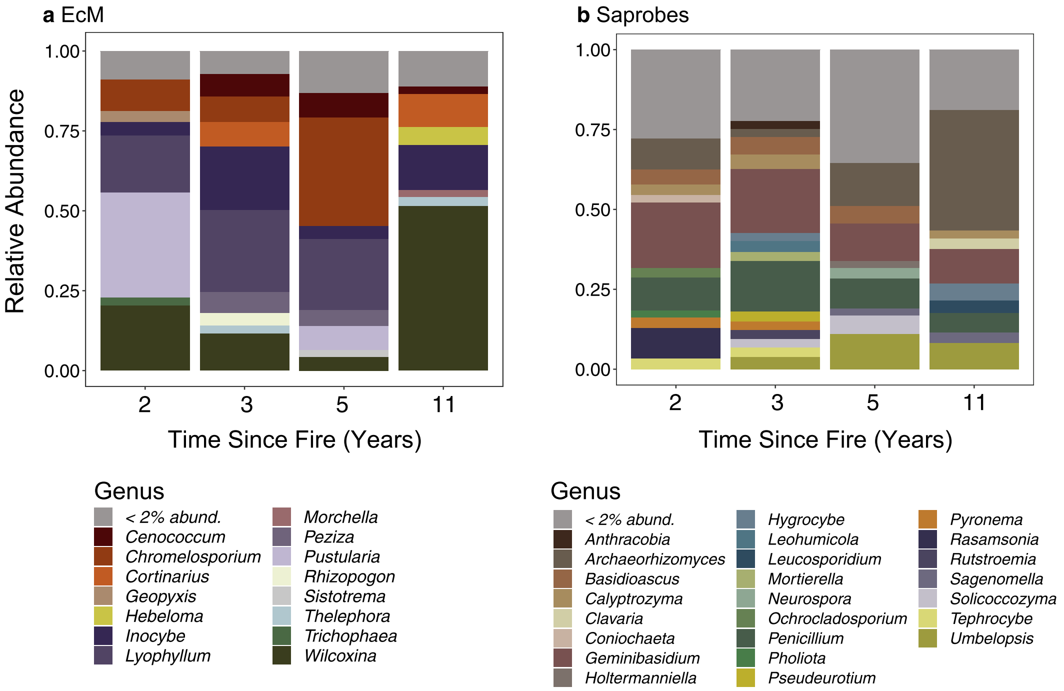

Fig. 7. Relative abundance at the genus level between fire years— Tripod Complex fire (2006, 11-years); Table Mountain fire (2012, 5 years); and Carlton Complex fire (2014, 3 years) and the North Star fire (2015, 2 years) in burned sites of (a) ectomycorrhizal fungi (EcM) and (b) saprobic fungi. Data for unburned sites are in Supplementary data 2, Fig. S6.

uptake of ammonium and nitrate (Hobbie et al., 2009), giving them an advantage in the burned environment, where ammonium and nitrate typically increase (Wan et al., 2001). However, in this study, we only measured total nitrogen, so we cannot state if and how these post-fire fungi respond to changes in other forms of nitrogen.

Earlier studies have classified post-fire taxa as pyrophilic fungi due to their ability to survive wildfires (El-Abyad and Webster, 1968; McMullan-Fisher et al., 2011; Petersen, 1985; 1970;; Raudabaugh et al., 2020; Seaver, 1909). Most of the dominant taxa in this study belong to the Pezizales order, which can produce large amounts of resistant propagules such as spores (Baar et al., 1999; Taylor and Bruns, 1999) and sclerotia (Moore, 1962; Smith et al., 2015). Although the dominant taxa may be resistant to high-severity wildfires, our study took place two years post-fire; therefore, we lack the resolution to make this statement. Additionally, it is possible that the dominant taxa could have dispersed into the burned area, and due to their morphological attributes, including exploration type (Agerer, 2001) and melanin production (Fernandez and Koide, 2013), they were able to survive the harsh, post-fire environment (Dickie and Reich, 2005). The removal of the aboveground vegetation and soil organic matter makes the post-fire environment a harsh system where fungi have to thrive under increased solar radiation and low soil moisture (Marcolin et al., 2019). Various pyrophilous fungi possess highly melanized cell walls or mantles (Koide, Fernandez, and Malcolm, 2014; Fernandez et al., 2016; Cordero and Casadevall, 2017). We speculate that this trait could be a morphological adaptation to fungal taxa associated with hosts that both experience frequent wildfires and are adapted to drier environments, such as *P. ponderosa*. In addition, melanin production in some EcM fungi, such as *Cennococum geophilum,* contribute to water stress tolerance (Fernandez and Koide, 2013). In our study, we saw the emergence of *Cennococum geophilum* three years post-fire. Given the observed lack of regeneration in these sites, we conclude that adaptations to the harsh, high severity burned environment are an important factor controlling EcM fungal community composition and dominance.

4.3. Basidiomycetes dominated the saprobic burned communities

In contrast to the EcM fungal communities, the saprobic communities were dominated by the phylum Basidiomycota, including pyrophilous fungi in the genus *Geminibasidium*, and *Penicillium*, similar to previous studies (Cowan et al., 2016; Whitman et al., 2019) and one Ascomycete in the genus *Archaeorhizomyces*. *Penicillium* species have been found to occur in higher abundance in areas burned at high-severity (Mikita-Barbato et al., 2015); thus, these genera might be taking advantage of the increased nutrients in the soil matrix (Whitman et al., 2019). *Archaeorhizomyces, Geminibasidium,* and *Basidioascus* are considered widely distributed fungi in the soil worldwide (Hewelke et al., 2020; Nguyen et al., 2015; 2013). Therefore, these genera might be adapted to various environments and can easily colonize disturbed soils where competition pressures have been reduced. Both *Geminibasidium* and *Basidioascus* are also xerotolerant and heat-resistant (Nguyen et al., 2013), making them capable of surviving high-severity wildfires and the harsh post-fire environment. Given that *Basidioascus* was previously rare (<2% relative abundance in the unburned sites), and that it was not detected in the oldest burned sites after five years, suggest that *Basidioascus* might be obligate pyrophilous fungi (Moser, 1949; Petersen, 1970), which are known to fruit exclusively on burned soil (El-Abyad and Webster, 1968; Seaver, 1909). Laboratory evidence using heat-treated soil has shown the heat resistance of *Basidioascus* (Nguyen et al., 2013), and *Basidioascus* has consistently been found in burned soils (Nguyen et al., 2013; Smith et al., 2017). However, the ecological role of *Basidioascus* and whether it is, in fact, an obligate pyrophilous fungus that only fruits on burned soils has yet to be determined

4.4. Long term changes to the soil nutrient affect post-fire EcM and saprobic communities

Consistent with (Cowan et al., 2016; Johnson and Curtis, 2001; MacKenzie et al., 2004; Neary et al., 1999), we found that wildfire leads to significant effects on the soil nutrients and the soil organic matter. The long-lasting alterations to nitrogen may be due to the loss of vegetation cover and, thus, the loss of annual foliage and branch inputs to the soil (Busse and Gerrard, 2020; Wilson and Maguire, 2009). Although we observed fluctuation of soil nutrients between the recently burned sites and the older sites (with time since fire), and although soil organic matter accumulation can contribute to soil nutrient accumulation, all burned sites were significantly low in organic matter and no significant new accumulation occurred with time since fire. On average organic matter depth was between 0 and 4.75 cm in the burned sites, which could notably influence the changes in soil nutrients. Post-fire increases in total phosphorus (Neary et al., 1999) have also been attributed to changes in pH via ash deposition (Xue et al., 2014). In this study, we did not measure ash depth or changes in soil pH; therefore, whether the higher levels of total phosphorus in the older sites are associated with a change in soil pH is undetermined. We suggest that future studies measure depths of ash deposition and changes in soil pH, as this has been shown to affect EcM fungi (Glassman et al., 2017b).

Although heat-induced mortality is likely the initial driver of change in fungal communities, the long-term effects on soil nutrients and the removal of aboveground vegetation and organic matter were important predictors of EcM and saprobic species richness and composition in the burned sites, similar to (Buscardo et al., 2011; Dickie and Reich, 2005; Owen et al., 2019; Vašutová et al., 2017). The most important driver for the EcM community was the depth of the organic matter. The soil organic layer contains the largest diversity of EcM (Courty et al., 2008; Jacobs et al., 2018; Neary et al., 1999) and saprobic fungi (Baldrian, 2017; Neary et al., 1999). Therefore, removing the organic layer during high-severity wildfires (Neary et al., 2005) leads to direct mortality of the soil fungal communities (Hart et al., 2005), thus selecting fungal species with fire adaptations and reducing overall species richness. We found no significant accumulation of organic matter in the burned sites, supporting the importance of the organic layer for restoring soil fungal communities. These findings support the importance of preventing high-severity wildfires because these fungi are not adapted to such intense heat and will not survive, resulting in a complete fungal turnover. An important caveat of this study is that we analyzed the differences in the mineral soil. Therefore, the effects of high-severity wildfire on the soil fungal communities, especially the saprobes, and their environmental drivers might be underestimated, as we did not sample the organic layer.

We found a high correlation between soil nutrients (total phosphorus and the ratio between total carbon and total phosphorus) and distance to the nearest *P. ponderosa* and the EcM community. Previous research has shown that fungal richness and diversity are higher near large (Glassman et al., 2017a) and mature trees (Cline, Ammirati, and Edmonds 2005), and studies have shown that dying trees transfer resources to nearby trees (Song et al., 2015). However, how long after death a host can release photosynthates to its surviving symbionts, or how long EcM fungi can survive on a deceased host, is not understood. Further research in this area could help develop post-fire management strategies as to when and where to inoculate seedlings for manual transplants and when and if we should increase post-fire retention of snags during post-fire harvesting. Ectomycorrhizal and saprobic fungi are critical drivers of the nutrient cycle; thus, they are inextricably linked to the soil environment. We found that this is specifically true for EcM species richness. Ectomycorrhizal fungal richness was significantly predicted by soil attributes, including total carbon, total nitrogen, and their respective interactions with wildfire, as shown previously (Gehring et al., 1998; Vašutová et al., 2017). Ectomycorrhizal fungi depend on their host for photosynthetically derived carbon (Smith and Read, 2009). Consequently, the removal of their host can significantly impede EcM growth and increases secondary EcM mortality. Furthermore, total nitrogen was a significant predictor of EcM species richness in agreement with previous studies showing that increase nitrogen leads to decreased EcM fungi (Hasselquist et al., 2012; Lilleskov et al., 2002). In contrast to EcM richness, saprobic richness was negatively associated with the interaction between time since fire (age of the fires) and treatment, suggesting that saprobesrespond to the high availability of decomposable material in the two-year burned sites. However, we did not measure biomass or stand density within our sites. We believe that future studies could benefit from including wildland measurements within their analysis, such as measurement of down logs, and dead woody fuel size classes, as they can potentially be used to understand the changes in the post-fire saprobic communities.

In this study, we aimed to consolidate the effects of high-severity wildfires on the soil fungal communities and address their changes with time since fire (succession) to determine if the effects were long-lasting. Although we were able to accomplish most of our goals, our study was not without limitations. A critical aspect of this study was the use of a chronosequence, substitute space for time, to address long term effects and succession. However, one of our fire sites (Table Mountain fire) was geographically distant from the other three fires, and although we maintained homogeneity in site characteristics (vegetation, elevation, soil, etc.), we did observe site effects on beta diversity. Nonetheless, we controlled for potential site effects in our statistical analysis by providing a random effect. In addition, our paired burned and unburned plots varied in distance with one another, where the NorthStar fire was 11 km away from its paired burned plots. However, we feel that having controlled for similar characteristics in the sites and environmental variables allows us to make comparisons between treatment and between the wildfires (time since fire).

4.5. Conclusion

In conclusion, our results indicate that high-severity wildfires have long-lasting impacts on the fungi of *P. ponderosa* forests, with both EcM and saprobic fungi requiring>11 years for both richness (EcM) and composition (EcM and saprobic fungi) to return to unburned levels. We suggest that these *P. ponderosa* forests are still under the direct and indirect stressors imposed by the wildfire, as evidenced by persistent changes in soil chemistry, fungal richness, and fungal composition. We found that *W. rehmii* (EcM) and *Geminibasidium* (saprobe) were the most dominant taxa in both the unburned and burned sites, showing the innate high capacity of dominant members of the soil EcM and saprobic community for resilience, as was shown in a *P. ponderosa* forest in California (Glassman et al., 2016). Since *W. rehmii* is known to associate with *P. ponderosa* and survive long-term alterations to the soil environment, *W. rehmii* may contribute to post-fire restoration management. We conclude that high-severity wildfire is the primary driver of changes to soil fungal communities in low-severity fire-adapted *P. ponderosa* forests. Thus, restoration of both the EcM and saprobic fungal communities after high-severity wildfires is linked to the recovery of the soil environment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Accessibility

The data that support the findings of this study (statistical analysis) are openly available in GitHub at https://github.com/pulidofabs/Fungi-PonderosaPine.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2021.118923.

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