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Fungal Diversity and Abundance and Nitrogen Accumulation during Forest Succession

Abstract

Succession following a disturbance event can radically alter a forest's ecosystem. Although many aspects of the community are affected, we chose to analyze the effect succession has on nitrogen levels and fungal diversity and functional groups. We looked at three plots that had each been burned once to study this relationship. At the three sites, we analyzed soil for percent nitrogen levels and did a survey of mushroom species composition, specifically focusing on the functional groups of fungi, mycorrhizal and wood-decomposers. We determined Shannon diversity indices for all the species across the three plots and ran a Chi-squared test to see if the proportion of each functional group was the same throughout the sites. Our soil data showed no significant difference in mean percent nitrogen levels across the three plots. Furthermore, there was no meaningful difference in species diversity, but there was a significant difference in the proportion of functional groups based on the burn year. Although our results were insignificant, other research has indicated that nitrogen levels peak at intermediate stages when organic leaf litter is high, but coarse woody debris is low. Furthermore, we concluded that early successional stages are associated with low fungal species diversity due to low leaf litter and availability of dead wood. Finally, we determined that younger forests have proportionately higher levels of mycorrhizal fungi than wood-decomposers due to the lack of coarse woody debris on which decomposers can live. Having such knowledge about nutrient availability and fungal succession is important for forest management, particularly for understanding the short and long-term consequences a prescribed burn can have on the ecosystem.

Introduction

Succession following disturbance events in a forest, such as fire, can alter the species composition and nutrient cycling ability of that forest. For example, fungal diversity and abundance have been found to be significantly lower immediately following a burn (Neary et. al 1998). Because of their ability to decompose and fix nitrogen, fungal species can affect the presence of such limiting nutrients.

Treseder et al. (2004) discovered that nitrogen was most available in older forests, with nitrogen levels being correlated with the presence of organic litter. Organic soil accumulates over time as plants begin to die and lose their leaves. The leaves in turn increase the amount of nitrogen available to reenter the system, thus leading to a high nutrient cycling capacity (Treseder et al. 2004).

Furthermore, high organic litter and subsequent nitrogen levels have been correlated with the presence of mycorrhizal fungi (Treseder et al. 2004). Mycorrhizal mushrooms make up an important functional group of fungi because of their ability to fix nitrogen from the soil and return it to the system (Treseder et al. 2004). In terms of succession, Read (1999) hypothesized that certain mycorrhizal mushrooms, such as Bolete Mushrooms (*Boletus sp.*), should become more dominant after the first 15 years of succession. Bolete Mushrooms are considered ectomycorrhizal fungi (ECM), a relevant subset of the mycorrhizal group (Allen et al. 2011). ECM fungi produce enzymes that are specialized for breaking down organic nitrogen and thus out-compete other types of mushrooms as more organic litter accumulates (Tresseder et al. 2004).

In addition to mycorrhizal mushrooms, wood-decaying fungi is an important subset of mushrooms that can be impacted by disturbance events. Previous studies have demonstrated that

Coarse Woody Debris (CWD) increases gradually during succession after early tree species are out-competed and begin to die (Sefedi et al. 2010). Further, areas with rich CWD have been associated with a high abundance and diversity of wood decaying fungi (Edman Et al. 2004).

For this study, we wanted to examine the relationship between succession time, nitrogen availability, and functional groups of fungi, specifically mycorrhizal and wood-decomposing mushrooms. Because organic litter accumulates over time and contains limiting nutrients, we expected to see a positive correlation between time and nitrogen levels.

We also predicted to see a difference between the proportion of mycorrhizal and decomposing fungi over time. We hypothesized that there would be a higher percentage of mycorrhizal mushrooms compared to decomposers in young forests because dead trees accumulate later during succession, and thus decomposers lack the proper habitat in early developing forests. Furthermore, because of the increased number of decomposers in later aged forests, we predicted to see higher species diversity in such forests.

Finally, we predicted the presence of ECM mushrooms to be correlated with organic litter accumulation because their enzymes give them a competitive edge in breaking down organic nitrogen. Therefore, we expected ECM fungi to be most dominant in older forests where there are larger organic litter layers.

Methods

We chose to survey three plots on University of Michigan Biological Station property that had each been burned once. In order to maximize the gradient in age between the plots, we chose plots that were burned in 1936, 1954, and 1998. For the 1936 and 1954 plots, we conducted five transects spaced 20 meters apart, going 100 meters down the plot. The 1998 plot had less square area and we therefore applied four transects spaced 15 meters apart, going 60 meters down the plot. The first and last transects were each 10 meters away from the edge of the plot in order to reduce edge effect.

We surveyed each plot one time on three different days (July 23rd, July 25rd, July 30th 2014). For the 1936 and 1954 plots, we collected five soil samples each day diagonally across the plot (10 meters up by 10 meters out, 30 meters up by 30 meters out, etc.). We used the same method to collect 4 samples from the 1998 plot. After collecting, we dried, ground, and analyzed the soil samples for total percent nitrogen levels. Finally, we applied a one-way ANOVA to compare the mean levels of nitrogen between the three plots.

To measure fungi abundance and diversity, we identified each individual mushroom that fell within two meters of either side of a given transect. We determined the four most common species of mycorrhizal mushrooms and the three most common species of decomposer mushrooms and used those species counts to determine the Shannon diversity index values for each plot. Additionally, we ran a one-way ANOVA on the three indices to determine if there was a significant difference in the values.

Finally, we categorized the different species of mushrooms according to functional groups: Mycorrhizal or wood-decomposing mushrooms. After grouping, we ran a Chi-Squared test to determine if the proportions of functional groups were the same between the three plots. We then broke down our mycorrhizal group to determine which species were considered ECM fungi and which plot contained the highest proportion of such fungi.

Results

The one-way ANOVA comparing mean percent nitrogen levels showed no significant difference across the three plots (F=.948, p-value=0.397, df=2,36). The 1954 plot had the highest percent nitrogen levels, with the 1936 plot next, and the 1998 plot showing the lowest mean percent nitrogen levels (Fig. one).

Preliminary analysis of the species composition of the three plots revealed Golden Chanterelle (*Cantharellus cibarius*), Aspen Bolete (*Leccinum sp.*), Birch Bolete (*Leccinum scabrum*) and King Bolete (*Boletus edulis*) to be the most common type of mycorrhizal fungi. Further, Oyster (*Pleurotus ostreatus*), Artist's conk (*Ganoderma applanatum*), and Smoky Polypore (*Bjerkandera adusta*) were the most dominant wood-decomposing mushrooms. We used those species to determine the Shannon diversity index values of the three plots. The 1954 plot had the highest diversity (H=1.823), and 1998 had the lowest (H=1.265). The 1936 plot was intermediate to those two with a value of 1.795 (Fig. two).

Despite differing diversity values, the one-way ANOVA comparing the three indices showed an insignificant difference between the three plots (F=3.967, p-value=0.08, df=2,6).

Additionally, we grouped the mushroom species according to functional groups, mycorrhizal or wood-decomposers. Unidentified species that fell into the above groups were also included. A chi-squared test comparing the proportion of mycorrhizal and wood-decomposers showed a significant difference in proportions between the two types of fungi across the plots (X^2=30.659, p-value=0.0001, df=1, fig. three). Mycorrhizal and wood decomposing mushrooms of the 1998 plot contributed most significantly to the final chi-squared value (Table one).

Finally, we identified all the mycorrhizal mushrooms we found as ECM fungi. Using the Chi-squared proportions for functional groups across the plots, we determined ECM mushrooms to be most dominant in the 1998 plot.

Discussion

Our original question sought to determine the relationship succession time has with nitrogen levels and presence of functional groups of fungi. We initially hypothesized that nitrogen levels would be linearly related to time, with increasing soil organic matter over time causing an increase in available nitrogen. Soil analysis revealed no significant difference in mean percent nitrogen levels across the three plots, and thus our hypothesis was disproven.

Although our results were insignificant, our general trends in mean nitrogen levels were mirrored by other studies. For example, the 1956 plot had the highest percentage of available nitrogen. Dighton et al. (1985) discovered that available nitrogen levels are the highest in intermediate successional forests and steadily decrease over time. They hypothesized that this decrease is due to a larger canopy presence, which prevents shade-intolerant plants from growing and actively cycling nitrogen into the system (Dighton et al. 1985). Other studies have confirmed this result, and have also demonstrated that heavier grazing and lower phosphorous availability in climax forests have been correlated with lower available nitrogen levels in the soils (Zackrisson et al. 2004).

Furthermore, Treseder et al. (2004) also found highest percent nitrogen levels in intermediate aged forests. They hypothesized that intermediate forests have a high level of organic leaf litter, but do not yet contain heavy CWD. Organic leaf litter is easy to decompose whereas the lignin in CWD breaks down very slowly, thus decreasing the cycling capacity of the forest (Treseder et al. 2004). Further, CWD increases with time and has been found to contribute only minimal levels of usable nitrogen to the forest when compared to leaf litter (Laiho 1999).

Despite insignificant results from our ANOVA test, we found lower percent nitrogen levels in the 1936 plot compared to the 1954 plot, and it is possible that a larger sample size would have resulted in a significant difference in mean nitrogen levels across those plots.

Finally, the 1998 plot had the lowest percent nitrogen levels, which is what we predicted to see. Other studies have found significantly lower levels of nitrogen in the soil between 6 and 15 years of succession (Visser 1994). Somsak (2009) hypothesized that a burn immediately converts organic nitrogen into a usable form. These first few years following disturbance are thus characterized by a rapid uptake of nitrogen. Leeching, however, following the immediate uptake results in lower nitrogen levels over the next ten years until a thick organic soil layer accumulates. Therefore, we should have expected to see lowest nitrogen levels in the 1998 plot, which is the trend our data followed.

In addition to soil nitrogen levels, we were interested in the relationship between successional time and fungal diversity. We predicted to see the lowest species diversity in the 1998 plot, with diversity increasing with time. Although our Shannon indices were not significantly different across the three plots, the 1998 plot had the lowest diversity.

Furthermore, when we organized the species according to functional group, mycorrhizal or wood decomposers, we found a statistically significant difference in the proportions of each group across the three plots. The 1998 plot had significantly less decomposing fungi than expected and substantially more mycorrhizal mushrooms than expected. We originally hypothesized that the increase in CWD over time would result in a higher presence of decomposers over time, and thus our prediction, that the 1998 plot would show low levels of decomposers, was confirmed.

We were finally interested in the subset of the mycorrhizal group, ectomycorrhizal mushrooms (ECM). Read (1999) hypothesized that ECM fungi should be dominant in an intermediate aged forest in which soil organic material is present. We therefore predicted to see the highest proportion of ECM mushrooms in the 1956 plots. As previously mentioned, we recorded Bolete mushroom and Golden Chanterelles across the plots, which have both been identified as ECM fungi (Pilz et al. 2003). Our results showed the highest levels of such species in the 1998 plots, which conflicted with our original prediction. Despite this contrast, other research has mirrored our results.

For example, Visser (1994) found that between 6 and 41 years of succession, the number of ECM species more than doubled in developing forests. This increase was associated with an accumulation of an organic litter layer, which ECM fungi are specialized to decompose (Treseder et al. 2004). Following 41 years, however, the number of ECM species leveled off, which Visser (1994) hypothesized to be due to the increased canopy presence preventing ECM growth. Furthermore, Treseder et al. (2004) hypothesized that ECM fungi decline in abundance in climax forests because of their inability to decompose tough substances like lignin, which accumulates over time. Although the 1956 plot is intermediate in age for our study sites, it is possible that the 1998 plot, aged 16 years, contained essential characteristics of an "intermediate forest", such as organic litter and low CWD, thus accounting for the high proportion of ECM species in that plot.

Overall, there are many factors that influence soil nutrient levels and functional groups of fungi over time. Although we found no significant difference in mean nitrogen levels across plots over time, the intermediate aged plot (1956) did show the greatest levels of nitrogen. This might be due to the high level of organic litter material that is easily decomposable, and the low levels of CWD that are hard to break down. In addition to nutrient availability, functional groups of mushrooms appear to change during succession. Specifically, there seems to be a low proportion of wood decomposers compared to mycorrhizal mushrooms at earlier successional states when CWD is low. Finally, ECM mushrooms thrived when CWD was low, and there was potentially a relationship between organic leaf litter and ECM abundance.

Understanding the relationship succession time has with nitrogen availability and fungus presence has important implications for forest management. For example, burns are often prescribed to forests in order to reduce the amount of fuel in the event of a natural fire. Therefore, it is essential to understand the long ranging effects burns have on nutrients and mushrooms in order to accurately predict the effects on the ecosystem.

Tables and Figures

Table One: Chi-Squared values of Proportion of Functional Groups of Fungi Across Plots

	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

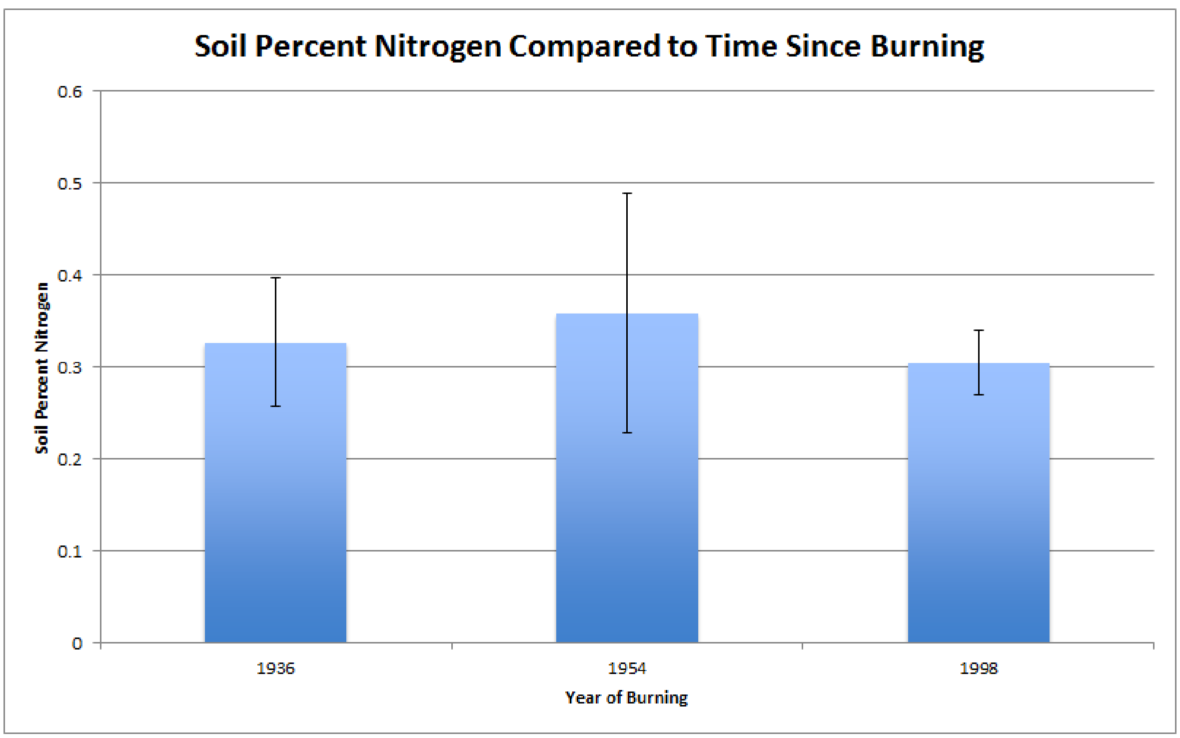

Figure One: Soil Percent Nitrogen Compared to Time Since Burning

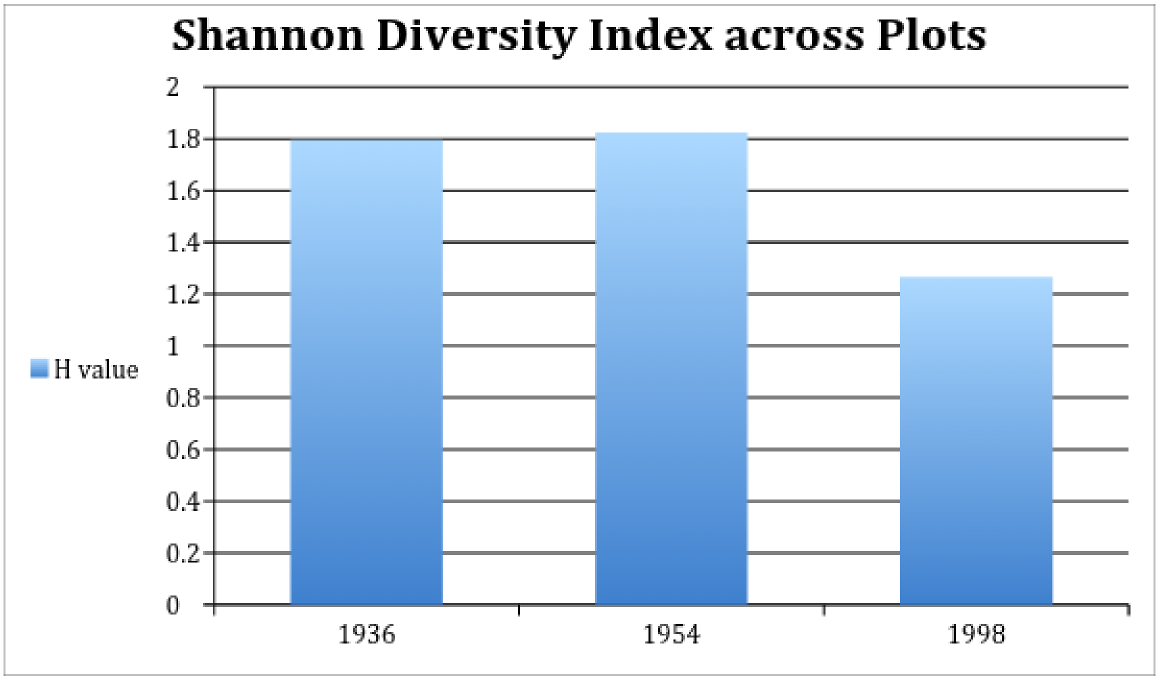

Figure Two: Shannon Diversity Indices across Plots

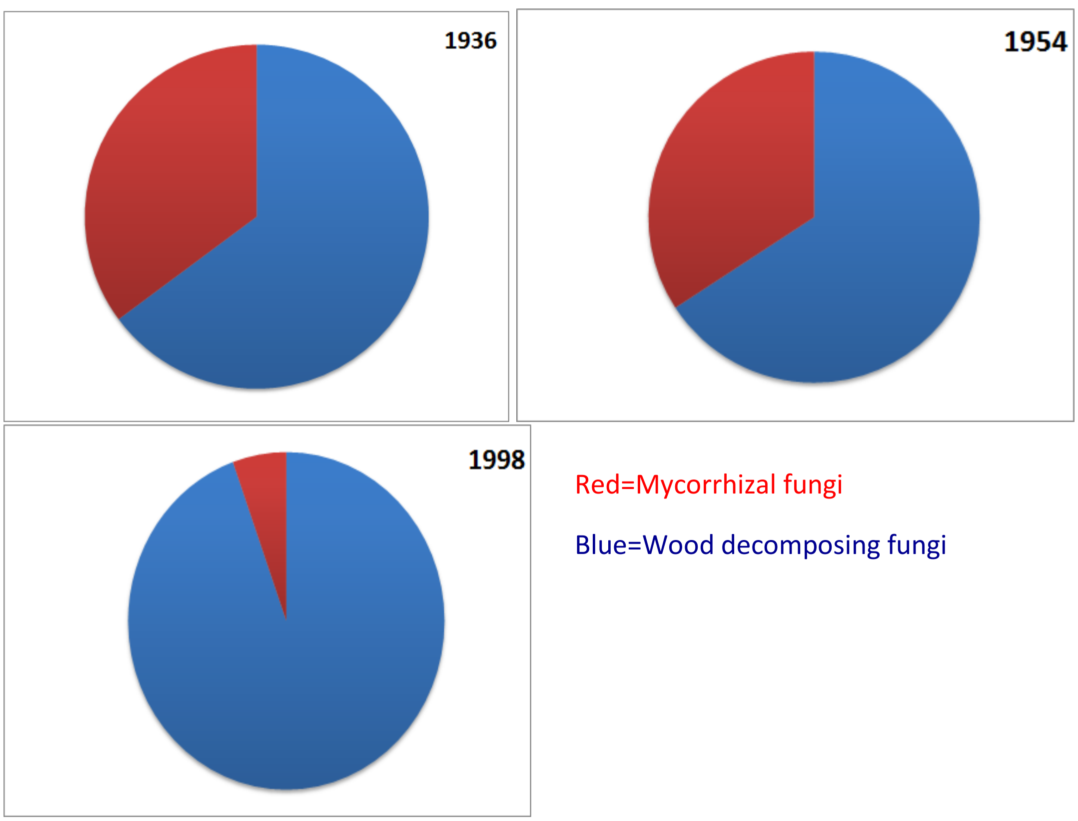

Figure Three: Percent Functional Groups of Mushrooms over Time

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