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THE CHANGING FUNCTIONS OF FUNGI IN FOREST SUCCESSION

<u>ABSTRACT</u>

The ecological roles of fungi in forest settings are incredibly diverse, but two of the most crucial services provided by members of this kingdom include fixation of nitrogen into forms useable by plants and decomposition of organic matter to allow the recycling of certain nutrients into the soil. As forests recover from disturbances such as burning, different nutrients become limiting to plant growth at different stages of succession—for example, nitrogen tends to be a limiting factor in younger forests. Our group hypothesized that nitrogen-fixing species of fungi (i.e. mycorrhizae) should be more abundant than decomposing (i.e. saprophytic) species in younger forests due to the competitive advantage in resource availability given to plants who had formed mutualistic relationships with mycorrhizae; in other words, we predicted that the primary function of fungi in a forest ecosystem would shift from mycorrhizal to decomposing as the forest increased in age. To test this, we performed a transect of three differently aged forests, represented by "burned plots" in various stages of secondary succession, and categorized each species of fungi found as either mycorrhizal or saprophytic. Additionally, we took soil samples from all forests to establish the relative ratio of carbon to nitrogen in each. Though no significant differences in soil nutrient percentages were found between the different plots, we found a significantly higher proportion of nitrogen-fixing:decomposing fungi in the youngest plot sampled. This result indicates that the primary function of fungi in a successive forest may indeed change from mycorrhizal to saprophytic (or, at least, less mycorrhizal) as the forest reaches different stages of recovery and that this change is strongly reflected in the composition of the fungal community, though several factors limited our ability to wholly assess the dominant functional group of fungi present in any particular forest. The capability to understand the changing dynamics of fungal function and distribution as compared to the specific needs of a particular ecosystem could be valuable for researchers attempting to describe the vast majority of fungal species that remain unknown yet still play unique, critical ecological roles in their environment.

<u>INTRODUCTION</u>

The kingdom Fungi encompasses a wide range of organisms including yeasts, molds, and mushrooms—many of which perform very unique, specialized ecological services for their particular ecosystem (Bjorn *et al.* 2006). These services include, but are not limited to, organic decomposition, nutrient cycling, pathogen distribution, and mineral transformation; as a result, fungi are critical to the establishment and prosperity of many types of ecosystems (Gadd 2007).

Though thousands of species of fungi have been classified and described, it is estimated that this represents less than five percent of the total species in the world; in addition, the functions and interactions of many of the species already described remain cryptic (Ostry *et al.* 2010). Gaining a better understanding of how sensitive certain types of fungi are to gradual changes in their ecosystem could aid in describing new species of mushrooms and clarifying the functions of others, tools which would undoubtedly be of use considering how essential members of this kingdom are to the success of their environments—particularly in forest settings.

The development of a forest community on a novel surface, such as after a disturbance event (e.g. burning), occurs in several stages. Initially, the soil contains a very low amount of organic material, and due to this feature the availability of useable nitrogen in the forest is a limiting factor to plant growth and net primary production in general (Cain *et al.* 2014). As time passes, more nitrogen is gradually deposited into the substrate via fixation and decomposition; at this point, other elements, such as phosphorus or carbon, become limiting to the composition and prosperity of the plant community.

Though the ecological services provided by fungi can be wide-ranging, two specific types are absolutely critical to the development of a forest ecosystem: nitrogen fixation and organic decomposition (Bjorn *et al.* 2006). The former service is accomplished in part by a subclass of

fungi called mycorrhizae, which harbor bacteria capable of converting inert atmospheric nitrogen into forms useable by other organisms (e.g. ammonium) (Paul *et al.* 2007). For this reason, mycorrhizae form a mutualistic relationship with the roots of many plants species, including trees, which provide the fungi with easy access to water and carbohydrates in return. A separate functional group of fungi, known collectively as the decomposers (i.e. saprophytes), grow on and inside decaying plants as they break down organic remains into re-useable forms.

Due to the constant flux of soil chemistry and organism composition in a forest undergoing secondary succession, different stages of forest development seem to require different levels of certain ecological services. Some of these services need to be performed by the members of its community in order for the community to thrive—but how strongly are these differential needs reflected in the relative abundance of certain service-providers? Our objective in this study was to determine whether the primary ecological function of fungi in a forest ecosystem changes significantly as the forest reaches different stages of development. Since soil nitrogen tends to limit forest growth early in succession, we hypothesized that, in younger forests, most of the fungal species observed would fall into the category of nitrogen-fixing mycorrhizae, as plants who had formed a mutualistic association with these fungi would have an advantage in resource availability over those who did not and therefore such mushrooms should be widespread. Conversely, given that as forests age elements such as phosphorus and carbon become more limiting than nitrogen, we predicted that saprophytic species of fungi capable of recycling these elements back into the soil would be more prevalent than nitrogen fixers in older forests.

METHODS

We selected three "burned plots" located on the University of Michigan Biological Station property, Pellston, Michigan (Fig 1). The roughly rectangular plots had been cut and burned at various points in the past and allowed to grow back with limited interference. The three plots we chose for our study were burned in 1936, 1954, and 1998—since a forest typically requires over 100 years to reach climax following a disturbance event, the temporal spacing between these plots was intended to represent different stages of recovery during secondary succession.

The plots varied in size but all were smaller than 10,000 square meters. In each plot, we set up a straight transect line from the northeast corner towards the northwest corner, ending the line 100 m from the northeast boundary. Following this, we performed 80 m transects directly south from the transect line, spaced 20 m apart and beginning 10 m away from the eastern edge of the plot to avoid complications associated with edge effects (Finney 1948). The exception to these dimensions was the 1998 plot, which had an 80 m (E to W) by 50 m (N to S) deer fence located in the middle. In this case, we altered the transect to fit within these dimensions. In total, the transects for the 1936 & 1954 plots covered 1,600 m^2 as compared to 800 m^2 in the 1998 section.

We counted all above-ground fungi within 2 m to either side of our transect lines and classified them as either mycorrhizal or saprophytic. We performed the classification using either direct identification of species or through characterization of particular features associated with one of the groups, such as extraradical hyphae or fruiting bodies for mycorrhizal species (Smith 2008). Additionally, we took five soil samples from each plot in a staggered distribution along

our transect lines in an effort to determine the total soil nitrogen and carbon compositions of the different plots.

We repeated the transects and soil samples three times per plot, on July 23rd, 25th, and 30th, 2014. After collection, we dried, ground, and submitted the soil samples for analysis of total nitrogen and carbon percentages by weight.

We ran three separate ANOVA tests in an effort to see whether the soil nitrogen or carbon contents or the carbon:nitrogen ratio were different in any of the plots. Additionally, we calculated the Shannon Diversity Index for each plot using the species of fungi that we were able to identify and used an ANOVA test to check for significant differences. Finally, we performed a chi-squared test to determine if the abundance of a particular functional group of fungi was significantly higher or lower than expected in any of the plots.

RESULTS

The mean soil nitrogen percentages ranged between 0.304% (1998 plot) and 0.358% (1954) (Fig 2). Mean soil carbon content followed a similar trend, with the lowest percentage (7.483%) occurring in the 1998 plot and the highest (11.273%) in the 1954 (Fig 3). The ratio of carbon:nitrogen appeared to increase with increasing forest age, the highest ratio (25.679) found in the 1936 plot and the lowest (24.468) in the 1998 plot (Fig 4). After conducting our ANOVA analyses, we found none of these differences in mean values to be significant (**N**: $F = 0.948$, df = 2, $p = 0.397$; **C**: $F = 0.839$, df = 2, $p = 0.441$; **C/N**: $F = 0.772$, df = 2, $p = 0.470$).

Fungal species diversity, as calculated using the Shannon Diversity Index, peaked in the 1954 plot ($H = 1.823$), followed by 1936 (1.795), and finally the least diverse plot, 1998 (1.265) (Fig 5). We also found the ANOVA test comparing the mean Shannon Diversity Index values

for each plot to fall short of significance (F = 3.967, df = 2, p = 0.080). The most common

species of fungi found in the 1936 plot were the mycorrhizal Golden Chanterelle (*Cantharellus*

cibarius), King Bolete (*Boletus edulis*), and the saprophytic Artist's Conk (*Ganoderma*

applanatum); in 1954, we found Golden Chanterelle and the decomposer Smoky Polypore

(*Bjerkandera adusta*) to be the most abundant; finally, we observed the Aspen (*Leccinum*

insigne) & King Bolete most often in the 1998 section.

In each of the three plots, we observed more mycorrhizal species than saprophytic and

the highest ratio of mycorrhizae:saprophytes in the 1998 plot. The Chi-Squared analysis

comparing the expected proportions of fungi functional group to the actual proportions found

indicated a significant different between the plots (X^2 = 30.659, df = 1, p < 0.0001) (Fig 6).

<div align="center">DISCUSSION</div>

The lack of significance in comparing any of the three soil nutrient categories between

the plots was inconsistent with our predicted model of nutrient accumulation in a forest

undergoing secondary succession following a disturbance, though some of the general trends did

appear to follow the expected pattern of nitrogen limitation early in succession and carbon

limitation later on. Soil nitrogen levels were found to be lowest in the 1998 plot, a result that

correlates with the expected general dynamics of succession, but the fact that we did not find the

nitrogen percentage to be significantly lower than the other plots suggests that this particular

forest had been disturbed long enough ago to be able to recover in terms of soil nitrogen content.

This finding is consistent with previous studies that found soil N recovery time in secondary

succession to be 15-20 years (Brown & Lugo 1990); additionally, the higher-than-expected N

content from the 1998 plot may be related to the significantly higher proportion of nitrogen-

fixing fungal species found in the plot, though cause and effect are difficult to assess in such a

situation.

The fact that soil carbon content followed a similar chronological trend as soil nitrogen is

unsurprising, as it has been shown that the rate of nitrogen accumulation in a successive situation

controls the rate of carbon accumulation (Knops & Tilman 2000). This may also explain the

lack of significance in the differences in carbon percentages between the soil samples.

Both soil nitrogen and soil carbon percentages peaked in the 1954 plot, a finding that is

consistent with prior studies that indicated the highest rate of nutrient turnover during forest

succession occurring in actively-growing, intermediate stage forests, which have a balance

between decomposing organisms and consistent plant uptake of recycled nutrients (Vitousek &

Reiners 1975).

The resulting C/N ratios calculated from these data, which we found to increase with

increasing forest age, appear to directly oppose our prediction of which elements limit plant

growth during different stages of succession. This finding may be due to the nature of burning as

a disturbance event, which is unique from other events that set in motion forest succession in that

levels of useable nitrogen immediately spike following the fire and then gradually taper off

before the relatively swift recovery (Antos *et al.* 2003). Additionally, different types of plants

require different ratios of carbon to nitrogen to grow; since a forest undergoing succession is

constantly experiencing a change in the composition of its plant community, it is reasonable to

assume that the relative proportion of carbon to nitrogen could continually increase as the

primary limiting nutrient of the ecosystem switches from the latter to the former (Prahl *et al.*

1994).

Our Shannon Diversity Index values indicate that the highest fungal species diversity occurred in the 1954 plot, which we utilized to represent the "intermediate" stage in forest recovery from disturbance. This result corresponds with our soil composition data, as an actively growing forest with high levels of available nutrients in the soil will typically contain higher species diversity than both younger forests, which are dominated by quick-growing "pioneer" species, and older ones, which tend to be composed mainly of a few stable tree species (Drury & Nisbet 1973). The results of our analysis on the diversity values fell just short of statistical significance, however, likely due in part to our inability to correctly identify every species of fungi encountered within our transect areas. Specimens of this type were grouped together as "general mycorrhizal" or "general saprophytic" and these two categories were not included in our calculation of the Shannon Diversity Index, a process which therefore reduced the number of species available to use in our calculation.

Our chi-squared test comparing the expected and actual proportions of mycorrhizal and saprophytic fungi found in each plot indicated a significant difference, with the most noticeable discrepancy occurring in the 1998 plot. Our transects of the youngest forest showed a higher proportion of mycorrhizal species present and a much lower presence of saprophytic fungi than expected based on null proportions from a compilation of all fungi found from the three plots. This difference corresponds with our initial hypothesis that plants forming mycorrhizae with fungi will be at a competitive advantage as compared to plants who have not formed such a mutualistic relationship and thus should have less access to nitrogen in the soil (though the soil nitrogen differences were not found to be significantly lower in the 1998 plot, this is not necessarily an indicator that nitrogen is not a more limiting nutrient for the forest, as previously discussed).

Our overarching question in this study concerns the primary function of all species of

fungi in a forest community as that forest progresses in its recovery from a disturbance event.

Based on the results of our particular method of assessing changing functional groups of fungi, it

appears that our hypothesis is supported in that the ratio of mycorrhizal:saprophytic fungi

decreases as forest age increases and that the mechanism behind this shift is a mutualistic

resource advantage amongst different trees. Such a finding appears to support Bruno *et al.*'s

(2003) hypothesis that facilitation can be as strong of a driver of ecological processes as any

other type of interspecies interaction, and also indicates that the diversity of fungi can be very

sensitive to the particular needs of the forest community in which they are found. Despite these

encouraging results, the main conclusion reached by our group is that our method of data

collection may have been too limiting to properly assess fungal dynamics in a successive forest.

We focused entirely on classifying above-ground fungal activity, which may not always be

representative of the total fungal community in a particular area and thus may not be an accurate

assessment of the dominant functional group present (Gardes & Bruns 1995). Additionally,

many species of fungi are not so easily classified into simple functional groups; some classes,

especially ectomycorrhizae, can perform nitrogen-fixation services as well as some

decomposition services for their ecosystem, a quality which may add confusion as to the primary

function of such a specimen (Read 1991).

Though these limitations represent significant hindrances in the conclusions of our study,

the versatility of fungi as ecological service-providers and their apparent sensitivity to the needs

of their community as opposed to merely their own requirements, a trait potentially facilitated by

the powerful ecological force of mutualism, remain intriguing areas for future study. A full

analysis of all fungi within an ecosystem (i.e. both above- and below-ground), coupled with the

ability to properly classify all the ecosystem services provided by each species and access to

many different types and stages of successive forests, could be useful tools in attempting to more

clearly define the overall role and importance of fungi in a community—findings which could be

of immense use in helping to define and characterize the members of this incredibly important
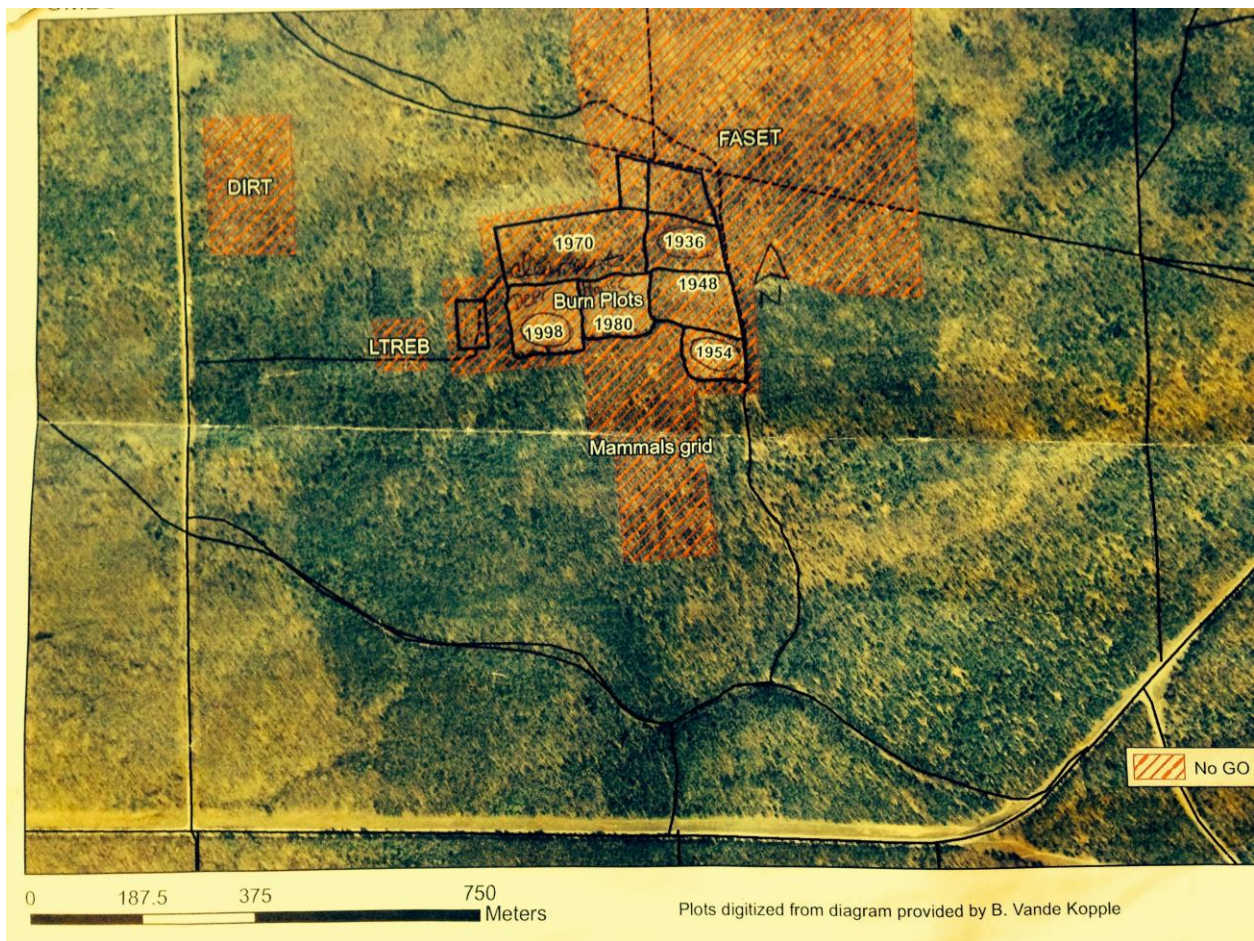
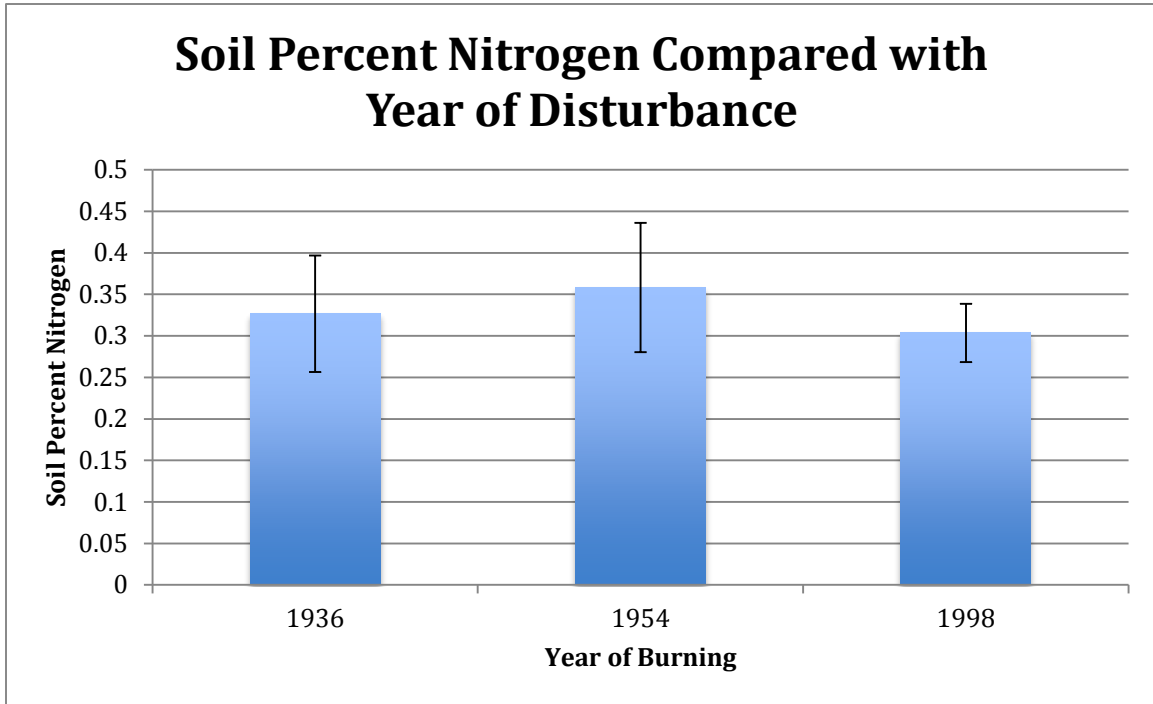yet persistently enigmatic kingdom.

FIGURES

Figure 1 - Map of UMBS Burned Plots

Soil Percent Nitrogen Compared with Year of Disturbance

Figure 2 – Soil Nitrogen Content vs. Forest Age

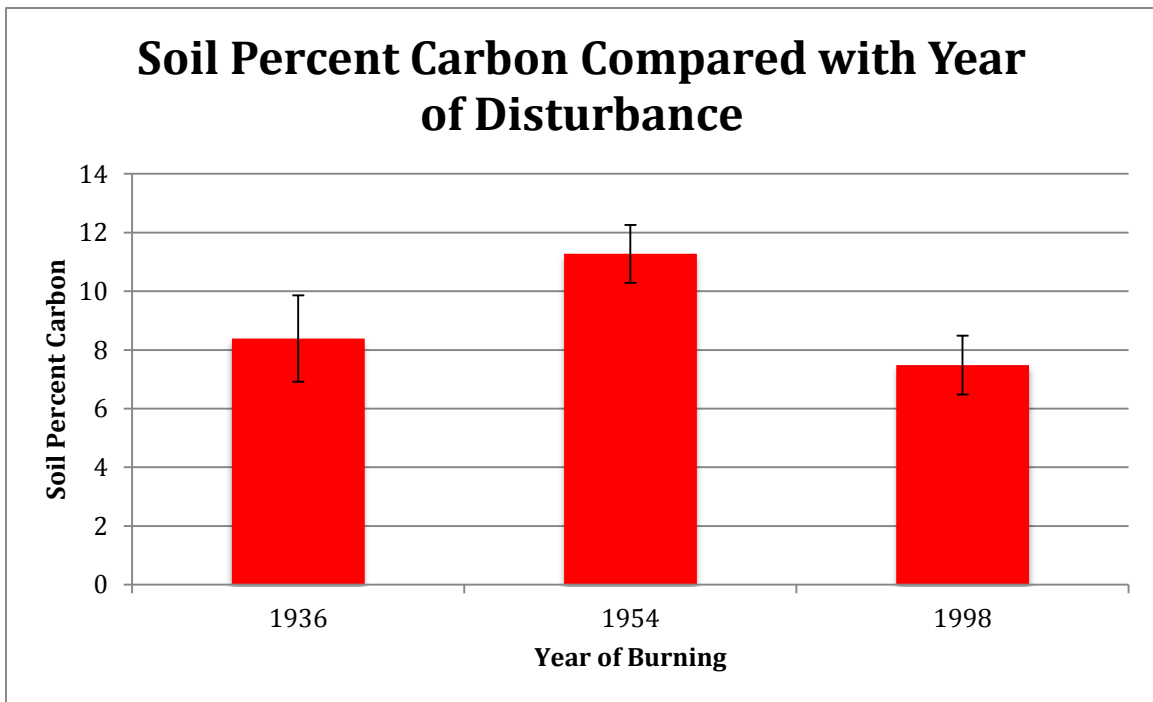## Soil Percent Carbon Compared with Year of Disturbance

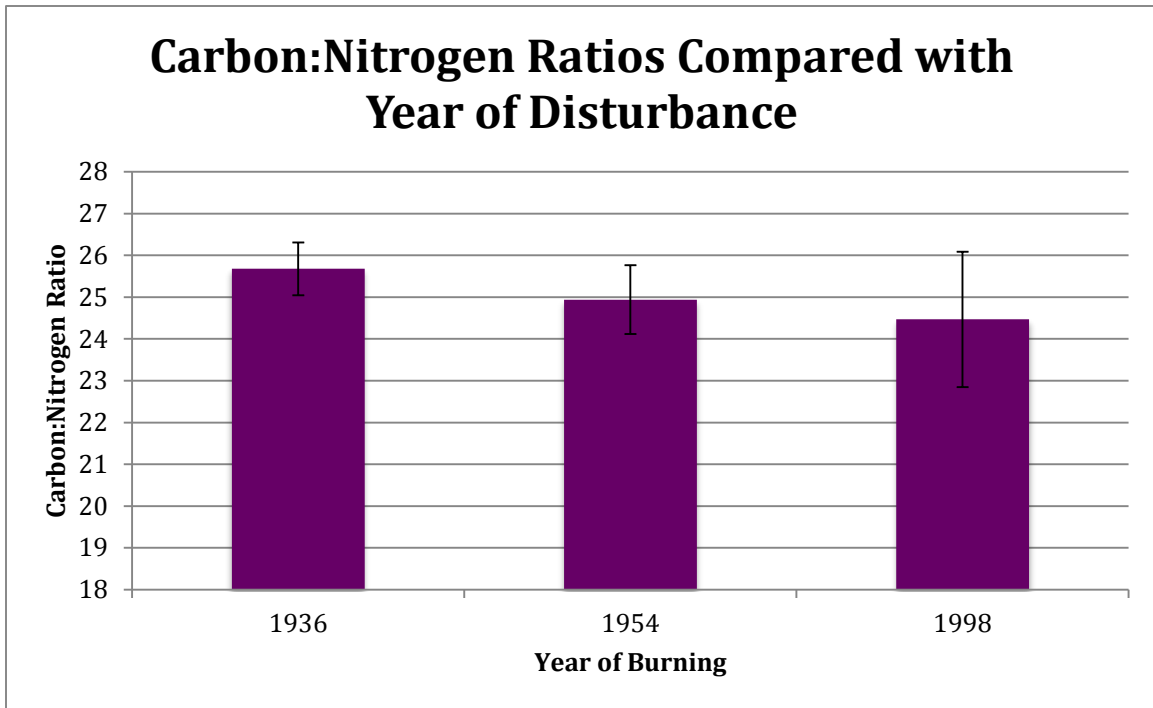Figure 3 - Soil Carbon Content vs. Forest Age

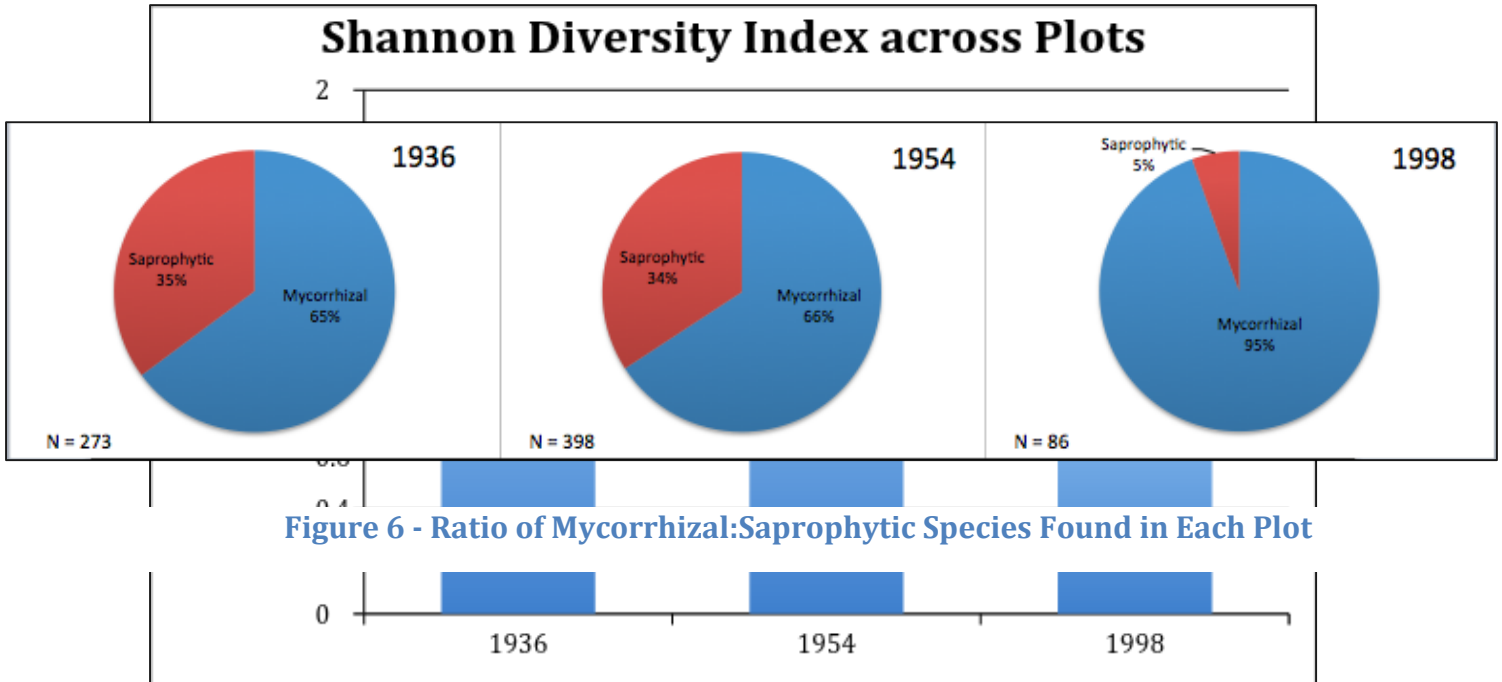**Figure 4 - Soil Carbon:Nitrogen vs. Forest Age**

Figure 6 - Ratio of Mycorrhizal:Saprophytic Species Found in Each Plot

Figure 5 - Diversity Index Values vs. Forest Age

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