

Microbial biomass, ammonium, and nitrate levels in the soil across a northern
hardwood/mixed conifer chronosequence

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Abstract

Wildfire is a disturbance that affects forest ecosystems and forest succession all over the world. Our study was conducted at a chronosequence that had been experimentally burned to simulate the effects of logging and wildfire on forest succession. We analyzed soil samples to determine microbial biomass (in the forms of amino N and total microbial N), ammonium content, and nitrate content in the soil across forest stands of three different ages. We found that microbial biomass was not correlated with stand age. We did find relationships between both ammonium and nitrate and stand age, as well as with microbial biomass and soil ammonium levels. The many roles of microbes in the soil are still not well known, but they do play an important role in nutrient cycling.

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Microbial biomass, ammonium, and nitrate levels in the soil across a northeastern hardwood/mixed conifer chronosequence

Abstract

Wildfire is a disturbance that affects forest ecosystems and forest succession all over the world. Our study was conducted at a chronosequence that had been experimentally burned to simulate the effects of logging and wildfire on forest succession. We analyzed soil samples to determine microbial biomass (in the forms of amino N and total microbial N), ammonium content, and nitrate content in the soil across forest stands of three different ages. We found that microbial biomass was not correlated with stand age. We did find relationships between both ammonium and nitrate and stand age, as well as with microbial biomass and soil ammonium levels. It is still unclear what factors influence microbial biomass, but we know that the microbes do affect the abundance of both ammonium and nitrate in soils.

Intro

Habitat loss and fragmentation due to anthropogenic disturbances are leading to biodiversity loss on a global scale (Fahrig 2003). In the northeastern United States, logging and subsequent wildfires from the late 19th century – early 20th century have altered the composition of boreal and temperate deciduous forests, with few patches of old growth forest remaining. Much of sustainable forestry today focuses on how to conserve ecosystems that are being logged or otherwise disturbed. Selective, carefully planned logging methods can preserve species diversity and maintain old growth forest ecosystems and ecological processes within those ecosystems (Boucher 2011, St-Laurent 2009). One study examined DEI, or the distance of edge influence on old growth stands, and concluded that when logging, it is important to leave large blocks of interconnected forest uncut (as opposed to creating isolated linear strips of stands) (Boucher 2011). Sustainable forest management has unfortunately only recently come into focus, following a history of destructive logging practices that permanently changed entire ecosystems and led to widespread wildfires. Studies have shown that frequently disturbed northeastern hardwood – conifer forests have a reduced capacity for annual carbon storage in comparison to forests that did not experience the same frequency of disturbance (Gough 2007).

Gough's study was done at the University of Michigan Biological Station (UMBS) across a chronosequence spanning 104 years and forest stands in six different successional stages (2007). The chronosequence has been clear-cut and burned at various time intervals to simulate logging and wildfire on the forest in order to explore the effects of these disturbances. In 1911, a natural burn (likely due to logging) swept over the entire forest. Since then, different plots of 1 – 2 hectares have been selected every ~20 years to be experimentally clear-cut and burned. The 1911 burn plot is considered a control group for the other plots; it has only burned once recently, while the other plots have experienced two separate burns within 100 years. Many studies have been conducted on soil C, soil N, and primary productivity in relation to both forest stand age and frequency of disturbance at the burn plot chronosequence (Gough 2007, White 2004). One aspect of the chronosequence that has not been explored, however, is how microbial biomass relates to total ammonium and nitrate in the soil, and whether any of these factors relate to forest stand age.

The term “microbes” refers to bacteria (which form bacterial colonies) and fungi; soil microbes in particular are key players in the ecosystem service of nutrient cycling, specifically nitrogen fixation, N mineralization, and nitrification (Hoorman 2010). N fixation is a process that converts atmospheric N_2 into forms available to the vegetation in the area, such as NH_4^+ (ammonium) and NO_3^- (nitrate). N mineralization is the process by which microbes break down organic matter into ammonium, and nitrification is the conversion of ammonium to nitrate. This step of the nitrogen cycle is vital so that plants can take up N through their roots and use it to make amino acids, proteins, nucleic acids, and chlorophyll, among other biological materials. Many climatic factors influence the rate and efficiency at which microbes fix nitrogen, the most important being soil pH (Hoorman 2010). Another form of nitrogen, ninhydrin amino N, can be used as an indicator of microbial biomass, because ninhydrin amino N only occurs in living cells as a component amino acids and proteins (Joergensen 1990, Friedman 2004).

In our experiment, we examined how microbial biomass varies across the UMBS burn plot chronosequence. We sampled the A horizon of the soil across forest stands of three different ages and analyzed them to determine the amount of ninhydrin amino N, total microbial N, ammonium, and nitrate in the soil. We chose to analyze the A horizon as opposed to lower horizons because studies have shown that a majority of microbial activity occurs in this layer, and drops off rapidly deeper in the soil (Agnelli 2004, Taylor 2002). Ninhydrin amino N, or simply amino N, refers to the amount of N assimilated into the microbial biomass in the form of amino acids (Friedman 2004). This measure can be used as an indicator of microbial biomass (Friedman 2004, Joergensen 2010). Total microbial N, in contrast, includes amino N as well as all ammonium and nitrate found within a microbial cell.

We used these nitrogen measurements to explore the following questions: Do nitrate, ammonium, amino N, or total microbial N soil content vary with forest stand age? Does soil ammonium have a positive relationship with amino N? Does soil nitrate have a positive relationship with amino N? We hypothesized that nitrate and ammonium would both be positively correlated with amino N, because microbes produce both ammonium and nitrate through the processes of mineralization and nitrification, respectively. We thought nitrate, ammonium, amino N, and total microbial N would all increase with stand age because the microbial communities would develop and grow in size over time.

Methods

Soil Sampling

We collected soil samples from the 1911 burn plot (our control), the 1954 burn plot, and the 1980 burn plot. The extracts were cored using a soil auger, and the E horizon was scraped away from the core so only the A horizon and surface leaf litter remained. The soil was sieved in order to extract leaf litter, particulate organic matter (POM), and roots from the mineral soil. We had 15 replicates at each plot, for a total of 45 soil samples. The samples were frozen with liquid nitrogen after being sieved.

Chemical Analysis

We used K_2SO_4 (potassium sulfate) to extract amino N, ammonium, and nitrate from each soil sample. To figure out the microbial biomass of each sample, we conducted a two-day $CHCl_3$ (chloroform) fumigation extraction. Chloroform fumigation lyses all the microbial cells in the soil, so that the N originally inside the cells gets filtered out with non-microbial N during the potassium sulfate extraction. We had a control group, which was non-fumigated, and a fumigated group. Each sub-sample within each group held 4 grams of soil. To

calculate microbial biomass, the mass of amino N found in the fumigated samples was subtracted from the mass of amino N found in the non-fumigated samples.

Statistical Analysis

We ran regression analyses comparing amino N mass and ammonium mass as well as amino N mass and nitrate mass. We also ran ANOVA tests comparing the mass of total microbial N and forest stand age, mass of amino N and stand age, mass of ammonium and stand age, as well as mass of nitrate and stand age.

Results

Average amino N did not vary across any the forest stands (**Fig. 1** – appendix; $F_{2,39}$, $p=.273$), nor did average total microbial N (**Fig. 2** $F_{2,39}$, $p=.863$). There was a slightly higher content of amino N in the oldest plot (104 years old). There was a significant difference in ammonium levels among the forest stands of different ages; in particular, the oldest stand had a much higher average ammonium content than either of the other two stands, which were relatively similar (**Fig. 3** $F_{2,39}$, $p<.000$). Average nitrate levels were statistically different from each other among all three forest stands (**Fig. 4** $F_{2,39}$, $p=.003$). Relative amounts of microbial N, ammonium, and nitrate are compared in **Fig. 5**; microbial N makes up more than half of the mass in each stand, and ammonium content is consistently much higher than nitrate content across all three stands. **Fig. 6** and **Fig. 7** show mass of amino N compared to ammonium content and mass of amino N compared to nitrate content, respectively. Linear regression tests showed that the amount of amino N and ammonium do have a linear positive relationship, while amino N and nitrate do not have a discernable relationship (ammonium: $R^2=0.365$, $P<0.000$, 95% Confidence Level; nitrate: $R^2=0.081$, $P<0.061$, 95% Confidence Level).

Discussion

These data suggest that within the forest we sampled, ammonium in the soil is strongly correlated with microbial biomass. We can expect to see ammonium levels increase with microbial biomass because microbes carry out N mineralization and denitrification in soil, converting atmospheric N_2 , N in organic matter, and nitrate to ammonium. We found nearly as much ammonium locked up in the microbes as we did in the mineral soil, suggesting that the microbes process large amounts of ammonium. However, we also expected to see nitrate levels increase with microbial biomass due to nitrification by microbes, yet we found no such trend in our data. This may be due to the uptake of nitrate by plants. Nitrate is one of the forms of nitrogen that plants most often take in through their roots. One study showed that nitrate was the dominant form of N used by plants in forests similar to the one we sampled, and that ammonium uptake by plant roots was negligible (Nadelhoffer 1984). We also found a high ratio of ammonium to nitrate across all the plots we sampled, which supports the work done by Nadelhoffer et al. in 1984.

There was a significant difference in the amount of ammonium in the soil of the 104-year-old stand and that of the two younger stands. The 104-year-old stand, our control plot, was only burned once, while the two younger plots were burned twice. The higher frequency of disturbance in the 61-year-old and 35-year-old plots may have led to a reduced ammonium content in the soil. Reduced soil ammonium in turn may be either a consequence or a cause of the reduced C storage and forest stand productivity examined by Gough in 2004.

We found that nitrate levels were significantly lower in the 35-year-old plot than the two older plots. Nadelhoffer showed that plants take up most of their N in the form of nitrate; therefore, it may be true that the trees in this plot are using up more nitrate than

the trees in the other two plots. This is logical, since the trees in the newest forest stand are young and are likely undergoing rapid early stages of growth corresponding with early successional stage. White et al. found in 2004 that net nitrification was at its lowest 18 years after disturbance, and increased from that point on. This could also explain low nitrate levels in the 35-year-old plot – nitrate may be a limiting nutrient for the forest at this point in succession. Additionally, the low nitrate levels across all plots could be due to the season in which the experiment took place. The summer is the time when most plant growth occurs in northern Michigan, so we could see an increase in nitrate uptake during this time of year relative to other times (Nadelhoffer 1984). Low nitrate levels could also indicate that nitrate is a limiting nutrient for plant growth in this forest across all stand ages.

Our data also suggest that microbial biomass does not vary with stand age, contrary to our original hypothesis. We see no significant increase in microbial biomass as the forest ages, so variation in biomass is likely influenced at least in part by other factors. These could include the plant species present aboveground, the amount of leaf litter and coarse woody debris on the forest floor, the soil pH, or the types of microbial communities present (bacteria, fungi such as brown rot or white rot). Though there is no significant difference in amino N (an indicator of microbial biomass) across the plots, we do see a trend showing more amino N in the oldest forest stand (104 years old, the control plot). This plot also had significantly larger ammonium levels in the soil, perhaps due to less frequent disturbance than the newer plots. It is possible that disturbance frequency affects soil ammonium levels, microbial numbers, and annual C storage (Gough 2004), since all of these variables are more abundant in the control plot than the experimentally burned plots.

Microbial communities are an integral part of forest ecosystems, yet their presence has been little studied thus far. The role they play in forest succession is not fully known, but we do know that they play an important part in cycling N through the forest, through the processes of fixation, mineralization, and nitrification. As we learn more about microbial activity in soils, we will be able to understand more regarding nutrient cycling and primary productivity in forests.

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Appendix

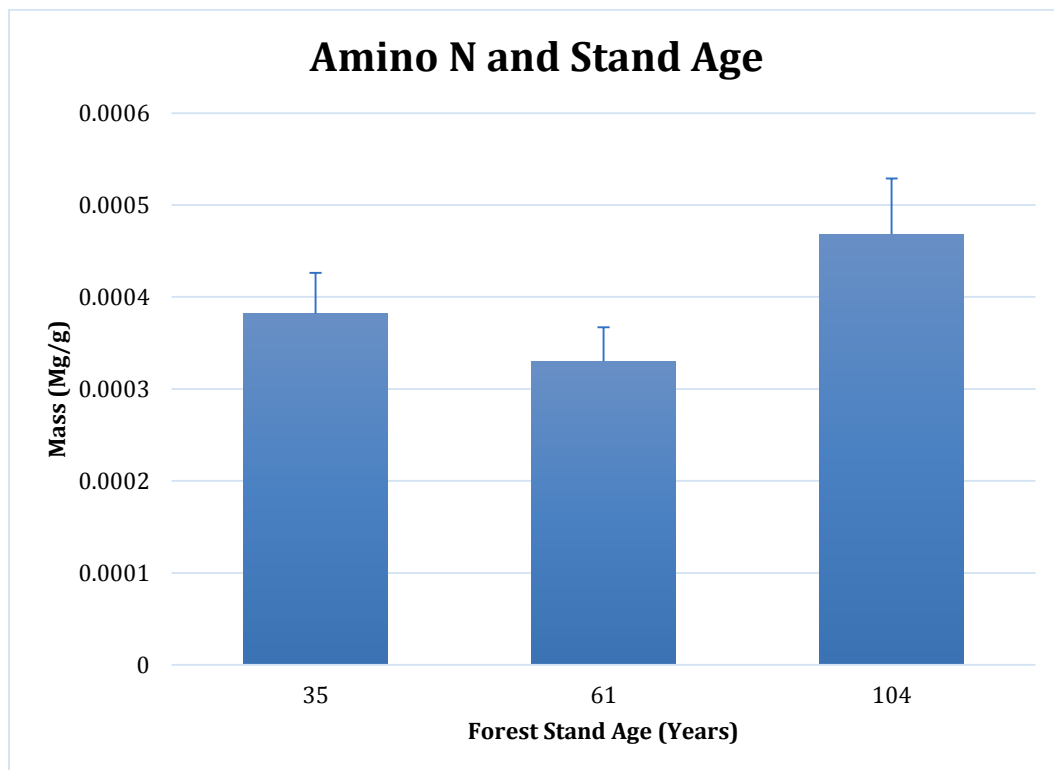


Fig. 1. $F_{2,39}$, $p=.273$

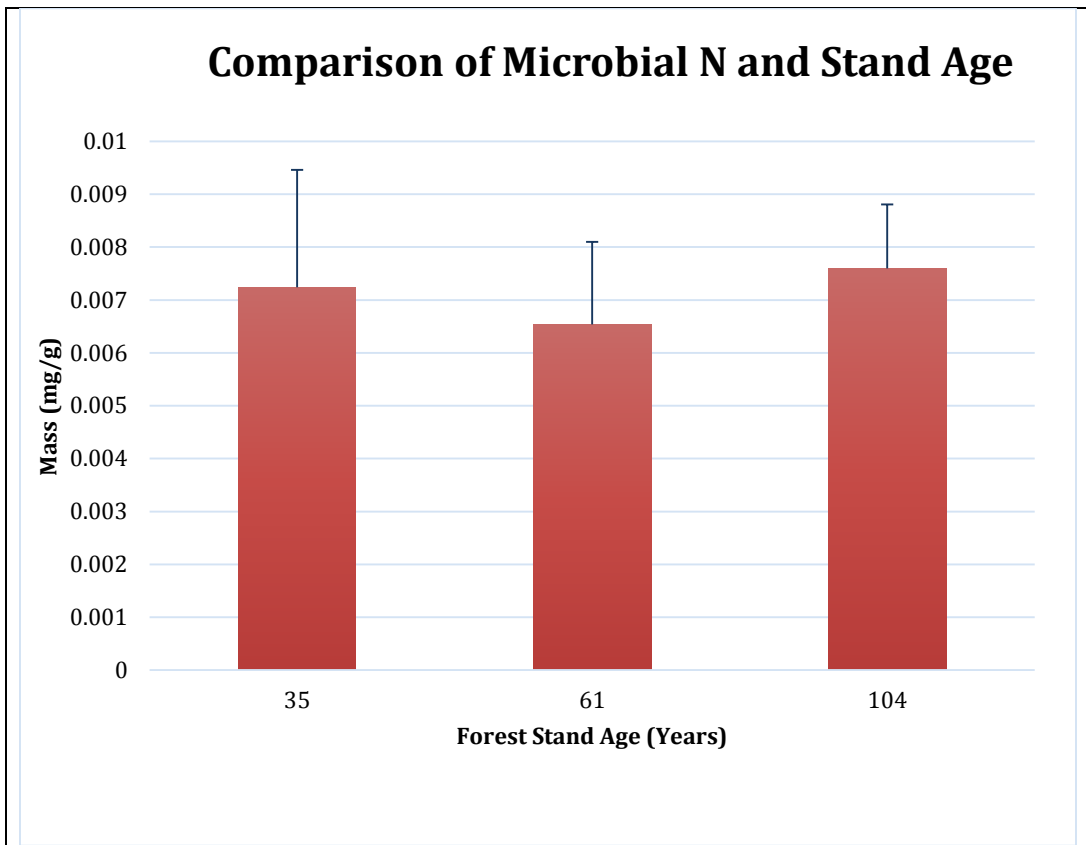


Fig. 2. $F_{2,39}$, $p=.863$

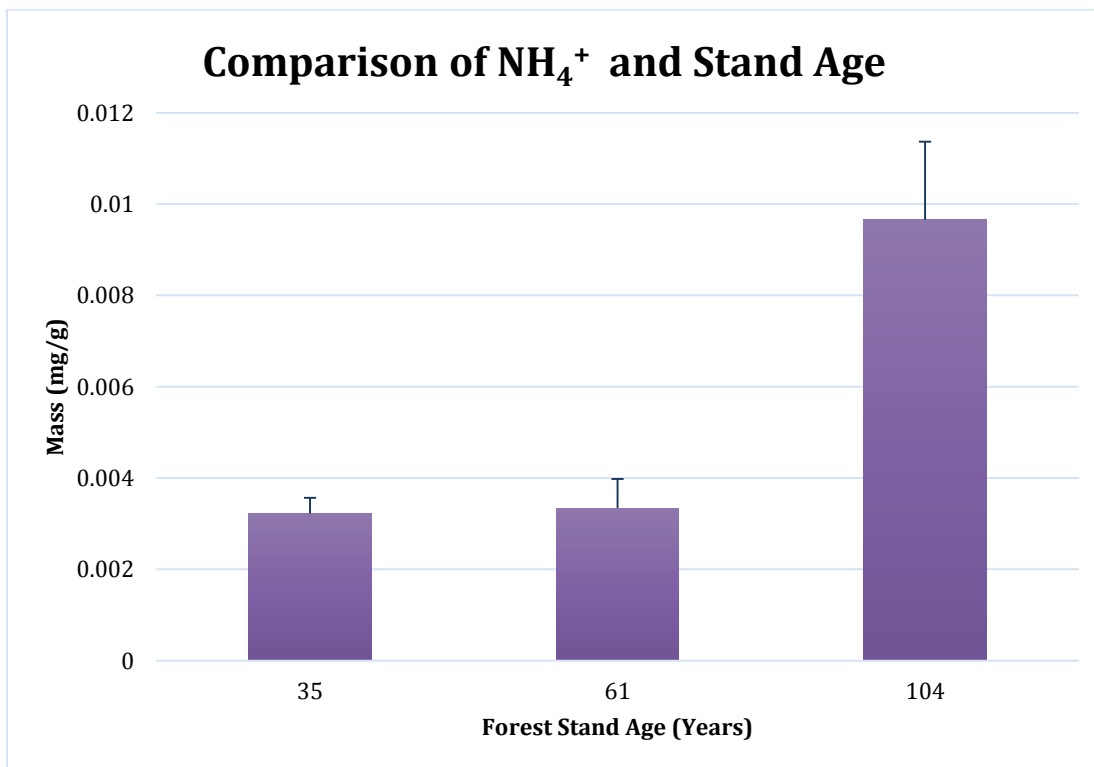


Fig. 3. $F_{2,39}$, $p<.000$

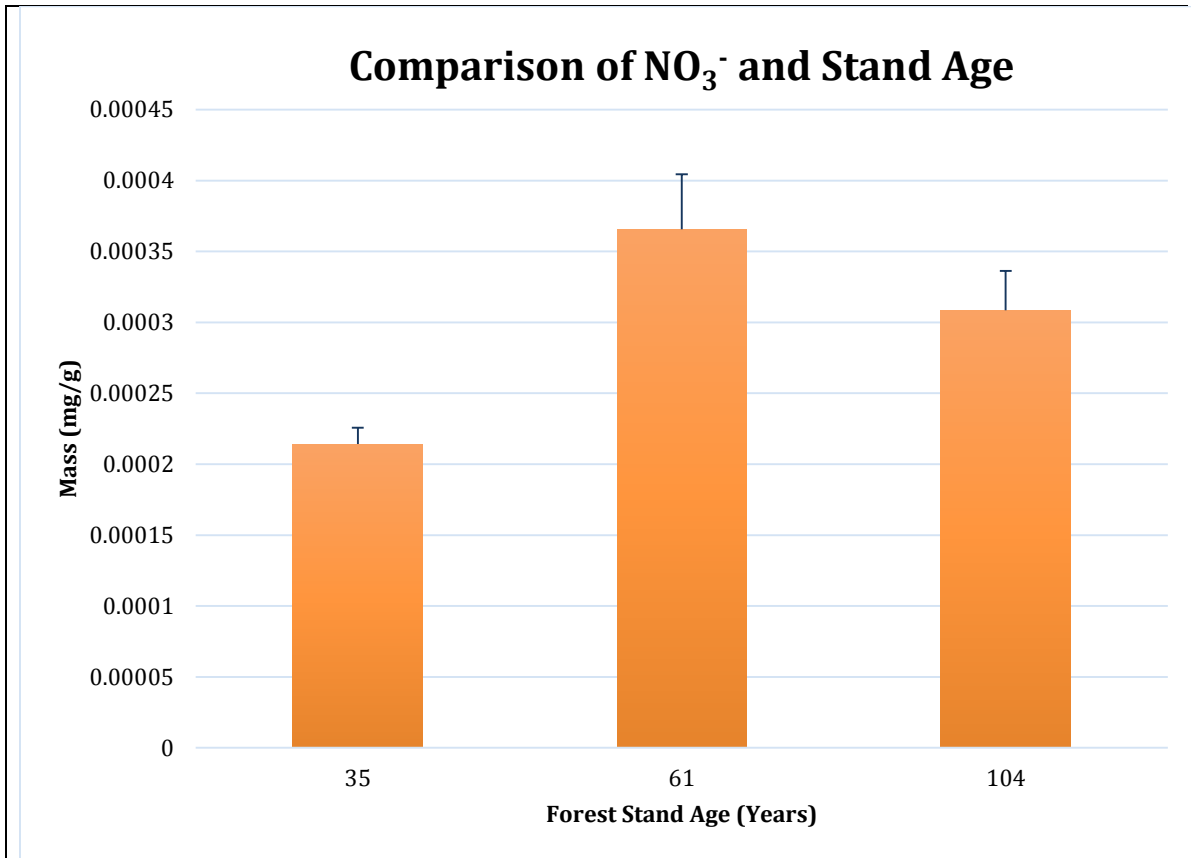


Fig. 4. $F_{2,39}$, $p=.003$

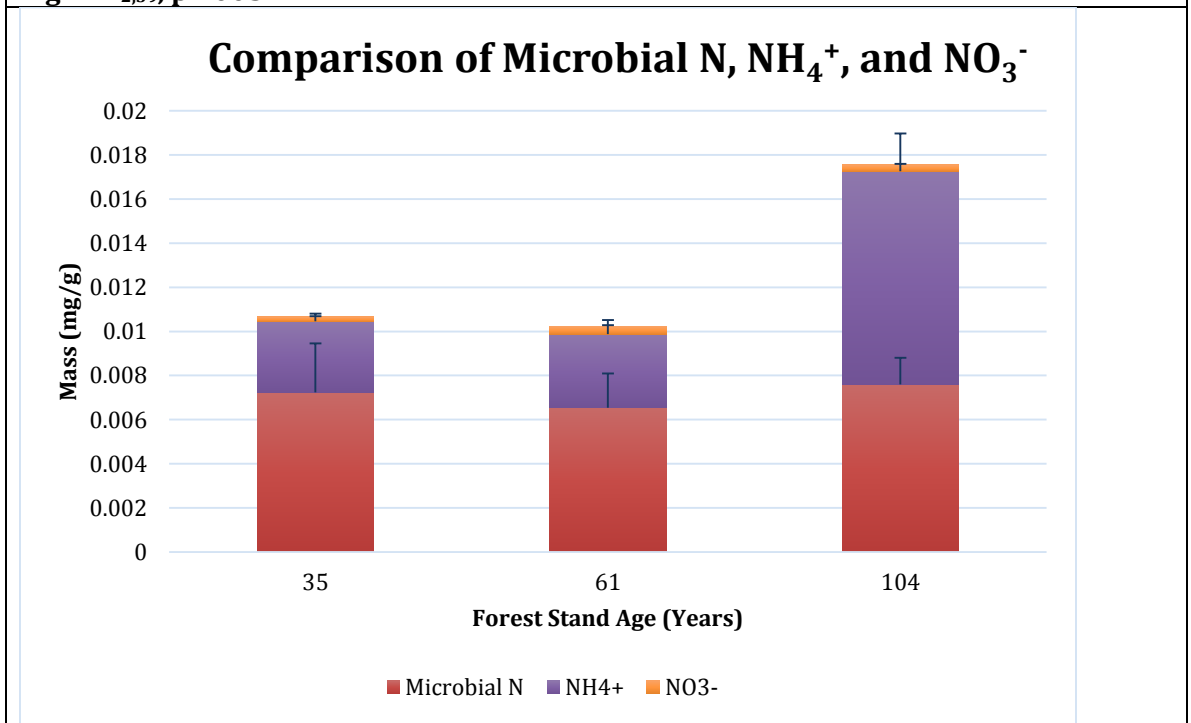


Fig. 5. Relative amounts of microbial N, ammonium, and nitrate. Microbial N ($F_{2,39}$, $p=.863$), NH₄⁺ ($F_{2,39}$, $p<.000$), NO₃ ($F_{2,39}$, $p=.003$)

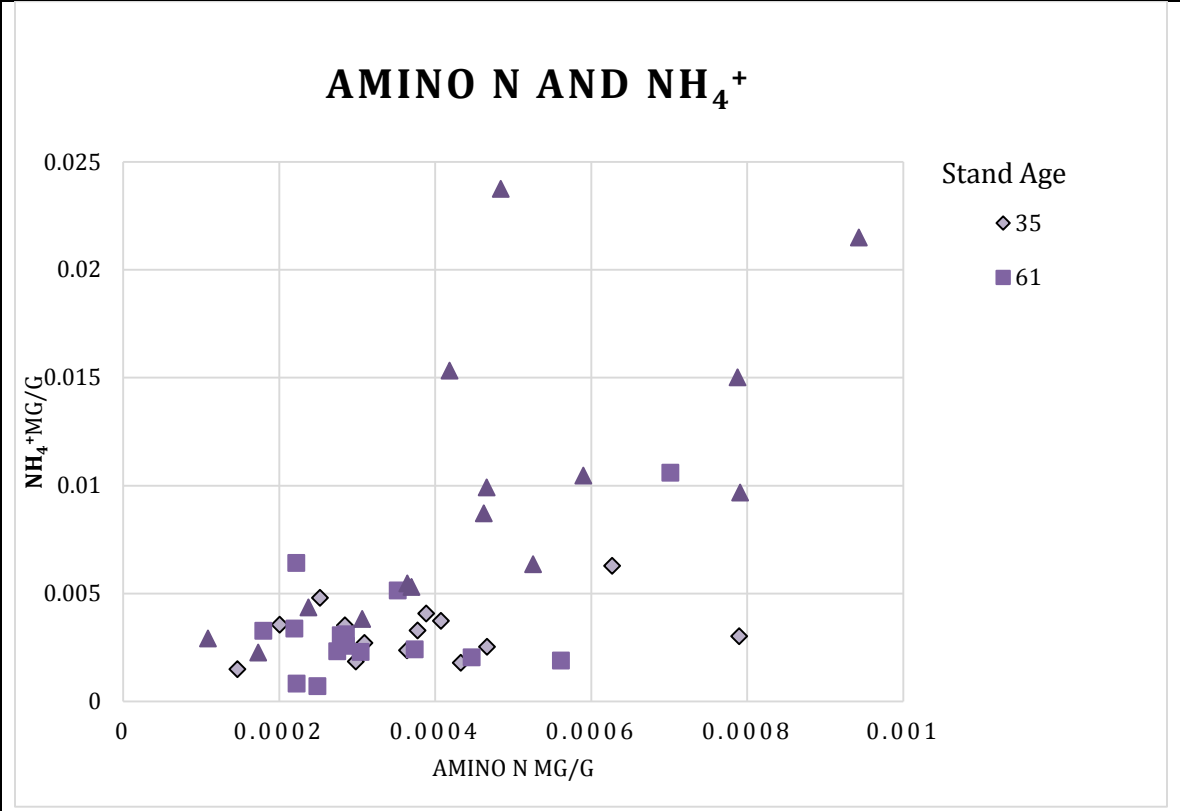


Fig. 6.
R²=0.365, P<0.000, 95% Confidence Level.

AMINO N AND NO3

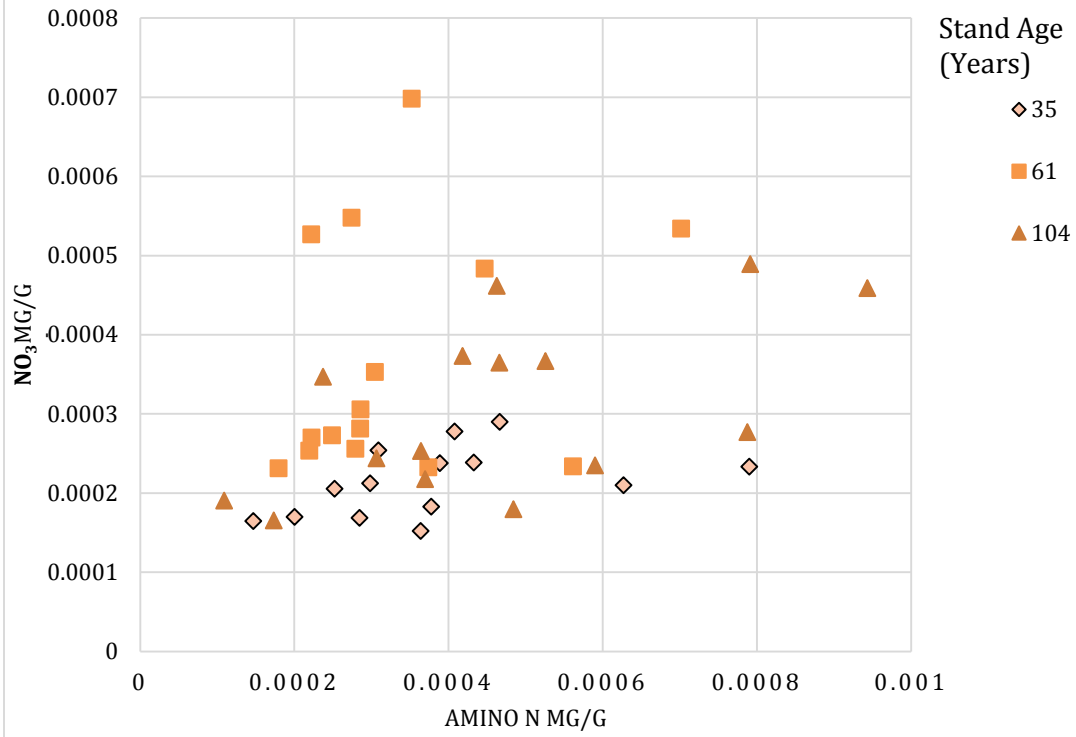


Fig. 7.
 $R^2=0.081$, $P<0.061$, 95% Confidence Level.