Summary of AshNet Biodiversity Data and Analyses

# Datasets

## Site-level data (n=8)

* Site Metadata (28 variables. Includes Ecozone, Forest Region, Province, Stand Age, Dominant Tree species, soil type and components, Qualitative Ash variables (type, amount, pre-treatment, year applied and storage))
  + Use dependent based on results, e.g. what makes more sense based on observed patterns (or one - off results)
  + Try to stick with continuous variables, stand-age, stand age at application etc.
* Climate Data (93 climate variables from model output)
  + Reduced to 19, just by finding variables highly correlated to groups of variables. Of these, thinking of using growing season length and Precipitation of Driest period to represent temperature and precipitation differences
* Ash Chemistry (Ca, P, K, Cd, Zn, Na for all. Incomplete data for Cd, Cr, Cu, Ni, Pb, TC, TN)
  + Ca highly correlated to Ni, P, K (just use Ca for these)
  + Include TC, TN, and use Cu to represent ‘toxic metals’ (weakly correlated to Cr, Cd, Pb)

## Sample MetaData

* Sample level data, treatment amount, block, replicate, treatment (n=458)

## Treatment Level Data

* Soil Microbial Biomass, Carbon, Nitrogen (n= 253)
* pH (n= 343)
* Soil Enzymes (n = 343)
  + Excluded lignases, Beta-Glucosidase at this time as no patterns, and mostly negative results throughout dataset.
  + Replicates pooled for these analyses
* ITS metabarcoding data (n=453)
  + Rarefied matrix
* 18S metabarcoding (n=452)
* F230 metabarcoding (n=403)
  + Poor sequencing distributed across samples
* BR5 metabarcoding (n = 455)
  + Not currently incorporated, very poor sequencing depth (<1000 for most samples)
* 16S metabarcoding (n = 171)
  + Subset of samples
  + HLB, ALN, ALS, only Organic soils
  + No ETM sequences
  + No duplicates

# Analysis

Initial examination of data shows a large amount of spatial variation within sites.

Analyses are being performed on metrics comparing treatments and controls for each treatment and soil type within blocks or between split-block pairs to account for this.

## Site Level Characteristics

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| --- | --- |
| Research Question | Tests |
| Does ash addition result in higher or lower pH, total C or total N of soils compared to controls? | Differences in Total C, total N and pH were calculated, and Bonferonni corrected, one-sample t-tests were performed for each variable for each site/mineral combination. |

## Enzymes

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| Research Question | Tests |
| Does ash addition result in different nitrogen, carbon and/or phosphate cycling at any sites? | Differences in enzyme activities were calculated, and Bonferonni corrected, one-sample t-tests were performed for each variable for each site/mineral combination. |
| Do the components of Ash (i.e., Calcium, TC, TN, Cu) increase or decrease soil nitrogen, carbon and/or phosphate cycling? | For each soil type, glm or lms with difference in enzymes as response variable, quantity of Ash components as independent variables. First, removal of “site noise” by developing a model which accounts for enzyme response to climate variables in only the control sites (i.e., growing season length, soil moisture) then prediction of the responses for treatment sites will allow for regression of the Ash chemistry variables against the residuals. |

## Metabarcoding (performed for each set of primers)

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| Research Question | Tests |
| Do sites with ash addition have different soil microbial communities than controls? | Visual examination of Bray-Curtis (or Jaccard) Distance based NMDS, supported by Adonis and the comparison of distances between site controls to the distances between treatments and controls. One-sided t-tests were used to evaluate the significance of differences between distances. |
| Do sites with Ash addition have a different diversity, or number of ESVs than controls? | The difference between control and treatment plot Shannon Diversity, Inverse Simpsons’s Diversity and Richness were assessed via Bonferonni corrected one-sample t-tests for each site/soil/treatment combination.  Visual analysis of Euler diagrams – is there less overlap between ash treated sites and controls, than between controls. |
| Are ash additions resulting in the increase or decrease of particular organisms across sites? | Visual analysis of Gain/Loss metrics calculated as % of sites to gain or lose a species compared to the control with Heatmaps. A filter was applied to the data to ensure that the direction of change was consistent. |
| Are changes in abundance of particular organisms related to particular ash components? | Multivariate GLM on the gain/loss matrix. May pool the 18S, F230, ITS in a single logistic regression, and a separate one for 16S. Information from this analysis will feed-back to inform filtering of heatmaps. |

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