**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 similar latitudes (Table 1) and have similar exposures as they both lie in a west northwest and east southeast position (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 (USDA Forest Service, 2013). 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—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 recent fire history on ectomycorrhizal communities associated with *Pinus ponderosa*.

**Sampling**

Sampling encompassed two fire affected and two fire unaffected sites in both ranges (total 8 sites). In the SCM, six trees were samples per site (total 12 trees in burned sites, 12 trees in unburned sites). Based on species accumulation curves from previous studies (Bowman and Arnold, 2018), we reduced sampling in the PM to five trees per site (total 10 trees in burned sites, 10 trees in unburned sites). Roots were collected by soil coring. Three root cores (5 cm diameter; 15 cm depth) were collected at the canopy dripline from each sampled tree corresponding to uphill, parallel, and downhill of the tree (SCM: 72 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.

Soil cores were collected from three trees per site (total 24 soil cores, 12 per range) and stored at 4°C before being processed within 72 hrs. 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 examined under a dissecting microscope. To verify host identification of roots collected in mixed forests (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.

Total genomic DNA was extracted 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 used primers ITS5 and LR3 for samples that failed to amplify during the initial PCR (Corrales et al., 2015). 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 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.

**Analysis**

*Species richness and diversity*

Diversity was analyzed using both Fisher’s alpha and Shannon’s diversity index (Table 2). 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). Data was pooled by site prior to calculating diversity (Table 2). Both diversity measures and species richness were calculated using the ‘vegan’ package in R (Oksanen, 2018). Diversity and species richness data was assessed for normality and heterogeneity of variance prior to analysis of variance (ANOVA) tests (data not shown). Species richness and Fisher’s alpha were both normal, but Shannon’s diversity index did not meet assumption of equal variance required for ANOVA. Therefore, the nonparametric Kruskal Wallis ANOVA was used to assess Shannon’s diversity index. All analyses were carried out in using R (R Core Team, 2018).

Species richness and diversity were analyzed as a function of fire history to assess differences between sites with a more recent burn history and those without. We also evaluated differences in diversity based on both geographical location and fire history to test whether responses to fire were distinct in each range.

*Community composition*

Community composition of EM communities was analyzed based on fire history, both within each range (Fig. 3a and b) and across both ranges (Fig. 3c), using an analysis of similarity (ANOSIM). Patterns were assessed visually using non-metric multidimensional scaling (NMDS) (Fig. 3). 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. Only one dataset failed to meet the equal variance assumption (Jaccard analysis of all data; Fig. 3c). Therefore, this dataset was analyzed using a non-parametric permutational multivariate analysis of variance (PERMANOVA).

Variation within the ectomycorrhizal community was partitioned between range (encompassing range differences in soil and climate) and fire history were assessed using a PERMANOVA analysis.

*Taxonomy*

No taxonomic differences were observed between communities in both ranges or between

**RESULTS**

*Species richness and diversity*

Overall, we found that site with a more recent fire history had more diverse EM communities. This trend was reflected in both ranges. On average, each tree had approximately 6.854 (± 2.056) species. Species richness did significantly differ by fire history with more recently burned sites having higher overall species richness, but there was no significant difference of species richness between the SCM and PM (ANOVA; fire history: F1,38 = 6.528, P = 0.015; range: F1,38 = 0.054, P = 0.8170).

Diversity was not significantly different between sites with different fire histories, ranges, or the interaction of the two variables (Fig. 2). Average Fisher’s alpha across all sites was 24.622 (± 8.667) with more recent fire history having, on average, 26.040 (± 11.496) and site without recent fire history having 23.203 (± 6.140).

*Community composition*

Both ranges showed difference in the EM community between sites with recent fire history and those without (Fig. 3a and b). EM communities in sites without recent burn history in both ranges are distinct although there is slight overlap, while sites with a recent fire history in both ranges are completely distinct with no overlap in community composition (Fig. 3).

*Taxonomy*

No major taxonomic patterns based on fire or range, although there are changes post fire.

**DISCUSSION**

**Figures**

Figure 1:

Figure 2:

Figure 3: Nonmetric multidimensional scaling (NMDS) analysis of EM community differences based on fire history. Each point is the EM community associated with a single tree (i.e. sample unit). NMDS plots are shown using the Jaccard diversity index. Statistics for both Jaccard’s and Morisita-Horn’s index are shown below. Green: trees in sites without a recent burn history; black: trees in sites with a recent burn history (15-20 years ago). a) Pinaleno Mts. (NMDS stress = 0.088, ANOSIM: Jaccard R = 0.495, P = 0.002; ANOSIM: Morisita-horn R = 0.484, P = 0.001); b) Santa Catalina Mts. (NMDS stress = 0.196; ANOSIM: Jaccard R = 0.139, P = 0.036; ANOSIM: Morisita-horn R = 0.120, P = 0.033); c) Both ranges (NMDS stress = 0.215; PERMANOVA: Jaccard F2,37 = 3.242, R2 = 0.153, P = 0.001; ANOSIM: Morisita-horn R = 0.098, P = 0.012); d) Venn diagram showing the percentage of community variation partitioned between range, fire history, and the interaction of the two. Analysis run using PERMANOVA and the Jaccard diversity index.