

# The effect of forest age on the relative abundance of mycorrhizal and saprophytic litter and wood decay fungi in burned plots in northern Lower Michigan

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## ABSTRACT

In this study, we explored how the age of a forest (i.e. time since disturbance) affects which species and functional groups of fungi are most dominant and the relationship between this and nutrient content in the soil. Using three burn plots at the University of Michigan's Biological Station, from the years 1936, 1954, and 1998, we counted the numbers of mycorrhizal fungi and decomposing fungi along a number of evenly-separated transects. We also collected soil samples to measure the percentage of nitrogen in the soil and the carbon to nitrogen (C:N) ratio in the soil. We conducted analyses of variance on the fungal diversity we observed and the levels of nutrients in the soil and we calculated a chi-squared test to measure the differences in the relative abundances of mycorrhizal and decomposing fungi in the burn plots. While we did not find statistically significant relationships between nutrients in the soil and the fungal diversity we observed, we did find that the 1998 plot had significantly fewer decomposing fungi than the 1936 and 1954 plots. We concluded that various stressors on recently disturbed ecosystems – such as a lack of large, fallen trees, or openings in the canopy – would make decomposing fungi less abundant in younger forests. These results are important because we know little about fungi, especially which fungi are threatened in North America. Also, disturbances and their effects on species diversity are important to study, given that climate change is predicted to increase the frequency of disturbances.

## INTRODUCTION

When forests undergo a disturbance, succession occurs and makes fungi important in order to convert atmospheric nitrogen into nitrogen that is usable by the recovering plant community. Secondary succession of plant communities occurs as a result of disturbances in an area of vegetation, and it is defined as the process of growth that follows the disturbance (Suzuki, 2002). Fungi, especially mycorrhizal fungi, play a key role in succession by making soil more suitable to plant growth by fixing atmospheric nitrogen. Nitrogen availability is a constraint on primary production and plant community composition during primary succession. Nitrogen is the most important determinant of primary production early in the succession process. Because fungi depend on other organisms for their growth, fungal succession is related to plant succession at various scales (Suzuki, 2002). The succession of pyrophilous fungi after a forest fire is considered a macro-scale succession, during which different kinds of seres of fungi integrate into a plant community (Suzuki, 2002). It may take a period of several years or more for the fungal community to return to its pre-disturbance conditions (Suzuki, 2002). There are some contradictory results that have been published on fungal diversity at later stages of succession. Frankland (1992) found that there was a decrease in the diversity of mycorrhizal fungi as the forest was reaching the final stage of succession (Suzuki, 2002). However, Hart et al. (2013) found that older trees were associated with more fungal taxa and had communities that were notably different in composition from younger trees.

At the University of Michigan's Biological Station, where we conducted this study, there are a number of burn plots. Because we know the years in which these plots were burned, we know the ages of the plots under study. We examined 1936, 1954, and 1998 burn plots in particular. There are a variety of fungi characteristic of aspen-birch ecosystem, the upper conifer ecosystem, and the northern hardwood ecosystem that are present in these plots (Ostry et al.,

2011). These types of ecosystems include various mycorrhizal fungi and saprophytic litter and wood decay fungi. Some of the mycorrhizal fungi in the area of study are Aspen Bolete (*Leccinum aurantiacum*), Birch Bolete (*Leccinum scabrum*), the King Bolete (*Boletus edilus*), Golden Chanterelle (*Cantharellus cibarius*), False Chanterelle (*Hyrophoropsis aurantiaca*) (Ostry et al., 2011). The saprophytic litter and wood decay fungi include Oyster Mushrooms (*Pleurotus populinus*), Artist's Conk (*Ganoderma applanatum*), and Smoky Polyphore (*Polyphorus adustus*) (Ostry et al., 2011).

Because fungi are so important to nutrient cycling in forest succession, we studied the connection between the numbers of and relative abundance of certain types of fungi (mycorrhizal and saprophytic litter and wood decay) in burn plots of different ages. The purpose of this study was to examine the numbers, types, and relative abundances of different types of fungi in the 1936, 1954, and 1998 burn plots on the University of Michigan's Biological Station's property, and to see if the age of the forest (as determined by the year in which it was burned) has an effect on which fungi we see. We hypothesized that there would be a difference in the numbers and relative abundance of fungi among the three plots. We predicted that because of the importance of nitrogen fixation early on in succession and because nitrogen is less of a limiting nutrient later on in succession, that we would see relatively fewer mycorrhizal fungi than saprophytic litter and wood decay fungi in the older burn plots.

## METHODS

The three burn plots used in this study were from the years 1936, 1954, and 1998. The University of Michigan's Biological Station has a number of burn plots, and we chose these specific years because they spanned a long period of time, and because other plots were clear-cut or had been altered with other environmental investigations. In order to determine from where to

measure transects, we measured the length and width of each burn plot. By measuring out the width of the transect, we could study fungi found in transects spaced 20 meters apart and extending the length of the plot. We included all fungi within 2 meters in either direction of the transect line, counted each fruiting body that we saw, and marked down whether it was a mycorrhizal fungi or a saprophytic litter or wood decay fungi. In addition, we collected soil samples from 2-4 inches deep in the ground at various points in the burn plots on each day of the study to analyze the carbon to nitrogen (C:N) ratio.

To test whether or not there were differences in the relative abundance of types of fungi between the three burn plots, we calculated a Shannon Diversity Index for the three plots, and then conducted a statistical analysis of variance (ANOVA) to measure the difference in the Shannon Diversity Index across the three plots. In addition, we ground the soil from the burn plots and tested them for stable isotope composition with an element analyzer, EA: Costech Elemental Combustion Analyzer. With these datum, we ran an ANOVA to compare the soil percentage of nitrogen compared to the time since the disturbance, and another ANOVA to measure the variance in C:N ratios for differently aged forests. Also, by creating pie charts of the functional groups – one for each burn plot studied – we could compare the relative abundance of mycorrhizal and decomposer fungi across the three plots. Furthermore, we conducted a X2 analysis to compare the functional groups across the plots.

## RESULTS

In the ANOVA comparing the soil percentage of nitrogen against the amount of time since the disturbance, (Fig. 2 –  $F=.948$ ,  $p\text{-value}=.397$ , degrees of freedom=2, 36), we did not find a statistically significant difference in the mean percentage of nitrogen in the soil across the three plots studied. The 1936 plot had a mean of .32% N, the 1954 plot had a mean of .36% N, and the

1998 plot had a mean of .31% N. For the ANOVA comparing the Carbon:Nitrogen (C:N) ratios for the different plots ( $F=.772$ ,  $p\text{-value}=.470$ ,  $df=2,36$ ), there was no statistically significant difference in the C:N ratios across the three plots (Fig. 3). For 1936, the C:N ratio was .470, for 1954 the ratio was 24.9%, and for 1998 the mean C:N ratio was 24.5%.

When we calculated the Shannon Diversity Index for the three plots (Fig. 4), we found that the Shannon Diversity Index in the 1936 plot was 1.86, 2.03 for the 1954 plot, and 1.94 for the 1998 plot. From these values, we ran an ANOVA to compare the Shannon Diversity Indices across the three plots ( $F=3.967$ ,  $p\text{-value}=.080$ ,  $df=2, 6$ ), and we found that there was not a statistically significant difference in the species diversity across the three plots studied.

In addition, after summing the numbers of mycorrhizal and decomposer fungi observed during the three days of study, we ran a chi-squared ( $X^2$ ) analysis (Fig. 5 –  $X^2 = 30.659$ ,  $df=1$ ,  $p<.0001$ ) on the relative abundance of mycorrhizal and decomposer fungi. For the 1936 plot, for mycorrhizal fungi,  $X^2 = 0.61$  and for decomposers,  $X^2 = 1.34$ . For 1954, mycorrhizal fungi had an  $X^2$  value of 0.511 and a  $X^2$  value of 1.127 for decomposers. In the 1998 plot, for mycorrhizal fungi,  $X^2 = 8.45$ , and for decomposers,  $X^2 = 18.61$ . Thus, there was a statistically significant difference between the 1998 plot and the other two plots in regards to the relative abundance of fungi from the two functional groups counted.

## DISCUSSION

None of our results regarding the percentage of nitrogen in the soil or the C:N ratio for the soil were statistically significant. This was not what we expected; this may be due to the severity of the initial disturbance. Also, nitrogen recovery typically only takes 15-20 years, so it is possible that the fungal communities in the older plots did not reflect nitrogen levels or C:N ratios because they were too old for nitrogen and C:N ratios to have a significant impact

(Vitousek et al., 1989). There are other factors, related to the age of the forest instead of the chemical composition of the soil, which may be more likely to explain our results. According to LeDuc et al., (2012), who did notice a link between organic N pools and ectomycorrhizal fungi (EMF) community composition, it is possible that the connection that some have observed between nitrogen levels in the soil and the composition of the fungal community is related to other factors, such as changes in belowground allocation, or root density and fungal exploration type. Belowground productivity – colonization by hyphae from living roots might – may lead to a competitive advantage to fungi that invest in mycorrhizal network, which will affect fungal communities over time (LeDuc et al., 2012).

While the C:N ratios and the lack of statistically significant results was not what we expected, later-stage plant communities are less dependant on nitrogen and become increasingly dependent on carbon. Also, the form of nitrogen is important in terms of how it affects which plant and fungal communities develop (Vitousek et al., 1989). Some hypothesize that the decline of inorganic nitrogen and the increase of organic nitrogen will influence changes in EMF taxa during forest development (LeDuc et al., 2012). The LeDuc et al. (2012) study, as well as past studies such as Twieg et al. (2009) suggest that inorganic nitrogen alone is not a predictor for the successional shifts in EMF community composition.

We found that fungal diversity was highest in the 1954 plot. Though our results were not statistically significant, they were close to being statistically significant, which suggests that perhaps with repeated trials, a statistically significant result could be obtained. The higher diversity we observed in the 1954 plot was consistent with our findings that the 1954 plot had the highest nitrogen levels in the soil. This reflects the intermediate disturbance hypothesis, which states that local species diversity is highest during the middle stages of succession, when the

biological community is actively growing and recovering, rather than immediately after the disturbance or when a climax community has been reached. According to Brässer et al. (2011), the heterogeneity of dead wood available for wood-decaying fungi may be more impactful on species diversity than resource availability, such as the amount of dead wood or the nutrients in the soil. However, the availability of dead wood with a greater diversity of decay stages tends to be highest in old-growth forests (Brässer et al., 2011). Both the intermediate disturbance hypothesis and the Brässer et al. (2011) study support that the highest diversity would not be found in the 1998 plot. Furthermore, the larger the part of a fallen tree that is in contact with the ground, the more fruiting bodies that will be found on and around that tree (Brässer et al., 2011). The trees in the 1998 plot are relatively small in comparison with the trees of the 1936 and 1954 plots, and fewer of them have were experiencing decay at the time of our study.

Our results for the relatively low number of decomposing fungi in the 1998 plot were statistically significant compared to the expected proportions of the functional groups for the three plots. This is, at least in part, due to a high ratio of mycorrhizal fungi to decomposing fungi in the 1998 plot. In addition, the lack of large-diameter, decaying logs could have affected the low abundance of decomposing fungi in the 1998 plot, since large, decomposing logs are important for maintaining fungal diversity in woodland habitats (Brazee et al., 2014). Root density may also be a factor affecting the abundances of mycorrhizal fungi and decomposing fungi observed in the 1998 plot. According to LeDuc et al. (2012), because root density increases with the age of the forest, fungi with shorter hyphal exploration types may be more efficient and thus have an advantage over fungi with longer hyphal exploration types. Because the 1998 plot is younger than the other two plots, root length may not confer an advantage that could support decomposing fungi.

Our results finding that the fungal species diversity was lowest in the 1998 burn plot was not surprising, because multiple studies have shown that severe fires alter fungal community composition, especially when the canopy of the forest has been effected (LeDuc et al., 2012). Post-fire communities of ectomycorrhizal fungi have few taxa of fungi, and the same may be true for decomposing fungi (Treseder et al., 2004). Diversity tends to increase with stand age due to both the persistence of taxa in young sites and also because of the gradual addition of new taxa into the community (LeDuc et al., 2012). LeDuc et al. (2012) also found that the greatest change in EMF community composition and diversity happened before the canopy closed after a disturbance. They found that the re-closing of the canopy after a disturbance takes 19-23 years. The 1998 plot is the only plot young enough to be affected by a still-open canopy.

We used three plots in our study, and collected data on three separate days. With an observational study, as opposed to a controlled experiment, it is important to consider possible environmental factors that could have affected the data and our interpretation of it. It is possible that we accrued some sources of error that could have been lessened by using more plots, or plots with greater variability in age. Also, the 1998 plot was fenced in and much smaller in size. The fence may have led to differences in fungal species diversity due to indirect effects of foraging. Differences in patterns of fungal dominance have been recorded in studies where deer have been excluded for over four years. Deer may be able to influence fungal community structure through their effect on the aboveground vegetation composition and structure (Brazee et al., 2014). The aboveground vegetation may affect the heterogeneity of tree types available for decomposing fungi, and may also affect the mutualistic relationships that form between mycorrhizal fungi and plants. In addition, fruiting body production is heavily dependent on rainfall, which varied over the course of our study and could have skewed our results. Short-term environmental changes in



temperature and precipitation can create variety in data that would not appear in a controlled experiment (LeDuc et al., 2014). Along with considering sources of error in our study, it is important to consider our assumptions and their effect on our interpretation of the data. We assumed that no fungi were coming or going from the population (i.e. growing or dying). This is a way to gain consistency in studies of diversity and populations, but this assumption may not have been a fair one given that rainfall was inconsistent and may have led to the fruiting or deaths of fungal bodies.

Our findings that the relative abundance of decomposing fungi were lower than expected in the earlier stage of succession, represented by the 1998 plot, are important for issues of forestry, protecting biodiversity, and understanding the possible effects that climate change's increased disturbance regime can potentially have on ecological communities. For example, wood-inhabiting fungi are responsive to forestry practices that aim to promote the structural attributes of old-growth northern hardwood forests (Brazee et al., 2014). Organisms like wood-inhabiting fungi that require woody substrates for their survival are important to understand if these species are to be protected through forest management (Brazee et al., 2014). The effects of disturbance on fungal communities are also important to understand because aboveground and belowground communities are tightly intertwined (LeDuc et al., 2012), and because the natural disturbance regime of the northern hardwood zone of North America includes windstorms that lead to openings in the canopy, which, in turn, affect fungal communities (Brazee et al., 2014). It is predicted that climate change will lead to increased disturbances, and so the response of fungal communities to disturbances is relevant and prompts further research. In addition, intense forestry has had substantial effects on the structure of forests, which then affects the long-term survival of a number of species, including fungi (Bässler et al., 2011). Because wood-decaying

fungi are so sensitive to disturbances in forest structure, they make for good subjects of study on how increased disturbances will affect fungal succession (Bässler et al., 2011).

## CONCLUSION

From our results, we conclude that fungal diversity does not differ significantly in differently aged forests. The levels of soil nitrogen percentage and the C:N ratio did not significantly affect the fungal communities observed in our study. However, the age of the plot does seem to have a significant effect on the proportions of mycorrhizal and decomposer fungi present, with older plots having a larger proportion of decomposer fungi compared to plots in earlier stages of succession. However, there has not been much research on how soil microbial communities may change after disturbances, and the driving factors behind the subsequent succession (LeDuc et al., 2012). In addition, studies on fungal communities in Europe recorded finding fewer red-listed species in disturbed plots than in old growth plots (Brässer et al., 2011). A red list of rare and threatened fungi does not yet exist in North America. Further research could work to determine what fungi species are threatened, and what their response is to disturbance. This, in turn, can be used in forestry and to protect the biodiversity of forest communities.

## TABLES AND FIGURES

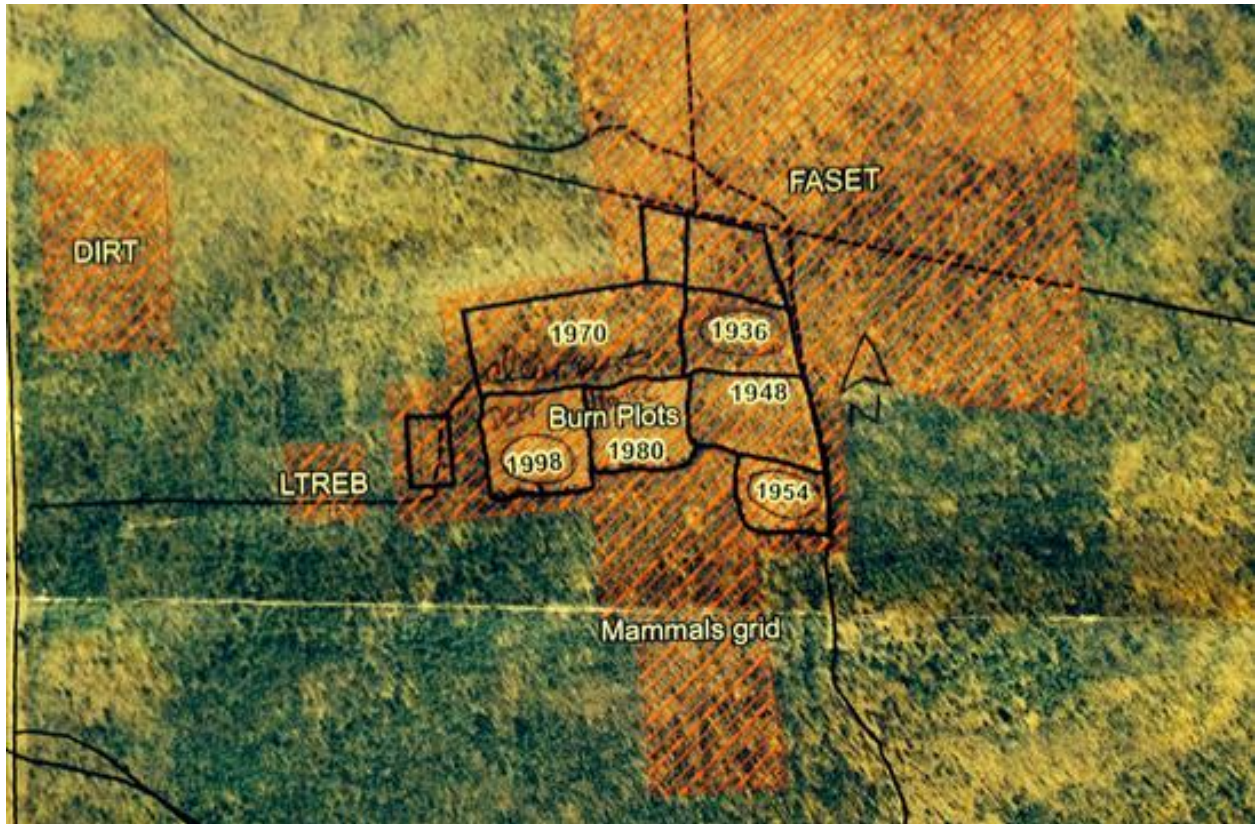


Fig. 1 – A map of the plots of various ages at the University of Michigan Biological Station. The plots included in this study were the 1936, 1954, and 1998 plots.

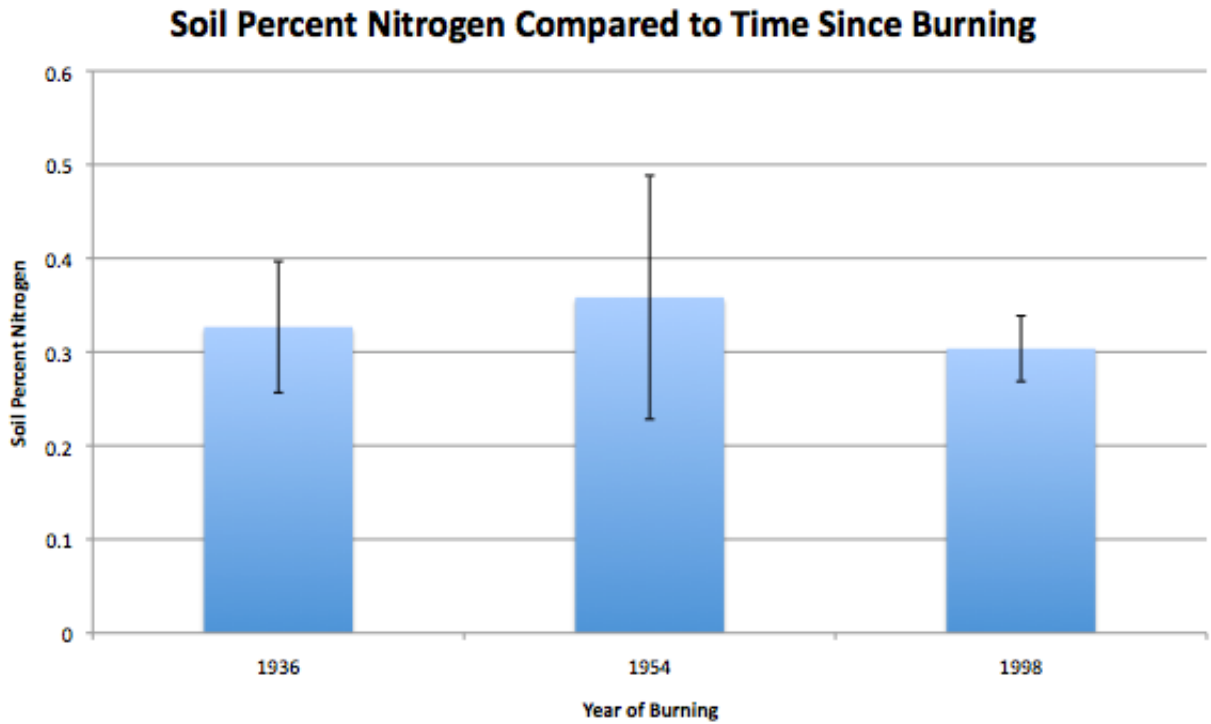


Fig. 2 – This graph shows the percent of soil nitrogen for the three different burn plots. There was not a statistically significant difference in the percentage of soil nitrogen based on the soil samples in our study.

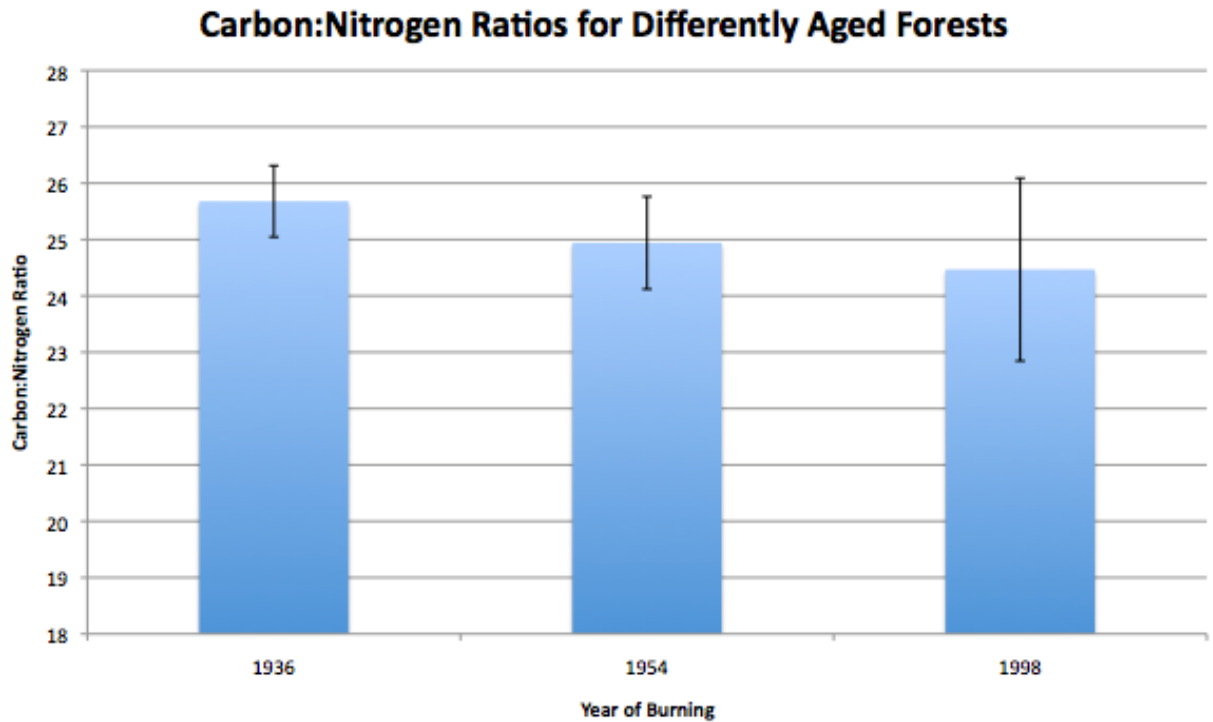


Fig. 3 – This graph shows the ratio of carbon to nitrogen in the three different plots. There was also not a statistically significant difference between the ratios of these nutrients in the three plots.

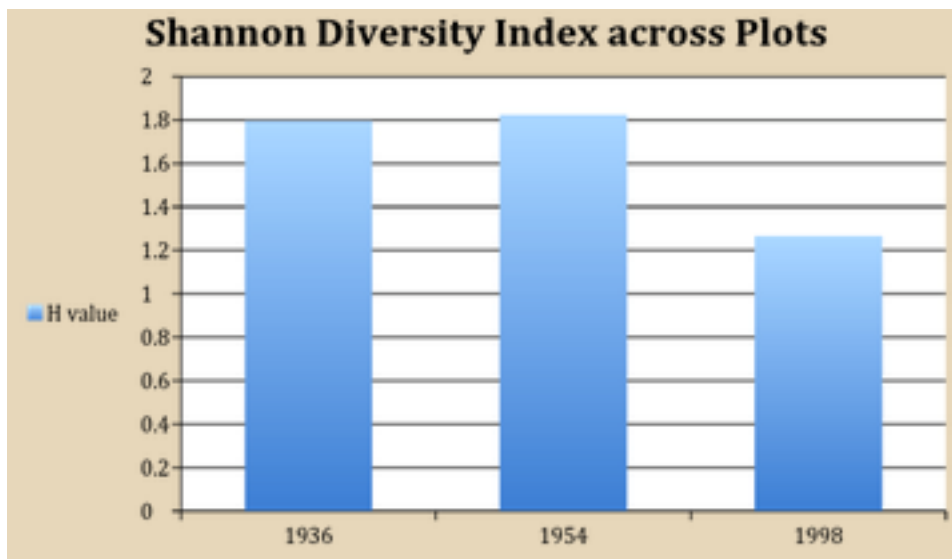


Fig. 4 – This bar graph illustrates the Shannon Diversity Index for each plot. Side-by-side, one can see that the 1954 plot had a slightly higher level of fungal diversity. However, the

differences in the Shannon Diversity Indices across the three burn plots was not statistically significant.

	1936	1954	1998
<b>Mycorrhizal</b>	$X^2=0.61$	$X^2=0.511$	$X^2=8.45$
<b>Decomposer</b>	$X^2=1.34$	$X^2=1.127$	$X^2=18.61$

Fig. 5 – This is the results of the chi-squared analysis that we ran on the numbers of mycorrhizal and decomposing fungi in the three plots. The 1998 plot showed a statistically significant difference in the relative abundance of decomposing fungi. The 1998 plot had a relatively low number of decomposing fungi.

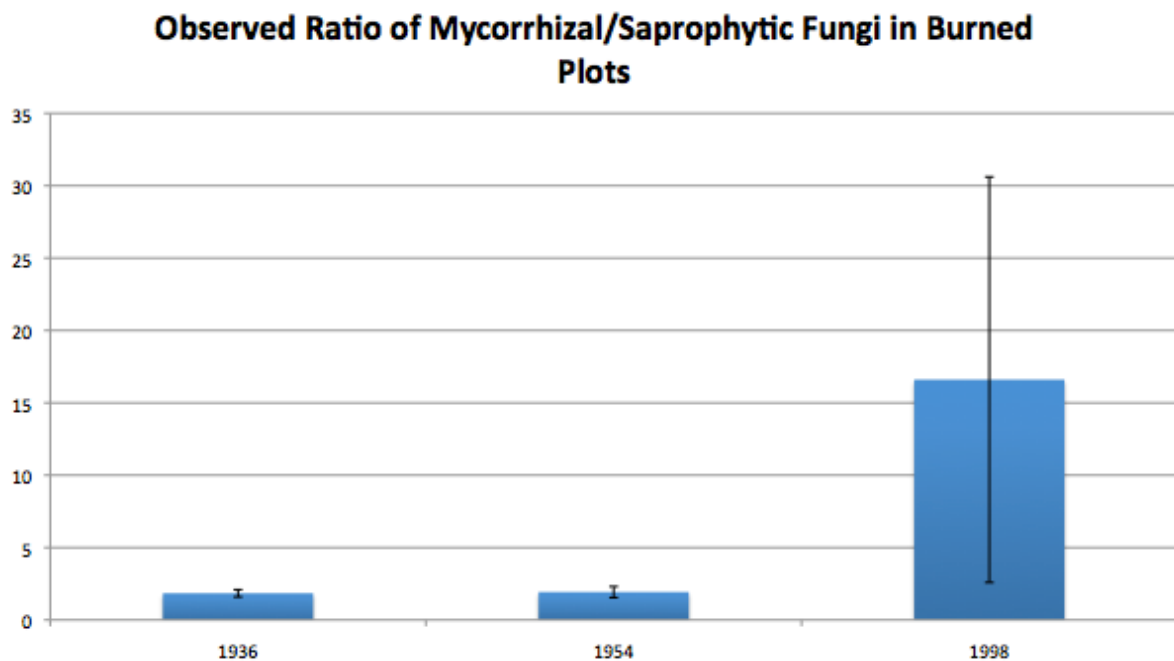


Fig. 6 – This graph shows the observed ratios of mycorrhizal to decomposing fungi in the plots studied. The 1998 bar indicates a high ratio of mycorrhizal to decomposing fungi.

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