**INTRODUCTION**

**METHODS**

Sampling spanned fire affected and fire unaffected areas in two mountain ranges, the Santa Catalina Mountains (SCM) and the Pinaleno Mountains (PM). Both ranges are located at approximately the same latitude (Figure 1; Table 1) (Shreve 1919). Located approximately 150 km apart, the SCM and PM have similar orientations, are similarly composed predominantly of gneiss and granite, and have similar plant communities (Shreve 1919; Shreve 1922). Portions of both ranges experienced severe wildfires between 2002 and 2004. In the PM, the 2004 Nuttail-Gibson complex burned both the northern and southern portion of the range affecting a total of 120.29 km2, and, in 1996, the Clark peak fire burned 26 km2 (USDA Forest Service, 2013). Prior to these two most recent burns, low severity fires were frequent, occurring approximately every 4.2 years (Grissino-Mayer et al. 1995). In the SCM, two fires—the 2002 Bullock fire and the 2003 Aspen fire—burned 123.7 km2 and 343 km2, respectively (Iniguez et al. 2008). These shared traits make the ranges optimal for a comparison study of the effects of a recent fire history on ectomycorrhizal communities associated with *Pinus ponderosa*.

**Sampling**

Sampling encompassed two fire affected (FA) and two fire unaffected (FU) sites in each range (total 8 sites). In the SCM, we sampled two FU sites in 2014 (six trees per site; total 12 trees) and two FA sites in 2016 (five trees per site; total 10 trees) (Table 1). We reduced sampling in the SCM FA sites and both the FA and FU sites in the PM to five trees per site based on species accumulation curves from previous sampling (Bowman and Arnold, 2018). For this study, we selected sites that had experienced only a low-intensity to medium-intensity burn from recent fires where the host forest was still standing. FU and FA sites were assessed using a combination of visual cues (e.g. presence of burn scares on trees, charcoal layer within soil horizon) and burn maps from the online tool Monitoring Trends in Burn Severity (MTBS) (2017).

Roots were collected by soil coring. Three root cores (5 cm diameter; 15 cm depth) were collected at the canopy dripline of each sampled tree corresponding to uphill, parallel, and downhill of the tree (SCM: 66 root cores; PM: 60 root cores). The litter layer was removed prior to coring and roots were transported in plastic bags in a cooler back to the lab. Roots were stored at -20°C before processing.

For all sites, soil cores were collected from three trees per site (total 24 soil cores, 12 per range) and stored at 4°C and were processed within 72 hrs after collection. Soil samples were sieved over a 2 mm mesh and dried at room temperature for 72 hrs. Chemical analyses were performed by Motzz Laboratories (Phoenix, AZ, USA: <http://www.motzzlaboratory.com/>).

**Sample processing**

Roots were gently cleaned with tap water over a 2 mm sieve. Root tips with evidence of EM fungal colonization were collected and examined under a dissecting microscope. To verify host identification of roots collected in forests with codominant Douglas Fir (*Pseudotsuga menziesii*) and/or Oak (*Quercus* sp.) (Table 1), we evaluated roots morphologically and molecularly as described in Bowman and Arnold 2018. We sorted EM root tips from *P. ponderosa* to morphotypes based on physical characteristics of the EM mantle. The number of live root tips per morphotype was recorded for each soil core. One or two representative tips of each morphotype per core were chosen haphazardly for DNA extraction. All other root tips were stored in cetyltrimethyl ammonium bromide (CTAB) at -80 °C. The remaining root samples were then air dried for 5-7 days, and their dry weight was recorded.

We extracted total genomic DNA from root tips immediately after sorting using the RedExtract-N-Amp plant PCR kit (Sigma-Aldrich, St. Louis, Missouri, USA) following the manufacturer’s instructions. The internal transcribed spacer region (ITSrDNA, including ITS1, ITS2, and 5.8S rDNA) was PCR-amplified using primers ITS1F and LR3. We did not use primer ITS4B because of the prevalence of ectomycorrhizal Ascomycota in preliminary surveys. Sequences were also run through UNITE and NCBI to ensure that amplified DNA was from target ectomycorrhizal species rather than soil fungi or root endophytes.

PCR products were visualized with SYBR green following electrophoresis on a 1% agarose gel in 1% TAE buffer. All samples that successfully amplified were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, California, USA). Bidirectional Sanger sequencing was performed by the University of Arizona Genetics Core using the Applied Biosystems Big Dye Chemistry Terminator v. 3.1 cycle sequencing kit. Once data were returned, we automatically assembled sequences, scored bases, and assigned quality scores via *phred* and *phrap*, coordinated by Mesquite v. 2.01+ (Maddison and Maddison, 2011; Ewing and Green, 1998; Ewing et al., 1998). Assembled sequences were manually edited in Sequencher v. 4.10.1 (GeneCodes Corporation). All sequences have been deposited to GenBank (Appendix #).

In total, we isolated a total of 9,844 root tips from 132 root cores. Of these, 514 root tips were selected from individual morphotype groups for sequencing in which 442 (86%) were successfully sequenced. Operational taxonomic units (OTUs) were assembled in the web-based Mobyle SNAP Workbench using the sanger\_otu\_clustering\_workflow (Monacell and Carbone, 2014; U’Ren et al., 2014). The assembly pipeline used removed chimeric sequences (Edgar et al., 2011); automatically trimmed the small subunit ribosomal DNA (SSUrDNA), large subunit DNA (LSUrDNA), and 5.8S sequences; and removed sequences lacking either the ITS1 or ITS2 region. Sequences were clustered into OTUs at 97% sequence similarity resulting in a total of 116 OTUs (Izzo et al., 2005; Smith et al., 2007). Of the 116 OTUs, 54 (47%) were singletons and 20 (17%) were doubletons.

**Analysis**

*Environmental analyses*

To determine whether environmental factors needed to be included in analyses, we analyzed climate and soil characteristics to assess for significant differences between the two ranges and between FA and FU sites within each range. As annual average precipitation (mm) and max annual temperature (°C) (Table 1) were co-correlated (linear regression; F1,39 = 117.10, p-value < 0.001, R2 = 0.74), we chose to use annual average precipitation to represent climate in our analyses as it showed a significant difference between ranges, while max annual temperature did not (Fig. S1). This was analyzed using a mixed effects model as part of an analysis of variance (ANOVA) in which range and fire history were fixed factors and site was treated as a random factor. As the purpose of this study is to compare diversity and community composition of EM within FA and FU sites between the ranges.

We conducted a PCA of the soil characteristics which showed significant differences between ranges or between sites with different fire histories (Table 2). PCA axis 1 which explained 50.22% of the variation, was used as the response variable in a mixed effects model ANOVA in which range and fire history were fixed factors and both site and tree were treated as random factors. Our analysis showed that soil did not vary significantly between ranges nor between sites with differing fire histories (Fig. S2). We, therefore, did not include soil in future analyses.

*Species richness and diversity*

Diversity was analyzed using both Fisher’s alpha and Shannon’s diversity index. Fisher’s alpha is robust to variable sample sizes and is used for communities that generally follow a logarithmic series distribution as is common microbial communities (Fisher et al., 1943). To complement Fisher’s alpha diversity index, diversity was also calculated using Shannon’s diversity index as it is a non-parametric diversity measure (Shannon, 1948). Diversity and species richness were calculated per tree using the ‘vegan’ package in R (Supplementary table S1) (Oksanen, 2018). Diversity and species richness data was assessed for normality and heterogeneity of variance prior to analysis (data not shown). Species richness and Shannon’s diversity were normal, but Fisher’s alpha index did not meet assumption of equal variance required for parametric analysis. After assessing the data, we removed those trees from the Fisher’s alpha analysis where the species richness was equal to the community abundance finding that this falsely inflated the Fisher’s alpha value (Supplementary table S1). After removal of outliers, Fisher’s alpha met all assumptions for parametric analyses. We assessed differences between FA and FU sites within each range for species richness, Fisher’s alpha, and Shannon’s diversity using a t-test. All analyses were carried out in using R (R Core Team, 2018).

*Community composition*

Community composition of EM communities was analyzed based on fire history, both within each range and across both ranges, using an analysis of similarity (ANOSIM). Patterns were assessed visually using non-metric multidimensional scaling (NMDS). To assess community differences, we used two different similarity indices: Jaccard (a presence/absence measure) and Morisitia-Horn (an abundance measure) (Magurran 2004). Data was assessed for normality and equal variance prior to ANOSIM (data not shown). The datasets that failed to meet the assumptions required for ANOSIM were analyzed using PERMANOVA.

*Taxonomy*

Sequences were assigned taxonomically using both NCBI and the UNITE database (UNITE community). Taxonomy was assessed using a chi-square analysis at the class level and genus level as a function of fire history within each range. Rare genera, i.e. those with less than four occurrences across all sites, were removed prior to analysis. Additionally, we used an indicator species analysis to assess whether particular taxonomic groups were associated more commonly with FA or FU sites within each range. The analysis was implemented using the ‘indicspecies’ package in R (De Cáceres & Legendre, 2009).

**RESULTS**

*Species richness and diversity*

Overall, we found that while species richness was marginally affected by fire history, EM diversity did not seem to vary based on fire history (Fig. 2). Both species richness (Fig. 2A) and Fisher’s alpha (Fig. 2B), on average, showed a general increase in FA sites compared to FU sites, and this trend was reflected in both ranges. Shannon’s diversity showed a similar pattern (Supplementary figure S3). On average, each site had 26.25 (± 6.16) species, with FA sites having 27.25 (± 7.5) and FU sites having 25.25 (± 5.44). Average Fisher’s alpha across all sites was 24.622 (± 8.667) with FA sites having 26.04 (± 11.50) and FU sites having, on average, 23.20 (± 6.14).

*Community composition*

Both ranges showed difference between FA and FU sites with the fire history more of the variation within EM community in the PM than the SCM (Fig. 3). When looking at differences in the EM community between ranges both in FA and FU sites, the community composition are not very similar (Fig. 4). EM communities in sites without recent burn history (FU sites) show slight overlap, while sites with a recent fire history (FA sites) in both ranges are completely distinct with no overlap in community composition (Fig. 4). Analyses using Jaccard similarity showed similar results as those using Morisita-Horn.

*Taxonomy*

At both the class and genus level, we found that EM taxonomy differed between FA and FU sites (Fig. 5 & 6). The most abundant genera overall were *Cenococcum*, *Russula*, *Tomentella*, and *Lactarius* (Fig. 6)*.* Both FA and FU sites within the SCM were not characterized by any EM fungi although a non-EM fungus of questionable status, *Sistotrema*, was unique to FA sites (Table S2). Within the Pinaleno Mts., FU sites were characterized by a species of *Lactarius*, an EM genus, and *Phialocephala*, a dark septate endophyte (DSE) (Table S2).

**DISCUSSION**

In this study, we aimed to determine whether 1) EM community diversity and composition would differ in FA and FU sites 15 years post-fire and 2) if similar patterns would be observed in geographically distinct, but environmentally similar forests. Here, we found no difference in species richness or diversity based on fire history although both were slightly higher in FA sites, but community composition did show shifts in FA sites compared to paired FU sites. Overall, we found similar patterns in both ranges demonstrating that even in geographically distinct forests hosting unique EM communities, EM species diversity and community composition undergo similar changes post fire disturbance. The main difference between the two ranges was the degree of shifts in EM community composition in FA and FU sites with the SCM showing a less distinct difference than the PM. Additionally, the taxonomic composition between FA and FU sites in the two ranges showed contrasting patterns. These differences could indicate that changes in taxonomic composition seem to be governed more by non-disturbance processes, such as historical contingency or annual average precipitation patterns.

Studies in which EM communities were assessed immediately post-fire have found that both diversity and species richness can increase compared to pre-fire status at those sites or similar sites that were not burned (Stendell *et al.* 1999; Baar *et al.* 2002). The slightly higher species richness and diversity we found in FA compared to FU sites could indicate that these sites are undergoing recovery to pre-fire conditions. A study in which recovery of sites post-fire was assessed found that approximately 15 years was sufficient for EM community recovery to near pre-fire diversity status (Treseder *et al.* 2004), although other studies have indicated that substantially longer is required for communities to fully stabilize post-fire (Visser 1995). Factors that could affect levels of species richness and diversity pre- and post-fire are the severity of the fire, that is temperature of fire and depth of soil horizons affected (Stendell *et al*. 1999; Dahlberg *et al.* 2001; Baar *et al.* 2002; Glassman *et al.* 2016). Although we were unable to measure these factors in our observational study, visual indicators and fire severity maps indicate that our FA sites probably underwent a low-severity fire which would have lower temperatures and low level penetration of soil horizons. Low level disturbances such as this can leave the EM spore bank and portions of the hyphal community intact decreasing recovery time (Dahlberg *et al.* 2001; Baar *et al.* 2002; Glassman *et al.*2016).

EM communities in the SCM and PM showed no similarity in FA sites (Fig. 4B) compared to a higher level of similarity in FU sites (Fig. 4A) indicating that despite overall similar shifts in community composition post-fire disturbance, the direction of the shift was dependent on the initial EM community pool. While the spore community was not assessed here, studies looking at disturbance of EM communities have shown that the dormant spore community serves as the initial inoculum post-fire (Dahlberg *et al.* 2001; Baar *et al.* 2002; Glassman *et al.*2016). Common genera associated with the spore bank are *Rhizopogon,* *Wilcoxina*, and *Tomentella* among others (Baar *et al.* 2002). While *Rhizopogon* and *Tomentella* were present in FA sites, these genera were also in FU sites and overall relative abundance was very low possibly supporting the recovery of FA sites to pre-fire conditions.

A major concern of this study was the temporal difference in sampling (Table 1). EM communities are known to undergo community shifts over relatively short time scales (i.e. seasonal), but year-to-year within the same season there can be little to no changes within the community (Koide *et al.* 2005; O’Hanlon 2012). Additionally, there is evidence that temporal shifts within EM communities is stronger in extraradical hyphae and less pronounced in root colonized EM (Koide *et al.* 2005). As we sampled within the same season of different years and only sampled EM colonized roots, the temporal effect on sampling should be minimal.

The findings outlined here support previous findings in studies of EM communities post-fire disturbance, but also demonstrate that the pre-fire community is an important factor in determining how these communities will respond to fire disturbance. While not assessed here, this could have important implications for speed and ability of forests to recover based on pre-fire diversity and composition of the EM community.

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**Figures**

Figure 1: Map of the Santa Catalina Mts. and sampling sites.

Figure 2: Species richness (A) and Fisher’s alpha diversity (B) as a function of both fire history and range. A. T-test, PM: T17.90=1.76, p-value=0.10; SCM: T18.68=1.81, p-value=0.09. B. T-test, PM: T7.09=1.87, p-value=0.10; SCM: T16.44=0.39, p-value=0.70. FA = fire affected, FU = fire unaffected.

Figure 3: NMDS of EM community across fire unaffected (FA) and fire unaffected (FU) sites in the Pinaleno Mts. (A) and Santa Catalina Mts (B) based on Morisita Horn similarity index. A. PM, stress=0.16, ANOSIM: R=0.54, p=value=0.001; B. SCM, stress=0.20, PERMANOVA: F=1.96, R2=0.09, p-value=0.02. Green = FU, black = FA; circles = SCM, squares = PM.

Figure 4: NMDS of EM community across fire affected (FA; A) and fire unaffected (FU; B) based on Morisita Horn similarity index . A. FA, stress=0.19, ANOSIM: R=0.33, p-value=0.001; B. FU, stress=0.15, PERMANOVA: F=3.23, R2=0.16, p=value=0.001. Green = FU, black = FA; circles = SCM, squares = PM.

Figure 5: Class level taxonomy of EM communities as a function of fire history. Each range assessed individually. A. PM, X26=22.48, p-value<0.001; B. SCM, X26=16.314, p-value=0.003. FU = fire unaffected, FA = fire affected.

Figure 6: Genus level taxonomy of EM communities as a function of fire history. Each range assessed individually. A. PM, X29=37.62, p-value<0.001; B. SCM, X213=34.61, p-value=0.001. FU = fire unaffected, FA = fire affected.

**Supplementary Figures:**

Figure S1: Average annual precipitation (mm) as a function of both range and fire history. ANOVA with site as a random factor, range: F1,4 = 99.21, p-value = 0.001; burn history: F1,4 = 3.59, p-value = 0.13; range:burn history: F1,4 = 7.67, p-value = 0.05. FA = fire affected; FU = fire unaffected.

Figure S2: PCA of soil characteristics as a function of both range and fire history. ANOVA with site as a random factor, range: F1,4 = 7.20, p-value = 0.06; burn history: F1,4 = 6.32, p-value = 0.07; range:burn history: F1,4 = 0.003, p-value = 0.96. FA = fire affected; FU = fire unaffected.

Figure S3 Shannon’s diversity as a function of fire history and range. T-test, PM.: T18.41=1.83, p-value = 0.08; SCM: T13.48=2.06, p-value =0.06.

Figure S4: NMDS of EM community across fire affected (FA; A) and fire unaffected (FU; B) based on Jaccard similarity index. A. FA, stress=0.19, PERMANOVA: F=2.92, R2=0.14, p-value=0.001; B. FU, stress=0.13, PERMANOVA: F=2.41, R2=0.12, p=value=0.002. Black = FA, Green = FU; circles = SCM, squares = PM.

Figure S5: NMDS of EM community across FA and FU sites in the PM (A) and SCM (B) based on Jaccard similarity index. A. PM, stress=0.15, ANOSIM: R=0.53, p=value=0.001; B. SCM, stress=0.21, ANOSIM: R=0.11, p-value=0.06. Black = FA, green = FU; circles = SCM, squares = PM.