Mycological Succession

Succession of Fungal Species in Forest Ecosystem and the Fixation of Nitrogen

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Abstract

The fungal species that are present after a secondary successional episode are not often examined. Our study focused on two functional groups, comprising of mycorrhizal and saprophytic fungi. Succession was examined on the UMBS burn plot property, using three separate plots 1936, 1954, and 1998. Our purpose was to determine whether the fungal diversity of each burn plot; whether the diversity changed over time after a secondary successional event. Researchers conducted numerous transects each 20m apart in order to search and identify various mushroom species but generally categorizing them into mycorrhizal or saprophytic functional groups. In each single transect, a soil sample was taken in order to quantify the carbon and nitrogen levels. ANOVA tests were ran on soil samples while a Shannon Index was ran on the specie diversity. Functional Diversity was measured by Chi Square.

Introduction

Succession can be described as the change in both abundance and type of species in a maturing forest once there has been a disturbance. The two types of succession consist of primary and secondary. Secondary succession is the removal of many organisms in an already existing habitat that is soon replaced by a new composition of plants. Generally, the soil nutrients seeds, and some roots, remain intact can serve as the foundation for the next generation of that disturbed ecosystem (Jasper 1990).

Fungi reside in their own kingdom. They are major players when it comes to nutrient cycling especially when breaking down complex carbons and fixing Nitrogen. Plant succession is different than the successional sere of fungi in that fungi need the plant and animal material as a substrate for their own growth (Reshi 2007). In order for fungal succession to take place, there needs to be sources of fresh CO2 compounds available (Suzuki 2002). The traditional imagery of fungal succession begins with the fixation of CO_2 from the atmosphere and surrounding nutrients from the soils. For this to happen, the main macronutrients needed in the plants and: potassium, phosphorus and nitrogen (White and Brown 2010).

As plants cannot harness atmospheric nitrogen into a usable form, they often share a mutualistic relationship with fungi. Mycorrhizal fungi benefit from the plants photosynthesis while the plant benefits from the fixated nitrogen. This type of fungi is found within two thirds of all plants (Hodge 2001).

Decomposing fungi are called saprophytic fungi because they convert dead organic matter instead into fungal biomasses, carbon dioxide, and other small molecules. Instead of a photosynthetic energy source, they get their energy by breaking down dead living matter (Fontaine et al. 2011).

These two functional groups of fungi will be present in regions where in nitrogen fixation is needed. Nitrogen 15 is a rare and stable isotope that is commonly measured in research for medicinal and agricultural purposes. With specific respect to fungi, Nitrogen 15 has been measured in various samples so as to trace how actively it has been fixating nitrogen. Movement up the food chain often accumulates N15 (Richardsa 2009).

To study the formation and decay of soil organic matter in the surface soils, Nadelhoffer and Fry (1988) examined two oak forests by assessing the abundances of the Nitrogen 15 and Carbon 13 isotopes. Two controls for the isotopic compositions were administered by using new litter inputs at various time sequences and using the overall isotopic fractionation during decompositioning. Fractionation is used to distinguish the difference between the true amount of carbon/nitrogen and the amount in the trees. The new litter inputs were found to lower the nitrogen and carbon levels the soil samples and yet increased decomposition. The overall litter fractionation during decomposition left residual nitrogen and carbon in the soil, while decomposition leveled out.

The University of Michigan's Biological Station burn plots from 1936, 1954, and 1998, were all experiencing secondary succession. The first purpose of our study was to examine how the age of the forest influences the two different functional groups of fungi at varying stages of secondary succession: mycorrhizal and decomposing fungi. The second purpose of study was to see the levels of nitrogen levels present in the soil. If there was not as much nitrogen left in the soil after the burn, then we expected to see more mycorrhizal fungi within the most recent burn plot of 1998. The older plots would have more detritus and thus we predict that there are going to be more decomposing functional groups present.

Methods

For both the 1936 and 1954 burn plot transects, there was a 90 meter tape laid out. At the 10m, 30m, 50m, 70m, and 90m tape markings, five transects were carried out from these points in order to search for fungi reaching within two meters of the 100 meter long

tape measures. In the 1998 burn plot, the procedure was to take transects instead at the 20m, 40m, 60m, 80m because these plots were differently sized. Fungi were searched for within 2 meters of this tape

Once fungal fruiting bodies had been found, they were then tallied and identified. Our method for identifying the mushrooms consisted of considering color, structure, and size. Fungi that had multiple fruiting bodies were counted as single bodies.

Soil samples were taken 2 - 4 inches below the organic layer, diagonally across each plot. This was done in order to promote the normal distribution. Samples are more likely to be representative of the entire system when each sample collected done so with consideration to even distribution. Each sample was dried in the UMBS research oven for 12 hours at 60 degrees Fahrenheit, then ground in the soil grinder. Samples were handled in an EA: Costech Elemental Combustion Analyzer by the Analytic Chemist, Jennifer Croskrey. The Elemental Analyzer provided Nitrogen and Carbon proportions in the soil.

Results

A one way ANOVA was conducted for just Nitrogen. This tests null hypothesis claimed that there is no statistical difference between the three averages of Nitrogen while the alternative hypothesis argued that there's a significant difference. Soil samples were first assessed for the total Nitrogen averages within each burn plot. The three means were compared at 0.32% for 1936, 0.36% for 1954, and 0.31% for the 1998 burn plot. We used a degrees of freedom at 2 and 36, which yielded an F statistic of 0.948. Our overall result for this test produced a p-value of 0.397. These results can be referred to back in Fig. 3.

An ANOVA two-way test was conducted in order to see the comparative measurements between Nitrogen and Carbon amounts in each soil across all the soil samples. This tests null hypothesis regards all means for the first population as equal. Another hypothesis is that the means are equal again for the second population. A last null hypothesis is that there is no interaction whatsoever between the two populations. Soil samples were assessed for the Nitrogen and Carbon levels. The average means included: 25.6% for the 1936 plot, 24.9% for the 1954 plot, 24.5% for the 1998 plot. We used our degrees of freedom again at 2 and 36, this time yielding an F statistic of 0.772. Our overall result for this test produced a p-value of 0.470. These results can be reffered to back in Fig. 4.

A last ANOVA test was conducted using the tally sheets in order to show trends using the Shannon Index. This test examined the statistical significance of our studied selected species. The distributions and abundances of studied fungal species were compared between each of the three burn plots. We compared species using the most commonly found species: Golden Chanterelles, Aspen, Birch and King Boletes, Oyster, Artist Conk, and Smokey Polyphores. A higher H value for the Shannon Index determines that a plot is more diverse and more equally distributed. Our null hypothesis argued that there was no relationship between fungal diversity across each plot. Indexes spanned from 1.86 in the 1936 plot, 2.03 in the 1954 plot, and 1.94 in the 1998 plot. Our degrees of freedom were again 2, and 36 while the F statistic came out to be 3.967. The p-value for this test was almost significant at 0.080. After using the ANOVA test to compare the three indexes, we concluded that there was no correlation between fungal diversity and the successional age. See Fig. 5.

A final test was administered on the proportions of mycorrhizal fungi and saprophytic fungi using a Chi Square test. Divided into their respective functional groups, we used the Chi Square in order to investigate whether our two categorical variables were significantly different from one another across all three plots. Our null hypothesis concluded that the amount of functional groups found in each burn plot were independent of one another, no relationship. Our degree of freedom was 1, while the X^2 statistic came out to be 30.659. This p-value was less than 0.0001. See Fig. 6, 7, and 8.

Discussion

Our p-value for the ANOVA comparison of Nitrogen levels across each burn plot proved to have no statistical significance. We ended up accepting the null hypothesis to be true, meaning that the averages were not comparatively different enough. We initially predicted that there would be less availability of nitrogen within the 1998 plot. As the plots got older, we predicted also that there would be an increase in availability nitrogen fixated into the soil. This was proven to not be the case within our system. However, it has been recorded in another study done by Zackrisson in 2004 about how nitrogen fixation increases with successional age as a whole. It is speculated amongst our colleagues that the reason for our inability to recognize this trend had to do with the ages of our plot samples. Perhaps our 1936, 1954, and 1998 plots were too close together in age. (See Fig. 3)

Our p-value for this ANOVA comparison between Carbon and Nitrogen ratios in the three burn plots was statistically insignificant. For this test, we ended up accepting the null hypotheses. Neither Carbon nor Nitrogen had significant mean differences in our

samplings. We accepted that there was no noticeable relationship or interaction between the ratios of Carbon and Nitrogen amount averages. The amount of Carbon in the soil does not determine the amount of Nitrogen present. We determined instead that functional groups of fungi might not be dependent on the soil composition so much as simply the age of the plot. Meaning that age within a plot allows for the increase of intricate root systems, which may determine the varying species of mycorrhizal fungi. However, this prediction was found to be backwards. In a study done by Marcel back in 1998, he asserted that instead it was the mycorrhizal fungal diversity that determined plant biodiversity and even ecosystem productivity. (See Fig. 4)

This method of bottom-up control within the ecosystem makes us reconsider our approach in better understanding this system. If we could conduct another research experiment, we would try to find older plots that provided more variability in the amounts of detritus and thus allowing for more fungal diversity. A 2009 study done by Alguacil gives an example of how diversity of arbuscular mycorrhizal fungi increased with the application of organic material. Older burn plots containing more types of detritus and more detritus in general would allow for a greater variety of fungi. On the other side of the spectrum, it takes about 10 years to replenish nitrogen (Yelenick, 2013). Ideally in our next study we would include a plot that was burned recently within 10 years, 50 years (for intermediance studies), and then at least 100 years for the last plot in order to acquire the increase the soil complexity of organic matter in order to a rise in mycorrhizal diversity (Treseder 2004).

The Shannon Index across each burn plot was provided by fig 5. These results yielded a p-value that was almost significant and yet the statistics argued that the

diversity between each plot was not significantly different. The Shannon Index measures for both specie abundance and specie distribution throughout an area. With respect to the Intermediate hypothesis, it was the 1954 burn plot that proved to be most diverse. This hypothesis predicts that diversity should be greatest when disturbance levels are intermediate. The higher the Shannon index, the more diverse the plot; figure 5 depicts that the Shannon Index was the highest at 2.03. Our findings for this highest diversity in the 1954 plot was consistent with how these corresponding soil samples had the highest nutrient content. Perhaps it could be due to too small a sample size that was not representative enough of the population that our findings were insignificant. Perhaps it could again that the age of the burn plots were not far enough apart in age to see this trend be significant.

After using the ANOVA test to compare the three indexes, we concluded that there was a correlation between the fungal diversity of fungi and the successional age of burn plots. It appears from figure 7 that as burn plots age, the amount of fungi in the mycorrhizal group decrease slight, while the total amount of saprophytic fungi increase. Our hypothesis was just this: that the amount of mycorrhizal fungi would be present in recently burned plots because of pioneer species and the ability to fixate nitrogen in subpar soils. The saprophytic functional group was predicted to increase in abundance over time as these plots grew in age due to the amount of litter and left over organic matter (substrate for which the fungi uses for growth (Reshi 2007)).

An application of our study could consist of when to schedule wild fires prescribed by the Division of Forestry in a given area. The DNR will administer controlled burns in a specific area in order to dampen the severity of each wild fire.

Although these controlled burns are not as often prescribed in the state of Michigan, the DNR is active in each state across the United States. In order to prescribe a control fire, it would be beneficial for those in charge to recognize both the successional process and that the nitrogen in each plot will be diminished for a time. Scheduled burns also diminish the potential for fungal specie distribution as they set the reset the nutrients in a given area back to either that of primary or secondary succession. Some endangered fungal species are known, but not all. Europe actively engages with identifying endangered fungal species on what is known as a "red list." A red list of rare and threatened fungi does not yet exist for North America. (Brazee et al., 2014) Rare species of mushrooms could be protected from further extinction through a future red list in the United States. Scheduled burns could be organized around this list, ensuring the protection of endangered fungal species.

Conclusions

Our findings lead our research team to the conclusion that fungal diversity does not differ significantly in forests that vary in age. It was also statistically significant to mention the leveling off of mycorrhizal fungi and the increase of decomposing fungi within a system as an area of secondary succession ages. Levels of soil nitrogen and carbon have no bearing on each other. Amounts of nitrogen and carbon in the soil do not share a relationship in differences across burn plots at varying successional ages. Succession also seems to have a significant effect on the proportions of mycorrhizal and decomposer fungi for each burn plot varying in age. Older plots tended to have a larger

ratio of saprophytic fungi while younger plots contained almost no saprophytic fungal species.

Tables and Figures

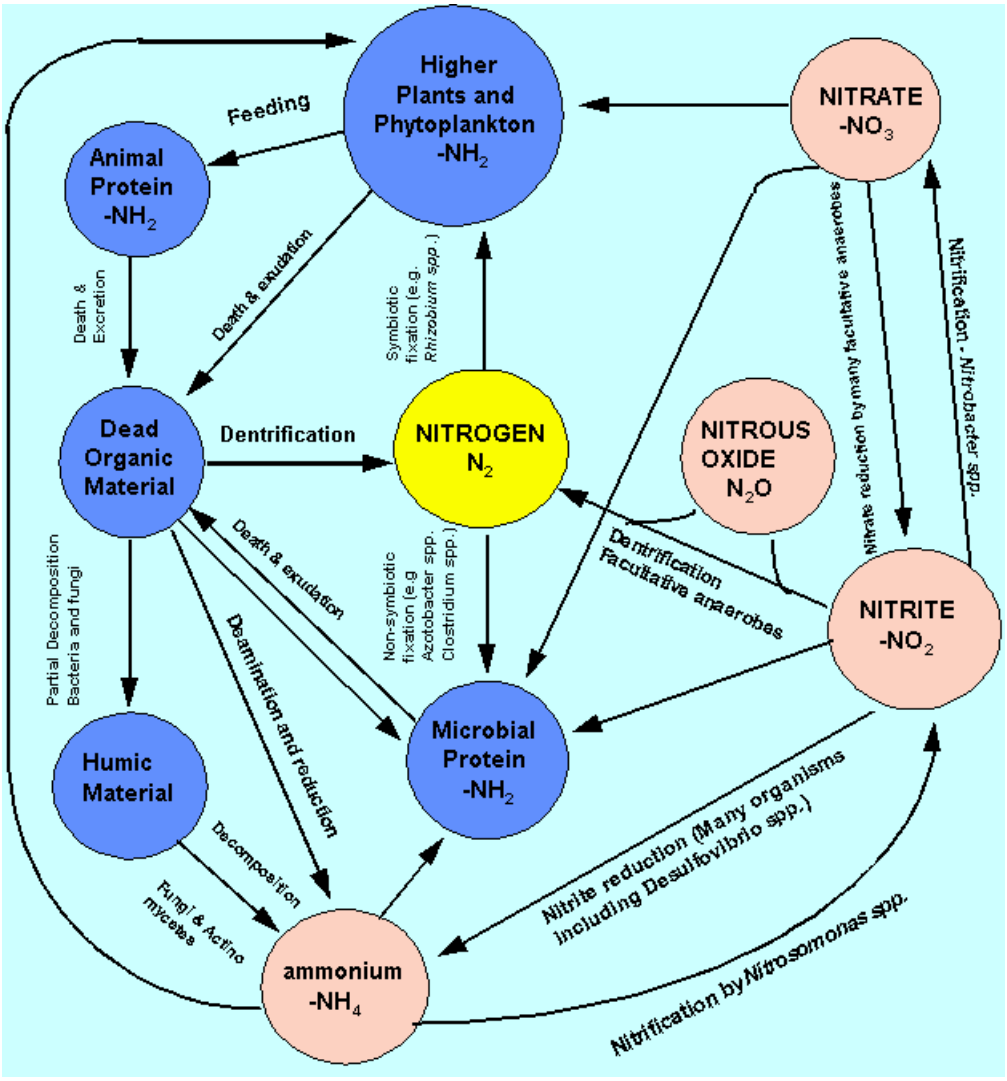

Fig. 1 Nitrogen Cycling by fungi takes detritus and converts it into NH_4.

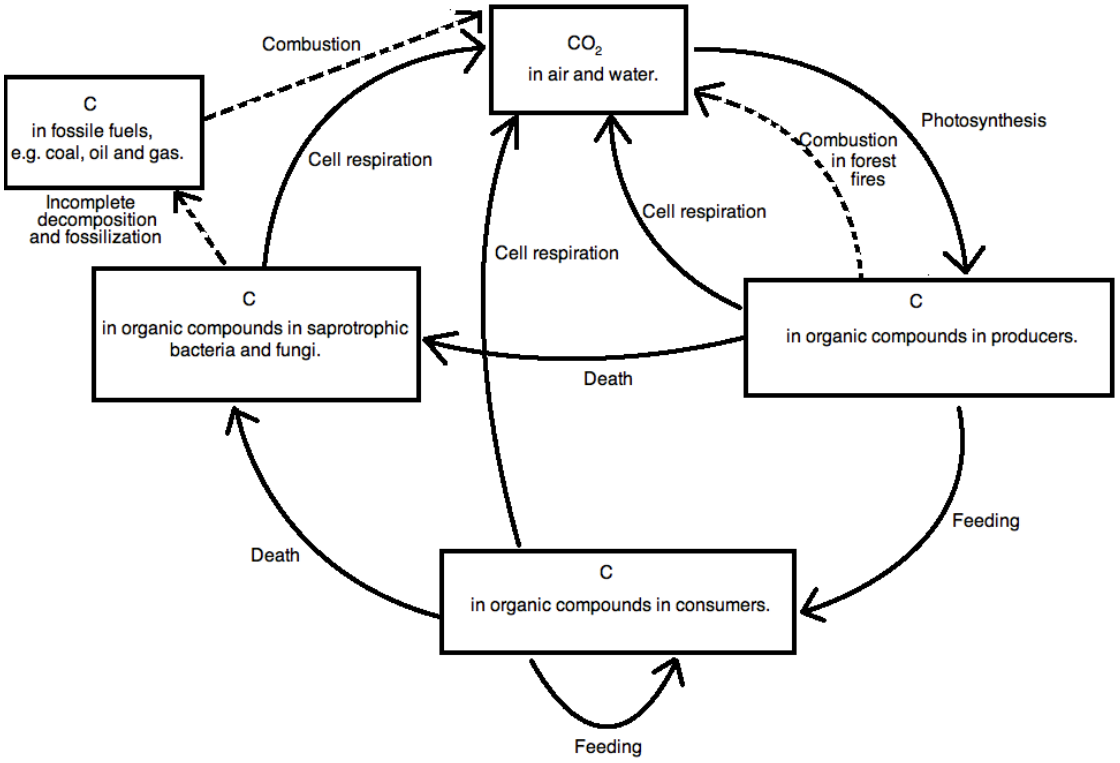

Fig 2. Saprophytic fungi harnesses the material of dead organic matter and releases it as either fixated carbon within the fungi itself or as CO_2.

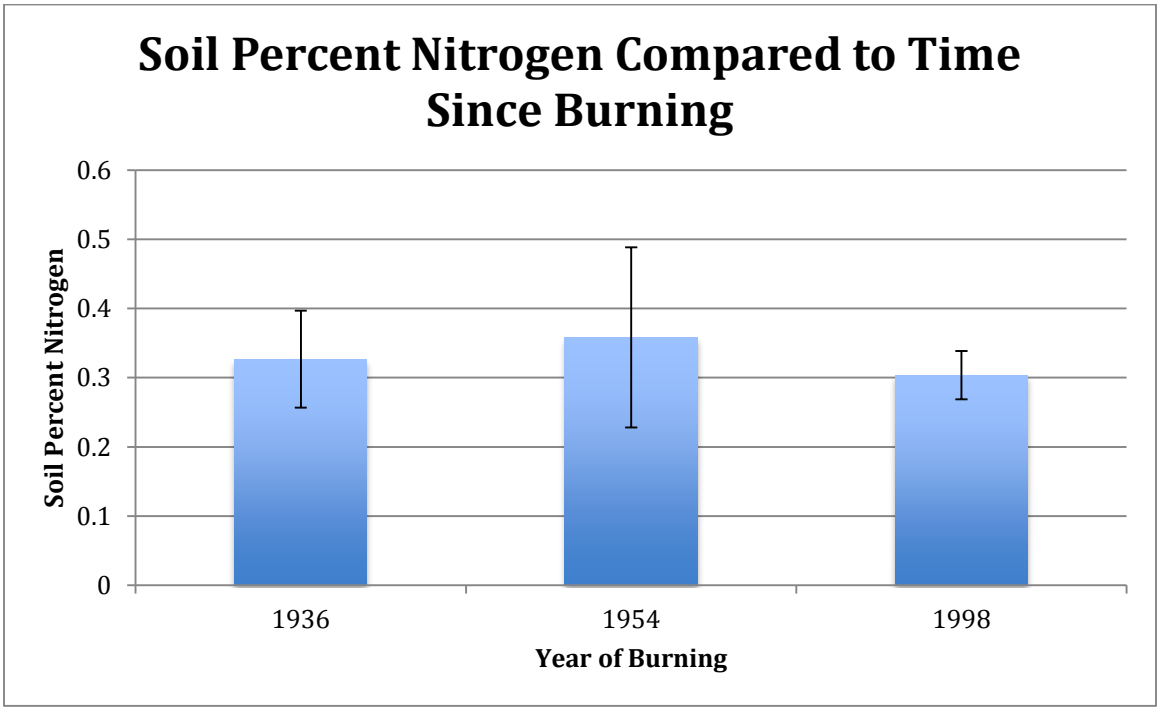

Fig 3. F = 0.948, p-value = 0.397, df = 2, 36. Statistically, Nitrogen averages are similar across the three burn plots.

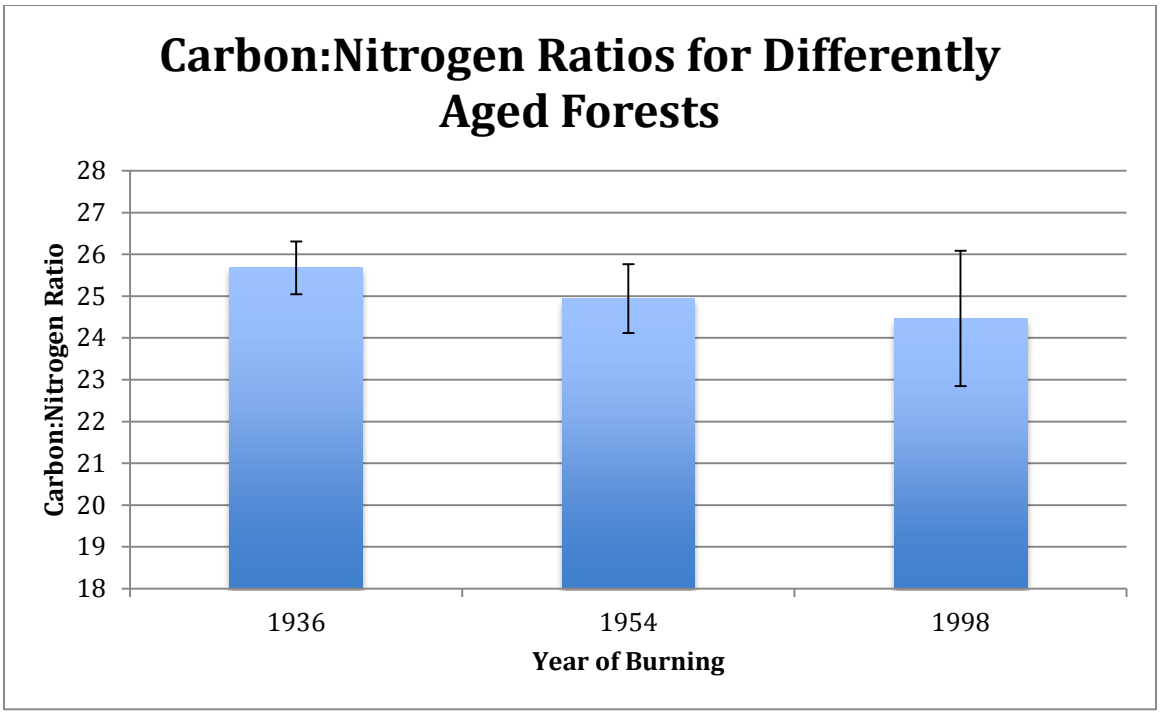

Fig 4. F = 0.772, p-value = 0.470, df = 2, 36. No relationship between ratios of Carbon and Nitrogen in each burn plot.

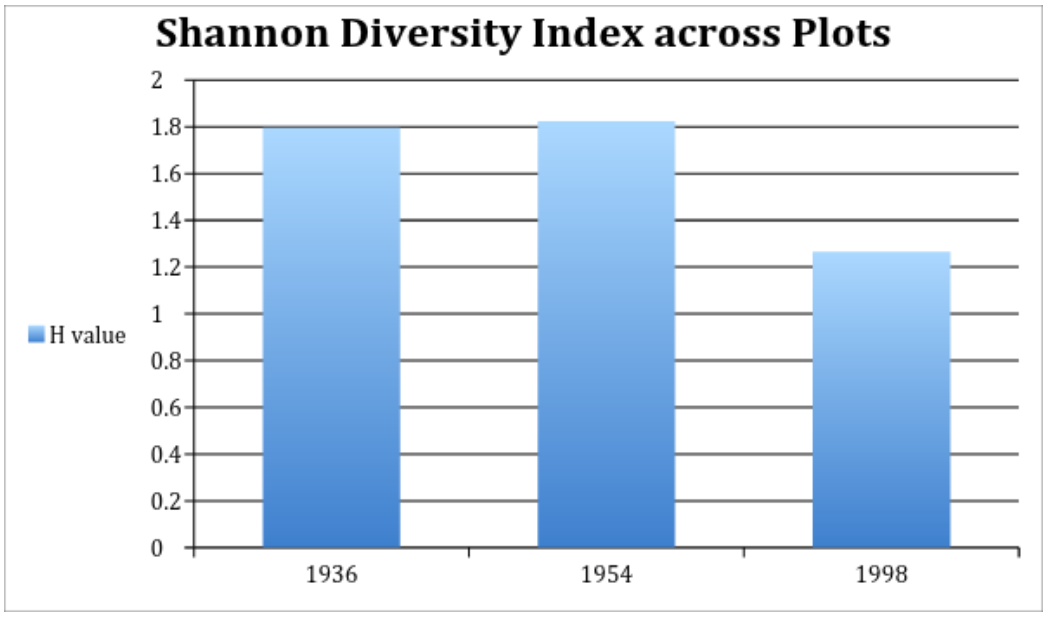

Fig 5. Specie richness and evenness is assessed and compared in each burn plot. No relationship between diversity and plot although p-value was close to significance.

	1936	1954	1998
Mycorrhizal	X^2=0.61	X^2=0.511	X^2=8.45
Decomposer	X^2=1.34	X^2=1.127	X^2=18.61

Fig 6. Chi Square Results prove that there is a correlation between functional groups and the 1998 plot. $X^2 = 30.659$, df = 1, $p < 0.0001$.

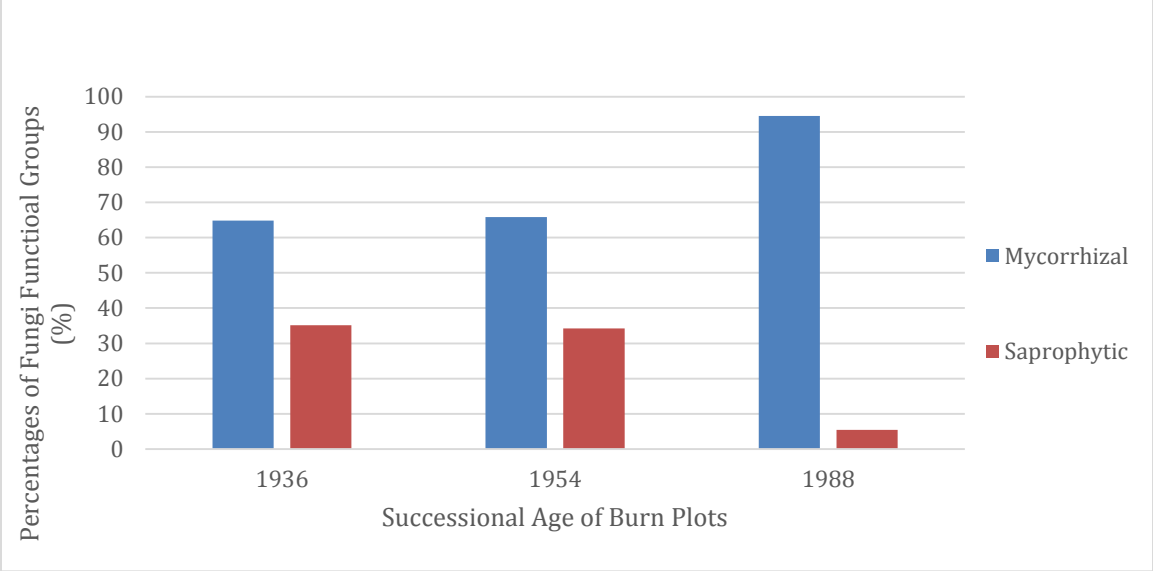

Fig 7. This plot expresses the functional group relationship to the age of succession in each plot. Decomposers for the 1998 plot were significantly less than expected.

References

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