

**Ammonium and Nitrate Content Availability of soils in Relation to Microbial Activity Across Forest Stand Age in Chronosequence**

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**Abstract**

Both ammonium ( $\text{NH}_4$ ) and nitrate ( $\text{NO}_3$ ) have been proven to be crucial building components of accumulating plant biomass in forests (Knute et. al, 1984 & Pastor et. al, 1984). We wanted to examine the volumetric relationships between the microorganisms that process these compounds and the amount of the compounds themselves throughout a secondary successional forest of different stand ages. We sampled 45 plots in the A soil horizon throughout the University of Michigan Biological Station burn plot chronosequence from 3 different forest age stands, comparing differences of  $\text{NH}_4$ ,  $\text{NO}_3$ , total microbial mass and amino N content between sites. We found significant differences between  $\text{NH}_4$  and  $\text{NO}_3$  content as well as a significant relationship between amino N and  $\text{NH}_4$  across stand age. In addition to  $\text{NO}_3$  and  $\text{NH}_4$  interactions and cycling properties with biomass accumulation and nutrient leaching, these differences in data could be related to the unique attributes of forests in different successional periods such as amounts of leaf litter, species composition, or soil pH (Knute et. al, 1984, Mcclung, G; Stenger 1995; Bauhus & Co t e, 1998).

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Both ammonium ( $\text{NH}_4$ ) and nitrate ( $\text{NO}_3$ ) have been proven to be crucial building components of accumulating plant biomass in forests (Knute et. al, 1984 & Pastor et. al, 1984). We wanted to examine the volumetric relationships between the microorganisms that process these compounds and the amount of the compounds themselves throughout a secondary successional forest of different stand ages. We sampled 45 plots in the A soil horizon throughout the University of Michigan Biological Station burn plot chronosequence from 3 different forest age stands, comparing differences of  $\text{NH}_4$ ,  $\text{NO}_3$ , total microbial mass and amino N content between sites. We found significant differences between  $\text{NH}_4$  and  $\text{NO}_3$  content as well as a significant relationship between amino N and  $\text{NH}_4$  across stand age. In addition to  $\text{NO}_3$  and  $\text{NH}_4$  interactions and cycling properties with biomass accumulation and nutrient leaching, these differences in data could be related to the unique attributes of forests in different successional periods such as amounts of leaf litter, species composition, or soil pH (Knute et. al, 1984, Mcclung, G; Stenger 1995; Bauhus & Co te, 1998).

## **Introduction**

An important limiting factor of production in forest ecosystems has been linked to organic nitrogen content in soil and rate processes of the nitrogen cycle (Knutte et. al, 1984 & Pastor et. al, 1984). The role of soil microbial biomass is well established as a major component in the cycling of soil nitrogen turnover, soil respiration and ecosystem productivity. (Marumoto et. al, 1982b; Van Veen et al., 1987; Duxbury et al., 1989; Jenkinson and Parry, 1989; Bauhus & Co  t  , 1998). Turnover is influenced by temperature, season, moisture, salinity, disturbance, leaf litter, stand age and other factors have implications for the growth and survival of forest trees and for net primary production in forest ecosystems (Bhabani et. al, 1995; Yuki et. al, 2013; Knute et. al, 1984, Mcclung, G; Stenger 1995; Bauhus & Co  t  , 1998). Both ammonium (NH<sub>4</sub>) and nitrate (NO<sub>3</sub>) have been proven to be crucial building components of accumulating plant biomass, and few studies have been conducted in northern temperate forests examining microbiomass relationships with ammonium and nitrate content throughout forest ages. We hypothesized nitrate, ammonium, amino-N, and total microbial N content is greater in older forest stands because microbial communities in older stands would have had longer times to develop. We also hypothesized there is a positive relationship between amino N and ammonium content in soil because ammonium indicates decomposition by microbes, and that there is a positive relationship between amino N and nitrate content in soil across stand age because microbes participate in nitrification. Understanding these relationships more thoroughly can help us understand microbial ecosystem functionality throughout time and mineralization rates that could relate to potential productivity, which is one important component to consider as a future carbon sequestration outlet with the shifting atmospheric CO<sub>2</sub> levels (Reich et. al, 2006).

## **Methods**

We took 15 random samples throughout forest stands of ages 35 years, 61 years, and 104 years, extracted by soil core. We determined to sample from the A layer because it has the most activity and microbe abundance (Agnelli et. al, 2004). We

separated the soil samples from leaf litter and other particulate matter by tweezing and sieving thoroughly. Soil was then measured into pair sets of 4 grams of fumigated soil and non-fumigated soil. Fumigated soil was stored in  $\text{CHCl}_3$  for 48 hours to kill microbes and to extract amino nitrogen, then transferred to 40mL of 3M  $\text{K}_2\text{SO}_4$  and shook for 60 minutes for homogenization in a benchtop shaker. We then filtered the solution through #42 GF filter paper into an  $\text{NH}_4$  and  $\text{NO}_3$  ninhydrin solution. We followed the same procedure for the non-fumigated samples with the exception of storing the soil in  $\text{CHCl}_3$  for 48 hours to avoid extracting amino N in the microbes. We then subtracted non-fumigated from corresponding fumigated samples to obtain an overall difference that has been shown to be reliable in estimating microbial volumes in soil (Joergensen & Brookes, 1980). We analyzed  $\text{NO}_3$ ,  $\text{NH}_4$ , Amino N and total microbial N amounts using ANOVA across the different forest stand ages and also regressed  $\text{NO}_3$  and  $\text{NH}_4$  with amino N to assess relationships between these components of the nitrogen cycle.

## Results

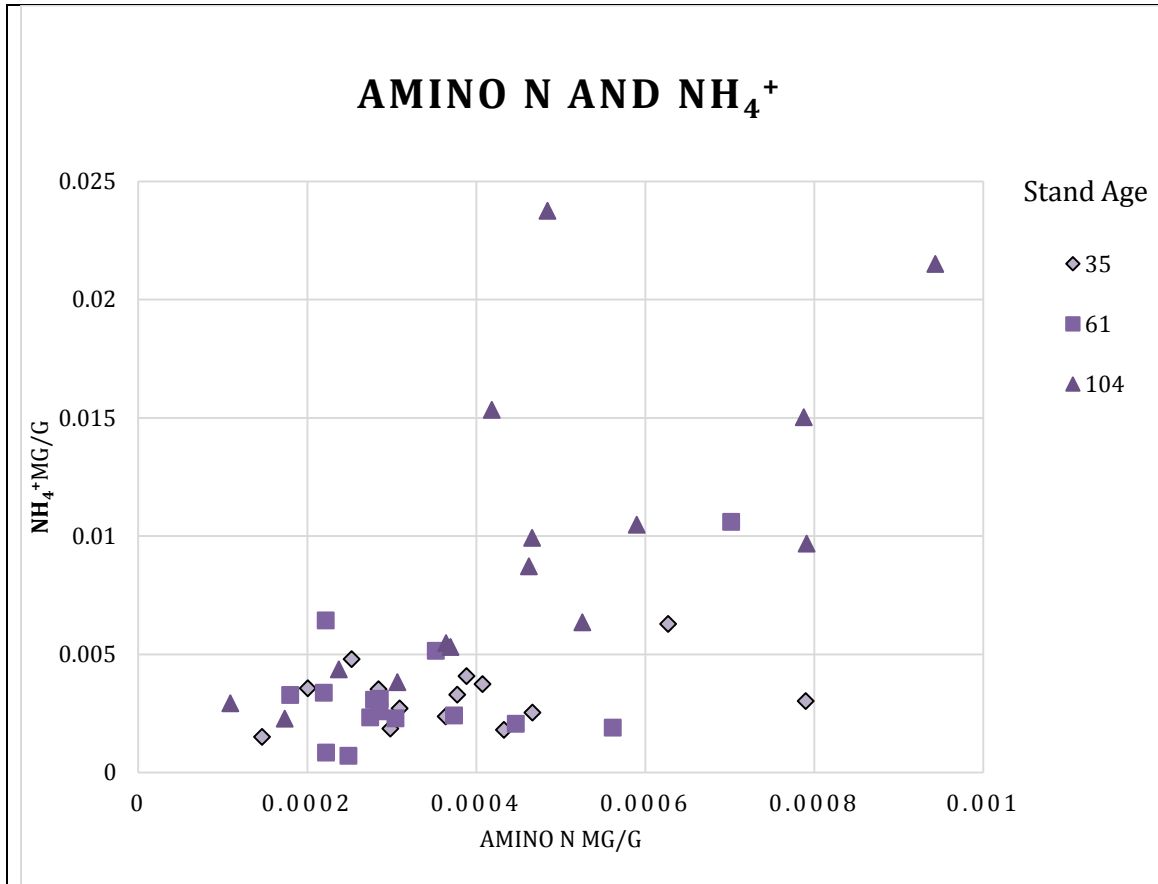
One-way ANOVA yielded significant results in  $\text{NH}_4^+$  ( $F_{2,39}$ ,  $p < .000$ ),  $\text{NO}_3$  ( $F_{2,39}$ ,  $p = .003$ ) across stand ages, but not total microbial N ( $F_{2,39}$ ,  $p = .863$ ) or amino N ( $F_{2,39}$ ,  $p = .273$ ). Regression of amino N and  $\text{NH}_4$  returned a significant relationship between the variables ( $R^2 = 0.365$ ,  $P < 0.000$ ,  $\alpha = .05$ ) while amino N and  $\text{NO}_3$  did not share statistically significant ( $R^2 = 0.081$ ,  $P < 0.06$ ,  $\alpha = .05$ ) relationship. All relationships can be further examined in figures of the Appendix.

## Discussion

Ammonium, a cation, is retained within the soils by cation exchange, whereas nitrate, an anion, is excluded from cation exchange sites and easily leached from most soils, which could account for part of the significance level difference (Robertson, G. Philip, and Peter M. Vitousek). Nitrate could also be used by plants to build biomass, attributed to the never ceasing production of biomass accumulations within forests and accounting for some nitrate loss, especially in the 35 year aged stand because of high productivity rates.

NH<sub>4</sub> availability followed a general trend of being highest in the oldest aged stand, which could be attributed to higher accumulations of organic matter on soil surfaces in older forests and possibly diversified communities of microbes (Smithwick, 2005). This trend can also be associated with increases in microbial biomass due to increased forest productivity with increased age, which is not statistically significant in our model but is notable in our data (Myrold et. al, 1989). Both thickness of organic matter and assessments of microbial communities would need to be addressed in different forest ages to further examine the possibility of these relationships. Nitrogen availability also tends to decrease as forests age (Bauhus 1998), a trend we did not see with our data. Both lacks of microbial accumulation and decreasing trends of nitrogen availability in our data could be credited to the age of our oldest stand being relatively young in the North American Temperate Zone. An age of 104 years may not be old enough to detect these trends which may be characteristic of old growth forests. Future studies would be encouraged to compare organic matter volumes on the soil horizon, soil pH, and types of microbial communities present to assess other relationships and possible trends across a larger range of stand ages. These examinations could more accurately explore other relationships and possible trends in nitrate, ammonium, and microbial mass allowing us to view the overall process of the soil focused nitrogen cycle throughout the forest aging process.

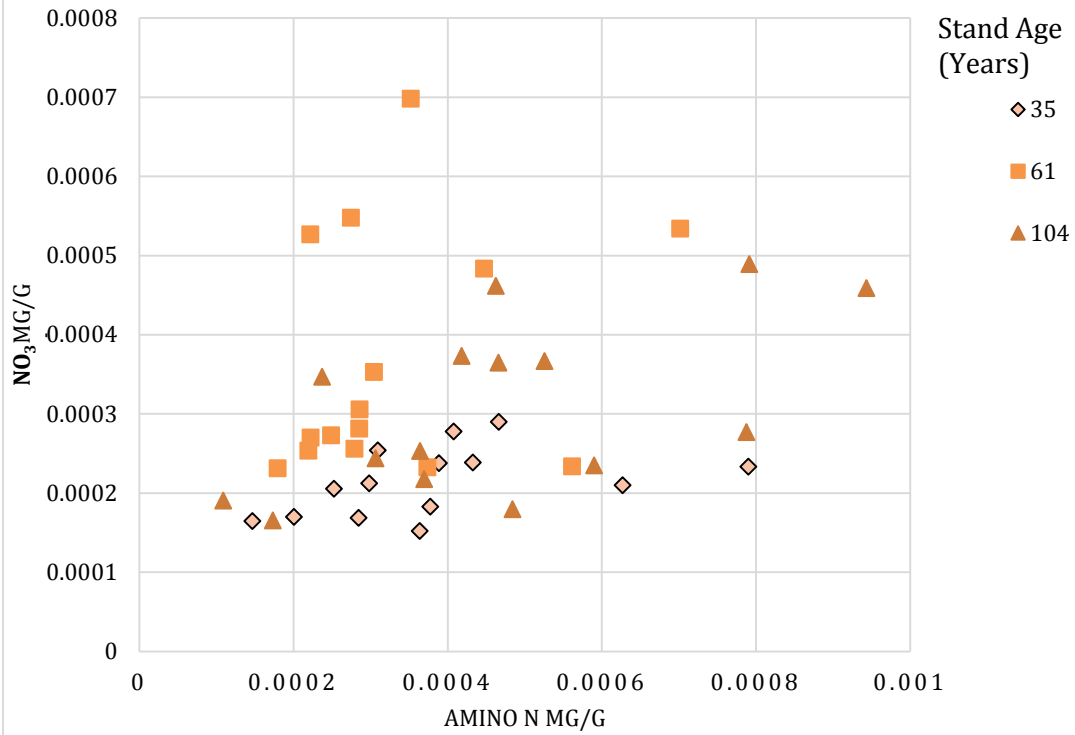
## Appendix



**Fig. 1.**

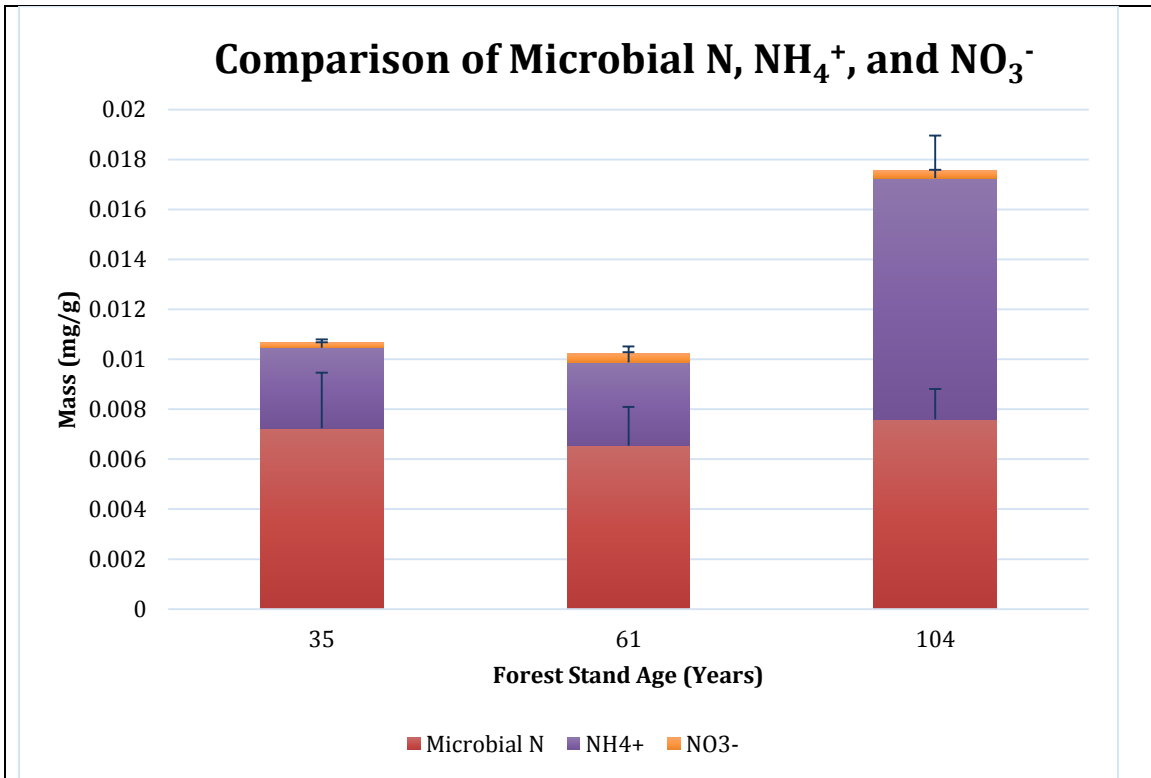
$R^2=0.365$ ,  $P<0.000$ , 95% Confidence Level. There is a significant relationship between amino N and  $\text{NH}_4$  in all pooled stand ages. Amino N is a good predictor of  $\text{NH}_4$  throughout stand age.

## AMINO N AND NO3

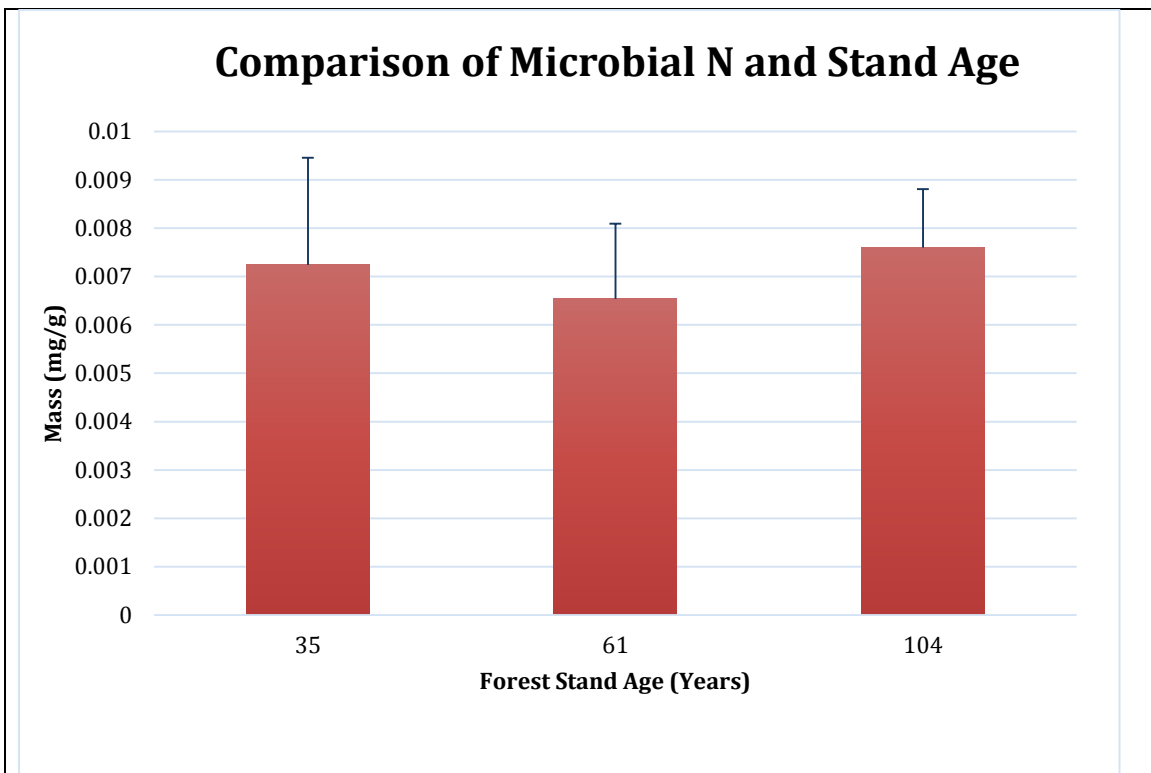


**Fig. 2.**

$R^2=0.081$ ,  $P<0.061$ , 95% Confidence Level. There is not a significant relationship between amino N and NO<sub>3</sub> in all pooled stand ages. Amino N is not a good predictor of NH<sub>4</sub>.

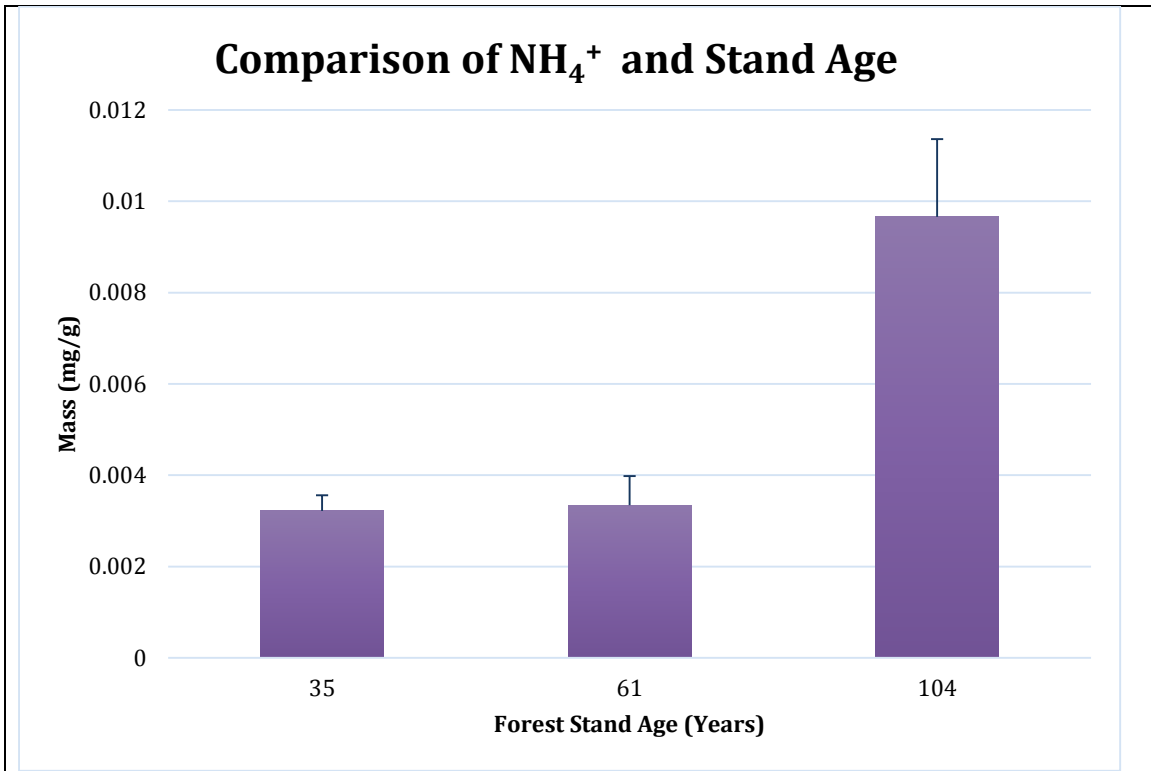


**Fig. 3. An overall comparison of Microbial N ( $F_{2,39}$ ,  $p=.863$ ), NH<sub>4</sub><sup>+</sup> ( $F_{2,39}$ ,  $p<.000$ ), NO<sub>3</sub> ( $F_{2,39}$ ,  $p=.003$ ). Although stand 104 had the highest amount of microbial N and NH<sub>4</sub>, it had the second highest amount of NO<sub>3</sub> (second to stand 61).**

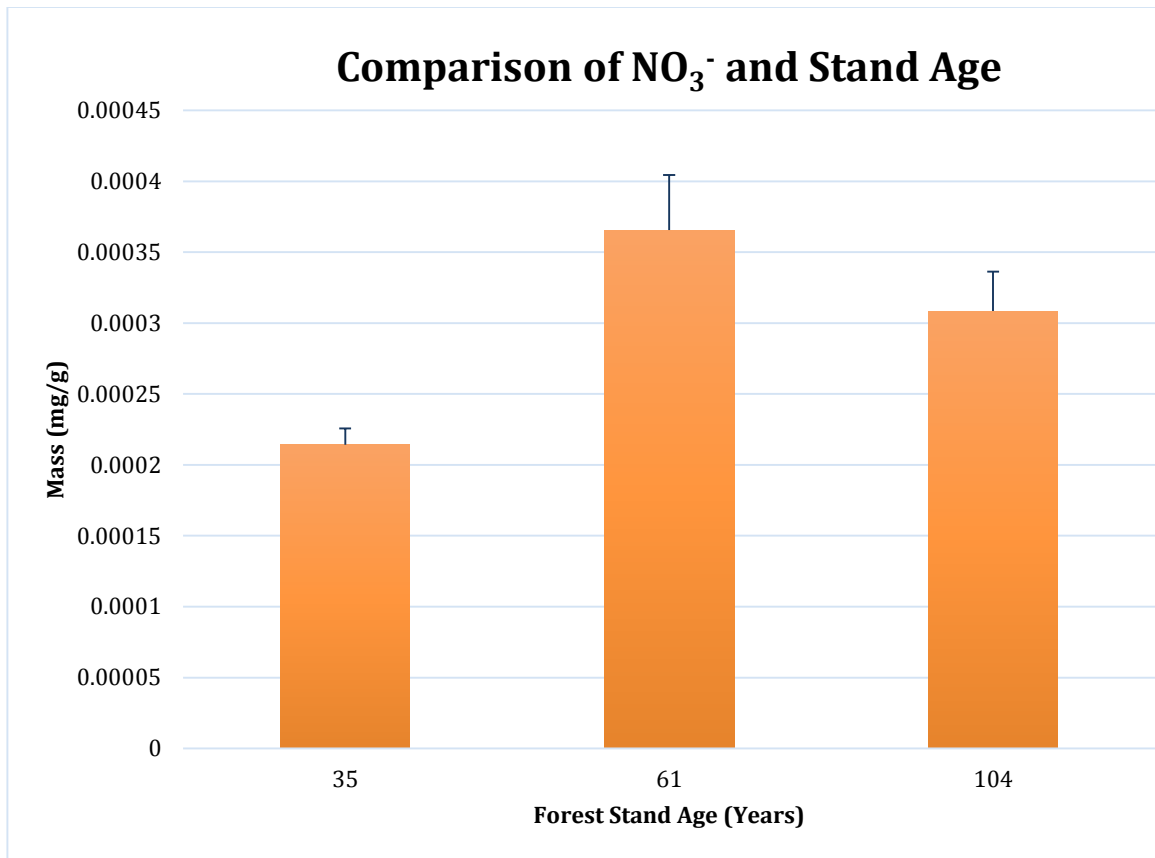




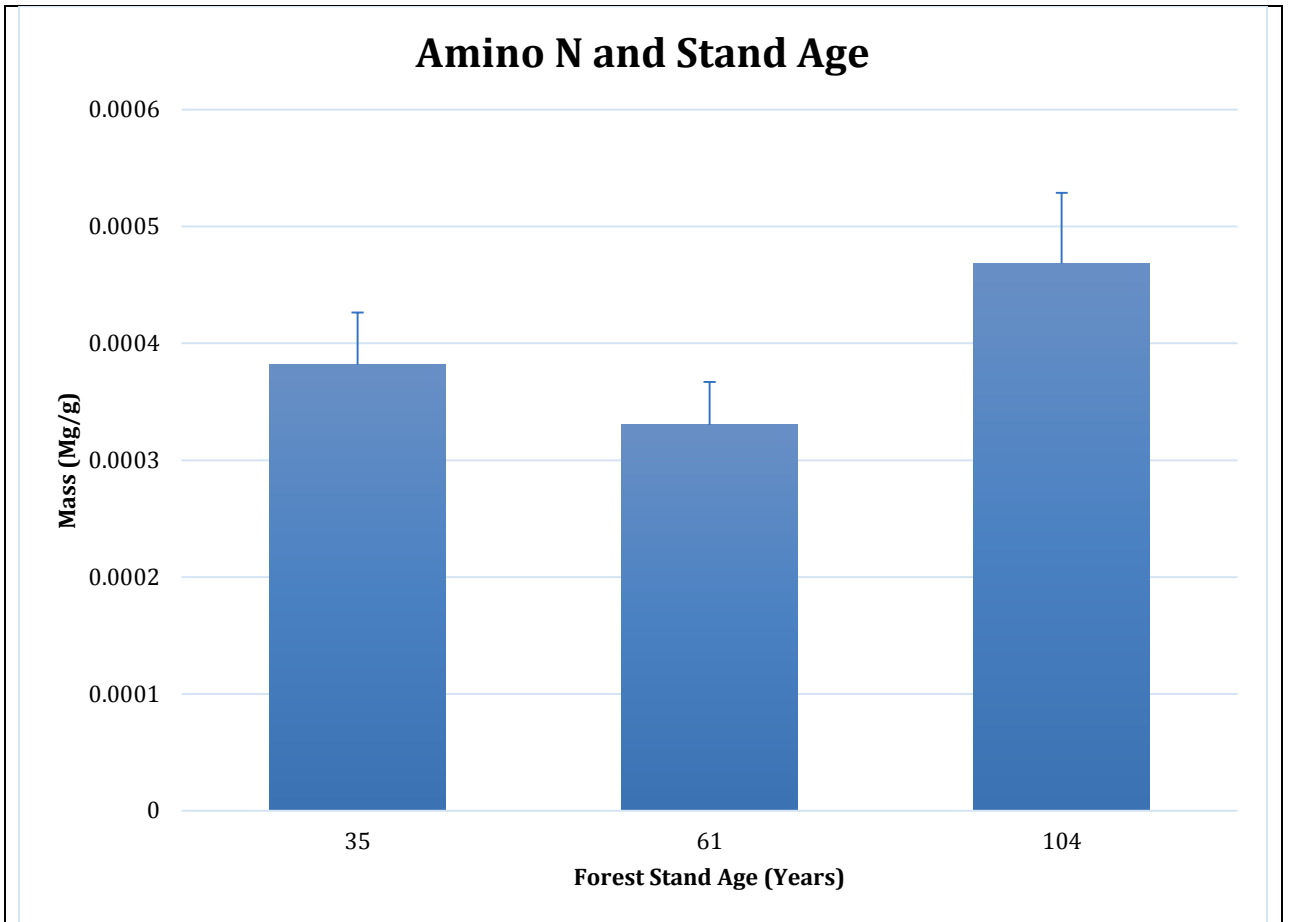
**Fig. 4.  $F_{2,39}$ ,  $p=.863$ . There was no difference between stand age and microbial N. Stand age does not affect microbial N (in the duration of our sampled time) because microbial N volume remains constant.**



**Fig. 5.  $F_{2,39}$ ,  $p<.000$ .  $\text{NH}_4$  changes throughout time in stand age.**



**Fig. 6.  $F_{2,39}$ ,  $p=.003$ . NO<sub>3</sub> fluctuates over stand age.**



**Fig. 7.  $F_{2,39}$ ,  $p=.273$ . There is no significant difference between amino N mass and stand age.**

## Works Cited

Agnelli, A., Ascher, J., Corti, G., Ceccherini, M., Nannipieri, P., & Pietramellara, G. (1980). Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA. *Soil Biology and Biochemistry*, 859-868. Web.

Bauhus, J., D. Paré, and L. Co té. "Effects of Tree Species, Stand Age and Soil Type on Soil Microbial Biomass and Its Activity in a Southern Boreal Forest." *Soil Biology and Biochemistry* 30.8-9 (1998): 1077-089. Web.

Das, Bhabani S., Gerard J. Kluitenberg, and Gray M. Pierzynski. "Temperature Dependence Of Nitrogen Mineralization Rate Constant." *Soil Science* 159.5 (1995): 294-300. Web.

Jenkinson, D.s., and L.c. Parry. "The Nitrogen Cycle in the Broadbalk Wheat Experiment: A Model for the Turnover of Nitrogen through the Soil Microbial Biomass." *Soil Biology and Biochemistry* 21.4 (1989): 535-41. Web.

Joergensen, R., & Brookes, P. (1980). Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 m K<sub>2</sub>SO<sub>4</sub> soil extracts. *Soil Biology and Biochemistry*, 22(8), 1023-1027. Web.

Marumoto, T., J.p.e. Anderson, and K.h. Domsch. "Mineralization of Nutrients from Soil Microbial Biomass." *Soil Biology and Biochemistry* 14.5 (1982): 469-75. Web.

Myrold, Davidd., Pamelaa. Matson, and Davidl. Peterson. "Relationships between Soil Microbial Properties and Aboveground Stand Characteristics of Conifer Forests in Oregon." *Biogeochemistry* 8.3 (1989): n. pag. Web.

Nadelhoffer, Knute J., John D. Aber, and Jerry M. Melillo. "Fine Roots, Net Primary Production, and Soil Nitrogen Availability: A New Hypothesis." *Ecology* 66.4 (1985): 1377. Web.

Nadelhoffer, Knute J., John D. Aber, and Jerry M. Melillo. "Seasonal Patterns of Ammonium and Nitrate Uptake in Nine Temperate Forest Ecosystems." *Plant Soil Plant and Soil* 80.3 (1984): 321-35. Web.

Pastor, John, John D. Aber, Charles A. Mcclaugherty, and Jerry M. Melillo. "Aboveground Production and N and P Cycling Along a Nitrogen Mineralization Gradient on Blackhawk Island, Wisconsin." *Ecology* 65.1 (1984): 256. Web.

Robertson, G. Philip, and Peter M. Vitousek. "Nitrification Potentials in Primary and Secondary Succession." *Ecology* 62.2 (1981): 376. Web.

Smithwick, Erica A.h., Monica G. Turner, Kristine L. Metzger, and Teri C. Balsler. "Variation in NH<sub>4</sub> Mineralization and Microbial Communities with Stand Age in Lodgepole Pine (*Pinus Contorta*) Forests, Yellowstone National Park (USA)." *Soil Biology and Biochemistry* 37.8 (2005): 1546-559. Web.

Veen, J.a. Van, J.n. Ladd, J.k. Martin, and M. Amato. "Turnover of Carbon, Nitrogen and Phosphorus through the Microbial Biomass in Soils Incubated with 14-C-, 15N- and 32P-labelled Bacterial Cells." *Soil Biology and Biochemistry* 19.5 (1987): 559-65. Web.