Background

As the field of environmental biology attempts to tackle anthropogenic climate change, it has become increasingly important to understand the mechanisms that affect the health of forest ecosystems in order to preserve remaining forested areas. Researchers have sought to understand the connections between succession and climatic changes to create potential models that can project the future of ecosystems based on variables such as succession, harvest and climate change. (Wang et al., 2015) Primary succession is the process by which a plot of land converts from a moss and lichen dominated landscape into a dense forest. Primary succession has 4 general stages, although these stages vary by biome and location, in which a barren landscape becomes dominated by pioneer species, after which an intermediate community can thrive, which results in a climax community. Secondary succession is the process in which an intermediate and climax community is perturbed by some force, either natural or anthropogenic, and reverts to one of the previous stages depending on severity of damage.

One major component to a forest's ability to continue their successional stages is the requirement of maintaining a mutualistic relationship with soil biotic ecosystems. The relationship between the forest and its successional stages requires a delicate balance between soil fungal and bacterial populations that facilitate the production of carbon and nitrogen, respectively, as the forest requires different ratios of carbon to nitrogen necessary for various stages from moss and lichen dominated grasslands to a dense forest. (Llado et al., 2017, Wallenstein et al., 2006). There is a relationship in the amount of fungi and bacteria that is present at the different stages of succession. As the different stages occur there is a gradual increase of fungi and an increase in bacteria followed by a gradual decline relative to the amount of fungi. Fungi is much larger than bacteria in biomass and out competes bacteria for space, reducing the available nitrogen in soil from bacterial communities. The amount of fungi directly influences the soils supply of available carbon and therefore facilitates the growth of mature trees. This relationship between fungi to bacteria ratios is a complex association that has correlational trends to succession. (Zhang et al., 2018)

Successional stages are therefore characterized as large tree growth from more available carbon in soil and this available carbon is directly related to the increase of fungi. The more available carbon in the soil, the more in biomass these trees are able to produce. Therefore total biomass is a metric that should correlate to the progression of succession. Stable nitrogen isotope leaf measurements are positively correlated with the relative amount of nitrogen fixing bacteria. As succession progresses, the amount of bacteria does increase, however, that increase relative to total area biomass that plots with larger trees can produce declines. Therefore, we should expect a logarithmic increase in leaf nitrogen through the successional stages. (Hobbie and Hogberg, 2003) if stable carbon and nitrogen isotope leaf measurements

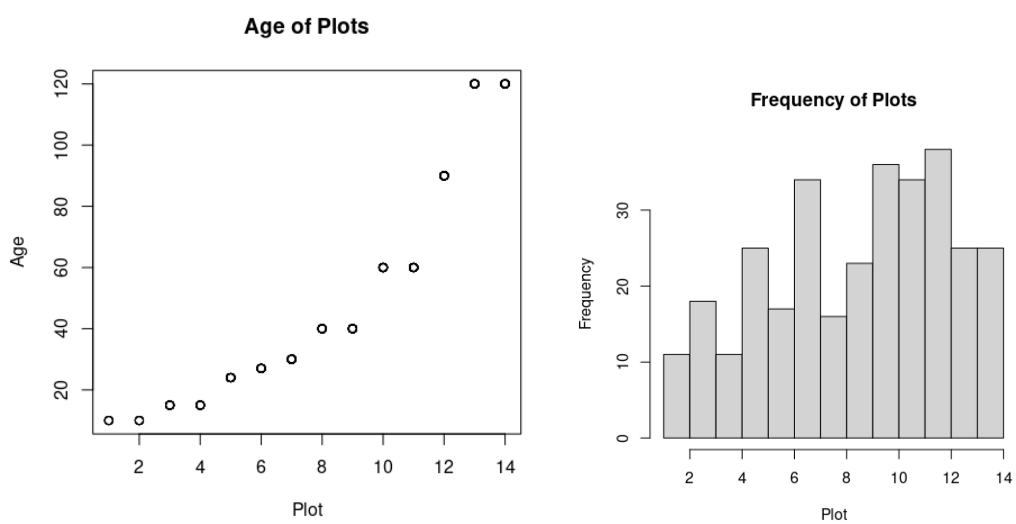

FIgure 1: A visual representation of succession set up as the base of the analysis, followed by a histogram of those plots.

are known to correlate to fungi/bacteria ratios, then there should be a statistically significant relationship between these leaf measurements and relative successional stages.

Methods

Data Analysis

The computation of the data in this project was done using R v.4.1.2 and RStudio 2023.09.1 build 494 on an Ubuntu 20.04 distribution linux-based operating system. Buzzard 2015 gives us access to several tree and leaf functional trait measurements of plant species residing within defined plots in Costa Rica's Guanacaste Conservation area. This data gives us access to age, plot, species, abundance, biomass, area and many leaf measurements, most notable being leaf carbon-nitrogen ratio, and stable nitrogen isotopes. By establishing a base-line of relative plot succession stage by tree age, we can establish which plot is in which relative successional stage.

Hypothesis Testing

In order to analyze this data and test our hypothesis, we can perform primary statistics of all leaf measurements. However, the two that we can expect to more strongly reflect their biotic soil communities, biomass as a reflection of available carbon in soil, an association represented by fungi and mature tree species. Additionally, leaf nitrogen isotope measurements reflect bacterial associations. Performing multivariate analysis with biomass and nitrogen isotopes as predictor independent variables should result in a trend that reflects the decreasing nitrogen availability through the successional stages.

Results

The analysis of establishing the successional stages, figure 1, shows the increasing age of plot left undisturbed, this data has already been ordered by plot numbers to reflect this. This is sufficient in establishing our relative successional stages in which plot number is in ascending order from youngest to oldest in 9 ages ranging from 10 to 120, with an n >= 11. In this dataset total plot biomass is also positively correlational to the plot, further supporting the establishment of the trend expected to that of successional stages, expecting denser forests through time.

First.Variable	Second.Variable	Correlation
biomass	basal.area	0.9830186
total.plot.biomass	total.plot.basal.area	0.9805984
age	plot	0.9296333
plot	total.plot.basal.area	0.5982379
plot	total.plot.biomass	0.5669969
age	total.plot.basal.area	0.5608699
age	total.plot.biomass	0.5204616

Table 1: Table showing relevant correlational variables.

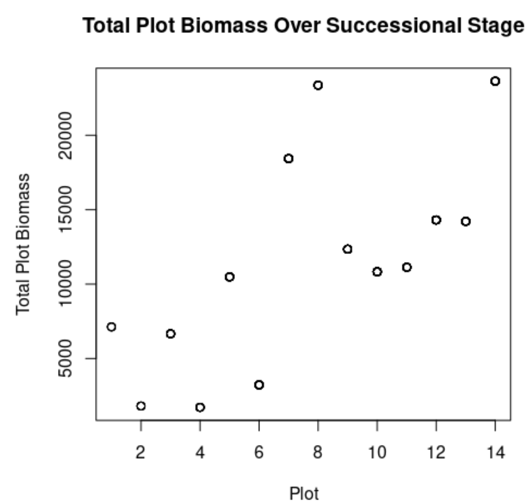

Figure 2: Plot showing relationship between total plot biomass over stages of succession.

Writing a simple program in RStudio allows us to provide a comprehensive and detailed list of any correlational association crossed between every possible variable combination, which then filters for any strong and weak correlations, table 1. These tables were generated with CRANs gt packages.

The testing for total plot biomass across plot found a statistically significant weak positive correlation, figure 2. The testing for leaf nitrogen stable-isotope measurements across the plots were found to not have any statistically significant relationship, figure 3. Additionally, there were other statistically significant correlations to either plot or age, however, this was for total plot basal area and average individual tree basal area.

Discussion

Linear regression of age across the plots represents successional stages in terms of age of forest left undisturbed, and these results were strongly positively correlated. The plots are already ordered, however the data will be represented in bins of each of the 14 plots. This ascending order made it simple as we can define the age of every plot, and assign it as an index in that order. This relationship then confirms the assumption within our hypothesis that the rest of the analysis can rely on, which is that these plots in order by age represent the relative succession stage.

Analysis of the total biomass within a given plot across each plot shows a weak positive correlation. Our assumptions rely on the total amount of biomass inside of a plot being representative of the total amount of carbon available in the soil, which is associated with fungal association of soil carbon production. The results seem to support this assumption as we can see a weak positive correlation suggesting that as these plots age they are being supplied with increasing amounts of carbon which facilitates growth. Additionally, the correlations with age or plot to basal area and total basal area seems to support this as well, and these variables follow the same logic with facilitation of growth supplied by more carbon as the trees, and the soils that facilitate their growth, age.

Unfortunately, there was no association between leaf nitrogen stable-isotope measurements and relative succession stage. The assumption was that as succession continues there would be an increase of nitrogen stable-isotope leaf measurements followed by a general plateau as bacteria communities grow

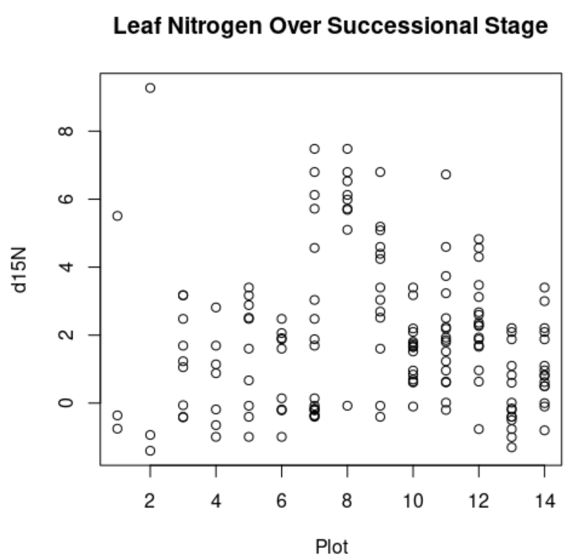

Figure 3: The measurements of leaf nitrogen stable-isotopes over relative successional stage.

and then are outcompeted by fungal communities. Additionally, research has shown that the leaf nitrogen stable-isotope is strongly correlational to bacterial community proliferation, suggesting that some factor is affecting the outcome of not having increasing stable-isotope measurements.

Conclusion

The goal of this analysis was to further establish previously researched ideas in both succession and bacterial/fungal associations. There was a hope to establish a strong connection between the successionary stages and the ability to predict fungal and bacterial associations through ecosystem features through those stages. Unfortunately, we could not find a predictor variable for bacterial associations, the known variable being leaf stable-isotope nitrogen as a known correlative. It would have to surmise that there are other variables not measured that have some impact disrupting that known association between leaf measurements and bacteria; or that bacterial communities have a unique pattern through succession that is not standard in this environment; or that leaf measurements are simply not correlational to succession regardless of known association with bacteria . Regardless, it would be of great use to acquire a predictor variable for relative stage of succession as it would assist in identification for forest rehabilitation.

References

Hobbie EA, Högberg P. Nitrogen isotopes link mycorrhizal fungi and plants to nitrogen dynamics. *New Phytologist*. 2012;196(2):367-382

Lladó S, López-Mondéjar R, Baldrian P. Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiol Mol Biol Rev*. 2017;81(2):e00063-16.

Wallenstein MD, McNulty S, Fernandez IJ, Boggs J, Schlesinger WH. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management*. 2006;222(1-3):459-468.

Wang WJ, He HS, Iii FRT, Fraser JS, Hanberry BB, Dijak WD. Importance of succession, harvest, and climate change in determining future composition in U.S. Central Hardwood Forests. *Ecosphere*. 2015;6(12):1-18.

Zhang K, Cheng X, Shu X, Liu Y, Zhang Q. Linking soil bacterial and fungal communities to vegetation succession following agricultural abandonment. *Plant Soil*. 2018;431(1):19-36.

Figures

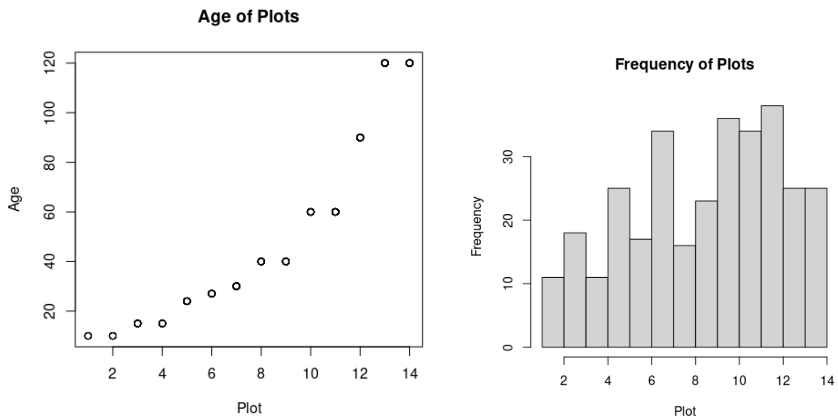

FIgure 1: A visual representation of succession set up as the base of the analysis, followed by a histogram of those plots.

First.Variable	Second.Variable	Correlation
biomass	basal.area	0.9830186
total.plot.biomass	total.plot.basal.area	0.9805984
age	plot	0.9296333
plot	total.plot.basal.area	0.5982379
plot	total.plot.biomass	0.5669969
age	total.plot.basal.area	0.5608699
age	total.plot.biomass	0.5204616

Table 1: Table showing relevant correlational variables.

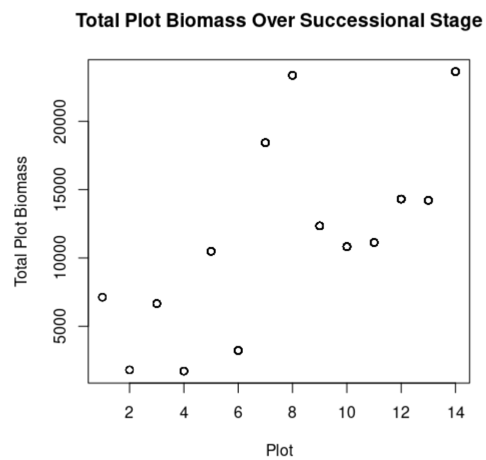

Figure 2: Plot showing relationship between total plot biomass over stages of succession.

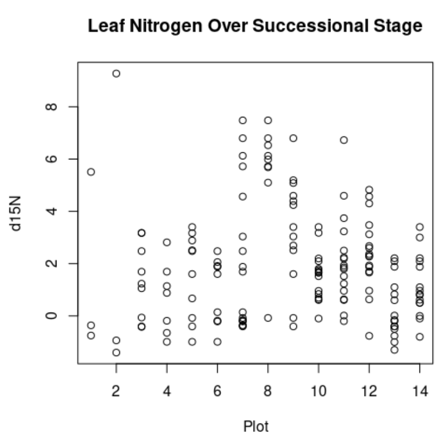

Figure 3: The measurements of leaf nitrogen stable-isotopes over relative successional stage.

Additional Citations

Github User: avrilcoghlan / LittleBookofRMultivariateAnalysis
The R foundation for Statistical Computing
RStudio, Posit Software, PBC
Data provided by Buzzard, et al. 2015,
https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2435.12579
Github link to analysis performed:
Code for formatted plots:
https://www.jungjulie.com/2019/04/15/how-to-create-publication-quality-figures-in-r/