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Klamath Mountain Samples: Fire and eDNA

Methods:

Wet Lab (more detailed on CaleDNA website):

1. DNA extraction from soil samples

2. Run three PCR reactions for each of five primers

3. Pool PCR’s and purify using magnetic beads

4. Run a PCR to add unique index’s to each sample

5. Send samples to be sequenced

6. Receive sequences, begin analysis

Data Processing:

1. Choose which file to use, decided on sum by taxonomy 70.
2. Rename samples to match metadata table
3. Decontaminate file
   1. Remove all taxa that have more than 5 reads present in the 2 blanks (PCR and DNA extraction)
   2. Remove all Human
   3. Remove all taxa with fewer than 5 reads
4. Reclassify taxa
   1. Using the Ruggiero et al. 2015: rename dataset from order up
5. Save and start analysis

Analysis

1. Import data into R studio
2. Follow : /Users/ericberaut/Google Drive/Fire.and.eDNA/Fire.and.eDNA.Plotscripts.R
3. Create alpha diversity plots, taxonomy bar plots, ordination plots, networks

Results:

Power point of final plots

I looked at three main research questions as I attempted to explore this data set: Does Fire increase species richness? Does Fire change community composition? Are there specific taxonomic groups affected by fire?

**Does Fire increase species richness?**

The simple answer, no. The data suggests that fire does not increase species richness. I determined this by making alpha diversity species richness plots. There was only one statistically significant difference in species richness between sites (fires) and it was only found with one primer. This was an increase in the species richness from the unburnt sites to the stanza site of the fungal communities. Due to the potentially differing baseline communities (the two sites are geographically isolated enough) we cannot say that fire drives this increase in species richness.

**Does Fire change community composition?**

The simple answer, probably. To determine if community composition changed, I created two types plots: taxonomy bar plots and ordination plots.

The taxonomy bar plots represent the relative abundance of rarefied data summarized to family. We can compare each sample to one another, as well as compare the sites to each other. In 18S and PITS, we see no real pattern across the sites. There are no unique differences between the unburnt samples and the rest. This indicates that fire does not change the composition of the dominant families in 18S and PITS communities. The CO1 and FITS communities showed more similarities within sites but still no differences between burnt and unburnt. This indicates that each site has somewhat unique CO1 and FITS composition but does not indicate a fire driven change. The 16S community looks consistent across all samples and sites, indicating the bacterial community of the entire area is relatively stable. It also does not indicate a fire driven change.

The ordination plots show the dissimilarity of the samples to one another. Each plot is one the primers and each data point is one of the samples. The points are colored by site and boxes are drawn in to show the grouping of the burnt and unburnt samples. For the 16S community, the boxes for the burnt and unburnt samples are diverging slightly. This indicates that unburnt samples are more similar to each other than they are to the burnt samples. Since the grouping appears to diverge vertically, we can infer that the variation across axis two can be explained by fire. This trend is present in the FITS data as well as CO1 and 18S, but weaker for these two. The trend is not present in PITS.

Since the PITS dataset looked unique, I wanted to do another test. Recoloring the PITS data points to match the 16S groups, I made another ordination plot. A side by side comparison shows that the PITS community is more correlated with the bacterial community than the site.

**Are there specific taxonomic groups affected by fire?**

The simple answer: maybe, but we can’t tell without more samples. To determine if there are specific taxonomic groups affected by fire I first had to choose taxonomic groups to look at. I went online and found bacteria and fungi that were shown to be fire responsive.

The first group was the family *Acidobacteriaceae*. It is present in oak tree rhizosphere microbiomes as well as being shown to respond to fire. In the data we see no clear trends in regard to burnt and unburnt comparisons. When looking at the time since last fire, we do see some trends. There is a decrease in variation in the years shortly following a fire (4,5), with variation increasing as time passes (10,16). The most variation is found in the unburnt samples. These results support the conclusions made in the literature (a decline in relative abundance following a fire) but must be taken lightly. Due to a low statistical power, these trends in the variation cannot be proven to be statistically significant without more samples.

I also looked at a few genera: *Arthrobacter* (bacteria), *Cadophora* and *Rhizopogon* (fungi). The plots I made to look at these are the original 16S and FITS ordination plots recolored to show presence absence of the genera. The original boxes surrounding burnt and unburnt data were overlaid to look for potential trends. No real grouping is shown, I could possibly look at t-tests to determine any significant differences in the presence absence. I would need additional samples to run stronger statistical tests for these genera.

Another way to explore specific fire responsive taxa is the network. I made a combined FITS and PITS network to look for any interesting interactions between the fungal and plant communities. One interesting connection is between the fungal genus *Rhizopogon* (shown to be fire responsive) and the plant genus *Ceanothus*. *Ceanothus* is a nitrogen fixing shrub shown to dominate post fire mixed evergreen sites.

FITS network: <https://public.flourish.studio/visualisation/248616/>

FITS and PITS network: <https://public.flourish.studio/visualisation/251259/>