Title: **Microbial Biomass, Soil Ammonium and Nitrate in a Secondary Successional Chronosequence**

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Abstract

We studied the microbial biomass, soil ammonium and nitrate in a secondary successional chronosequence. We wanted to know if nitrite, amino-N, or total microbial N content in soils vary with forest stand age. We also wanted to know if there was a relationship between amino-N and ammonium and if there was a relationship between amino-N and nitrate. To assess the soils, we collected a total of 90 samples from the burn plot chronosequence on the University of Michigan Biological Station (UMBS) property. Soils were collected from the 1911, 1936, 1948, 1954, 1980 and 1998 burn plots. Soils were sieved and picked through to remove fine roots and other debris such as twigs and pebbles that could interfere with our date. Soils then were taken to the lab to be homogenized and weighed out. The soils were then separated into fumigated and non-fumigated samples and KCL extractions and chloroform tests were run on the samples. We found that there was no significance across the chronosequence in nitrite, amino-N or total microbial N related to forest age stand. We also found no significant relationship between amino-N and nitrate but we did find a significant relationship between amino-N and ammonium. Future studies should be run to understand how microbes are affecting nutrient cycling in secondary successional chronosequences and the types of microbes present.

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Abstract:
We studied the microbial biomass, soil ammonium and nitrate in a secondary successional chronosequence. We wanted to know if nitrite, amino-N, or total microbial N content in soils vary with forest stand age. We also wanted to know if there was a relationship between amino-N and ammonium and if there was a relationship between amino-N and nitrate. To assess the soils, we collected a total of 90 samples from the burn plot chronosequence on the University of Michigan Biological Station (UMBS) property. Soils were collected from the 1911, 1936, 1948, 1954, 1980 and 1998 burn plots. Soils were sieved and picked through to remove fine roots and other debris such as twigs and pebbles that could interfere with our date. Soils then were taken to the lab to be homogenized and weighed out. The soils were then separated into fumigated and non-fumigated samples and KCL extractions and chloroform tests were run on the samples. We found that there was no significance across the chronosequence in nitrite, amino-N or total microbial N related to forest age stand. We also found no significant relationship between amino-N and nitrate but we did find a significant relationship between amino-N and ammonium. Future studies should be run to understand how microbes are affecting nutrient cycling in secondary successional chronosequences and the types of microbes present.

Introduction:

Microorganisms have critical roles in the functioning of soil in nutrient cycling, structural formation, and plant interactions both positive and negative. (Harris, 2009). Because of this, it is important to understand how microbial biomass and forest stand age are related to forest productivity or if they even are. There have been many studies throughout the years on microbial biomass or forest succession but the processes and changes that occur during forest re-growth are less studied and not fully understood.(Susyan, Wirth, Ananyeva, Stolkinova, 2011) There is little knowledge of the succession of soil-borne microbial communities. (The ISME journal, 2011). One such study that has been done to try and understand the process of microbial biomass and forest re-growth was a study done at the University of Michigan Biological Station (UMBS) in 2003. The focus of the study was to compare the soil N availability and biomass accumulation in a series of plots undergoing secondary succession following disturbances of logging and burning, similar to that which occurred throughout the Upper Lakes region over a century ago.” (White, Zak, Barnes, 2003). The study focused on the availability of N because it is well documented that soil N availability often limits the productivity of forests in the Upper Great Lakes region (Pastor et al., 1984; Zak et al., 1989).

In this study, we compare the amount of nitrogen (N) in the soils to the different aged forest stands undergoing secondary succession. The objectives of are study are to (i) quantify the amount of N in the soils of each of the burn plots. (ii) Compare the N levels in the soils from each burn plot. To address are objectives, collections for this study were made from the 1911, 1936, 1948, 1954, 1980 and 1998 burn plots on the University of Michigan Biological Station which is located at the tip of northern Lower Michigan. The experiment was conducted inside each of the burn plots to show if microbial biomass and forest stand age has an effect on forest productivity. We hypothesized that nitrite, ammonium, amino-N and total microbial N content will be greater in older forest stands because microbial communities in older stands will have more time to develop. We also

hypothesized that there will be a positive relationship between amino-N and ammonium as well as a positive relationship between amino-N and nitrate.

Methods and Materials:

Sampling

We sampled soil from the 1911, 1936, 1948, 1954, 1980 and 1998 burn plots located on the University of Michigan Biological Station (45.5598, -84.7138) at the tip of Norther Lower Michigan. In each burn plot, 3 random locations were set up to sample. For each of the 3 locations inside of the burn plots, 5 samples were taken from each. Samples were collected using a steel cylinder, which is approximately 2 inches in diameter. The steel cylinder was placed on the ground and hammered in using a rubber mallet. The steel cylinder was hammered into the ground to collect the O and A horizons in the soil. The O and A horizons in the soil were collected because the most microbial activity and productivity occurs in these layers. Once a sample was collected, the O layer, which contains organic matter such as leaves and pine needles, were put inside a labeled brown paper bag. The A horizon of the soil was put inside of a Ziploc bag and then put inside of the labeled brown paper bag. This process was done for each of the 5 samples collected from each of the 3 locations on a burn plot. There were 15 total samples collected from each burn plot. In total there were 90 samples collected over all of the burn plots. Once the 15 samples were collected, they were brought back to a sieving station set up near the burn plots. Before the A horizon could be sieved, the sieves had to be cleaned. To clean the sieves they were brushed down with a wire brush then washed into a tub of water and then they moved to a second tub of water to be washed again. Once the sieves had been washed, they were rinsed down with DI water. They were then set out on a table to dry. Once the sieves were dried and ready to use, they were wiped down with Ethanol to ensure that all bacteria had been killed. This same process was used in cleaning the other equipment for the soils which was, plastic boats to hold material and plastic sheets to catch falling debris from the sieves. Tweezers and lunch trays were wiped down with ethanol. The cleaning process was to be repeated each time a new sample was to be sieved. The sieves that were used were 2.5mm. For each of the soils sieved, small roots and POM (small pebbles, stones and wood) were to be separated into plastic boats using tweezers. Once the soils, small roots and POM were separated, they were put into separate labeled plastic bags. This process was used for all of the 90 samples that were sampled across the burn plots.

Fig 1: Soils samples being collected using the cylinder and mallet

Fig 2: Soil samples being seived

Lab preparations

To prepare are samples for testing, we thoroughly cleaned all of the tools that were going to be used in testing the soil samples. 90 glass jars that can hold approximately 4 milligrams of soil were washed using a scrub brush and soap. Once the glass jars had been washed, we then rinsed them in DI water three times. The jars were then put in acid for 24 hours to kill any bacteria that may interfere with are data. We then labeled 90 plastic tubes for each of the samples collected at the burn plots. 4 milligrams of soil were measured for each plastic tube. To measure and transfer the soils for the plastic tubes, a work area was cleaned and set up. The tables were covered in aluminum foil and tapped down. The aluminum foil was then cleaned with ethanol 3 times. For each sample, the soils were homogenized in order to create an even layering throughout the soil and to prevent heavier particles such as sand from falling to the bottom. Each soil sample was placed on a piece of aluminum foil that was cleaned with ethanol 3 times. Once the homogenizing was complete any POM material left in the soil sample was taken out. 4 milligrams of soil was then measured. To measure the soil, scoops from the top, middle and bottom were taken repeatedly until 4 milligrams was reached. A scale was used in weighing the 4 milligrams. To weigh out the 4 milligrams a glass beaker was set onto the scale and then reset to zero. Once the scale was set to zero, a labeled plastic tube was set inside the beaker and then the scale was again reset to zero. Soil was then added to the plastic tube until 4 milligrams was reached. Once 4 milligrams was reached, we then repeated this procedure for the 90 glass jars. The remaining soil was placed into a separate labeled plastic tube. The exact weights of the 4 milligram tubes were then recorded. Weights of the 4 milligram tubes ranged from 4-4.3 milligrams. Plastic tubes were not to contain any less than 4 or more than 4.3 milligrams or the testing and analysis would be ruined. The soils were placed in a total of 180 containers, 90 in plastic tubes and 90 in glass jars. The soils were separated into two categories which were fumigated and non-fumigated.

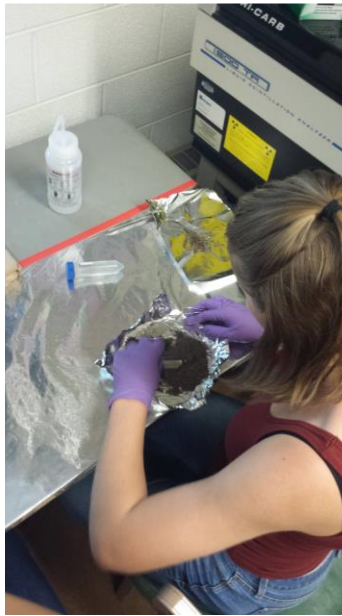

Fig 3: Soils being homogenized in the lab

Lab Testing

In this study, we used Chloroform Fumigation Extraction (CFE). This procedure estimates the size of microbial C and N pools. The materials needed for CFE are; ethanol-free chloroform (3x75 ml), Aluminum foil, 2 dessicators with maintained vacuum pump, 16 liters of 0.1 M K_2SO_4 with dispenser (17.427 grams/liter), 2x2 l beakers, steering bars and steering plates, 20 L Carboy. 48 Whatman filter paper; Extract rack (made of wood); 80 mm funnels; Shaker (180 strokes/ minute); Boiling chips; 130 x 20 ml scintillation vials (5 per sample); 52 x 50 ml screw-top glass tubes with Teflon lined caps (2 per sample); 26 x 125 ml glass vials with lids (1 per sample– for fumigated samples only); 52 x 125 ml plastic specimen containers (2 per soil sample); 26 x 250 ml screw-top bottle (1 per sample to collect unfumigated filtrate). Before we began the testing on the soils we had to prepare 2 dessicators. Once this was complete, testing began on all 180 soil samples. The procedure for the testing was: 1) Weigh fumigated and control samples in the proper containers according to the table **1**. Cap the vials containing the samples and store them in the +4 °C fridge**. 2.** Pour 75 ml of ethanol-free chloroform into one 125/ 250 ml Erlenmeyer; 1 Erlenmeyer/ dessicator. Cover the bottom of the Erlenmeyer with 1 layer of boiling chips. **3.** Randomly place the fumigated samples (uncapped) into dessicators. Add the Erlenmeyer containing the chloroform and cover the dessicator. **4.** Evacuate dessicators until chloroform boils vigorously (but not spill out). Repeat the operation twice, allowing the air to enter the dessicator each time to facilitate the homogenous distribution of the chloroform throughout samples. The dessicators are evacuated a third time until the chloroform boils vigorously for ~2 min. Keep the dessicators in the dark at 25 °C. Repeat the operation twice a day for 48 hours. **5.** After 48 hours, remove the chloroform flask and evacuate the desiccator(s) 8 times 3 minutes to evacuate residual chloroform. **6.** Extract fumigated and control (unfumigated) samples using 0.1 M K_2SO_4 at a ratio of 5:1 extractant to dry soil ratio according to table 1. **7.** After shaking, filter the soil solution using a pre-rinsed (with 0.1 M K_2SO_4) No 42 filter paper. Collect the filtrate on a 125 ml plastic specimen container. Using disposable pipets, place the filtrate into 4 containers for the controls and 3 containers for the fumigated samples according to table 2.

Record the exact volume placed in each container. **8.** Keep all extracts in sealed, labeled vials in -20 °C freezer until 2 days prior to analyses.

Calculations

Calculations on the soil samples were run through multiple ANOVA and regression tests. ANOVA Tests were run on Microbial N, NH_4^+, NO_3^- mass (mg/g) compared to forest stand age. Regression tests were run on the relationships between amino-N and NH_4^+ and amino-N and NO_3^-.

Fig 4: Chloroform testing

Fig 5: KCL extractions

Results:

Amino N and NH_4^+ in Soils:

To compare Amino N and NH_4^+ to stand age we used a regression test with a 95% confidence interval. In this test we found that there is no significant difference between Amino N and NH_4^+ compared to stand age. (Fig 1). If we disregard stand age, we found that there is a significant difference in NH_4^+ compared to Amino N. The p-value is less than 0.05 which indicates that there is a significant difference in NH_4^+ and Amino N disregarding stand age.

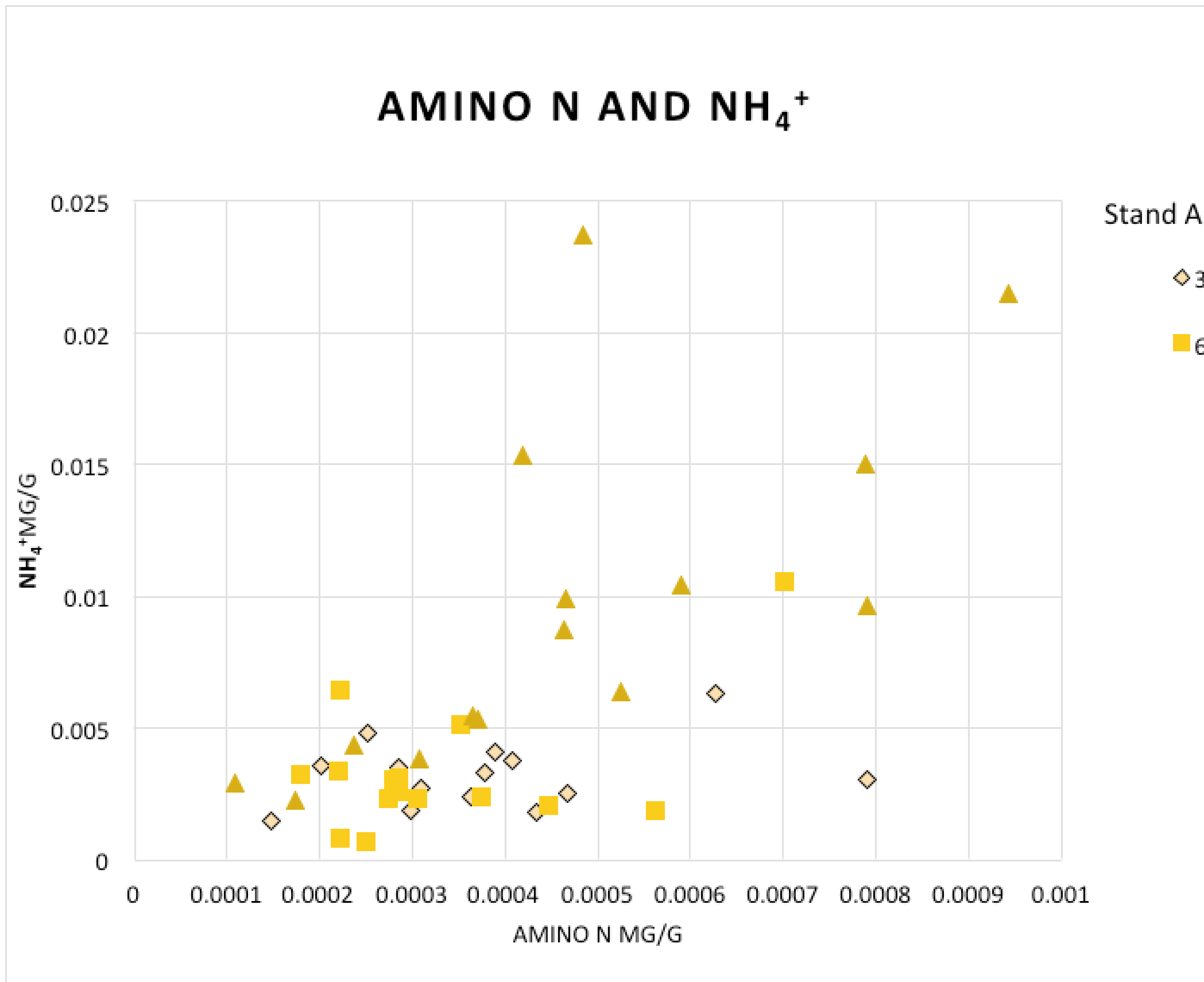

Fig 6
R^2= 0.365 P<0.000, 95% Confidence Level

Amino N and NO_3 in soils:

To compare Amino N and NO_3 to stand age we used a regression test with a 95% confidence interval. In this test we found that there is no significance between Amino N and NO_3 compared to stand age. The p value is less than 0.061 but greater than 0.05 which indicates that there is no significant difference of Amino N and NO_3 compared to stand age.

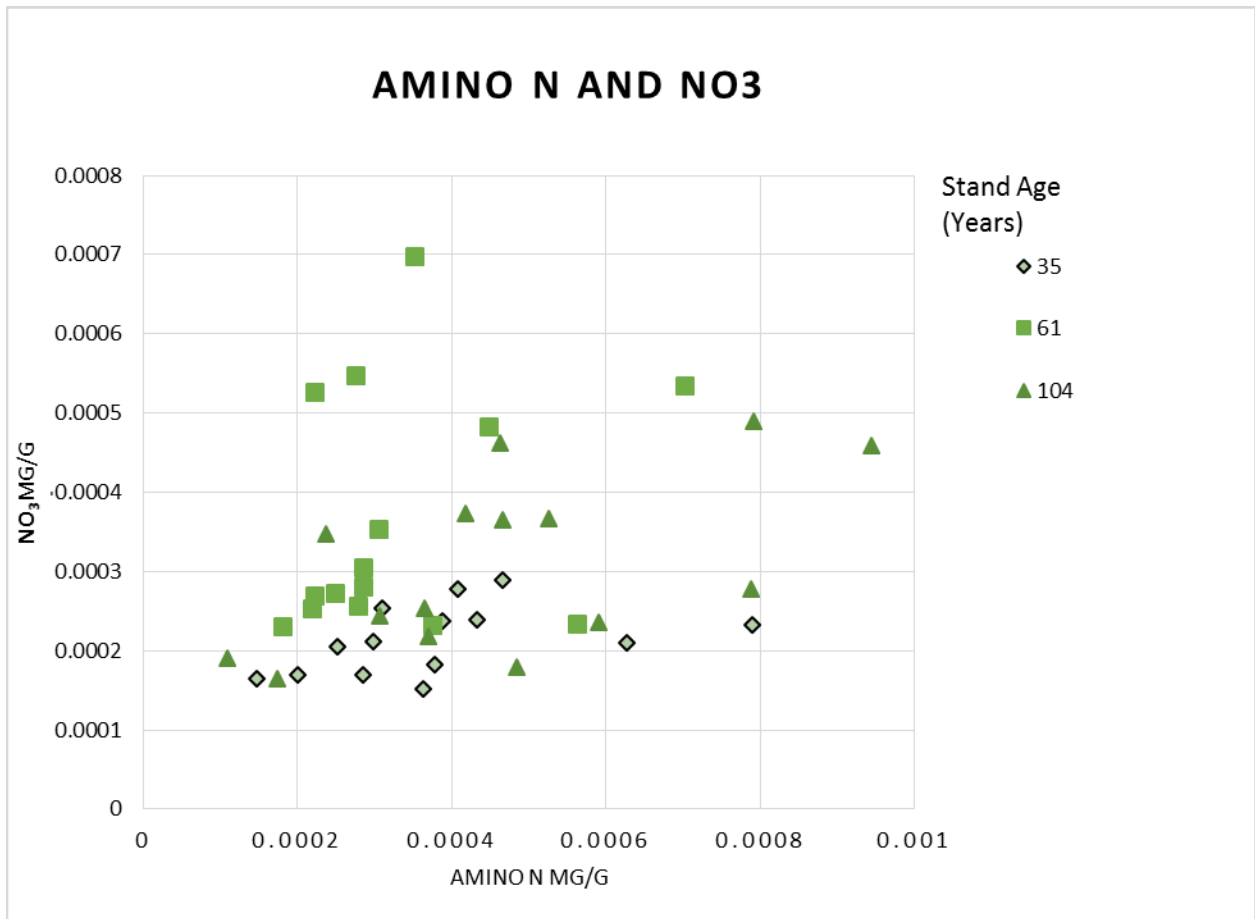

Fig. 7
R^2=0.081, P<0.061, 95% Confidence Level.

Amino N and Stand Age:

To compare Amino N and stand age, we used an ANOVA test. (Fig 3). Our results showed that across the stand ages the mass (mg/g) of the Amino N were similar. Are p value was equal to 0.273 which shows that there is no significant difference in the amino N and stand age.

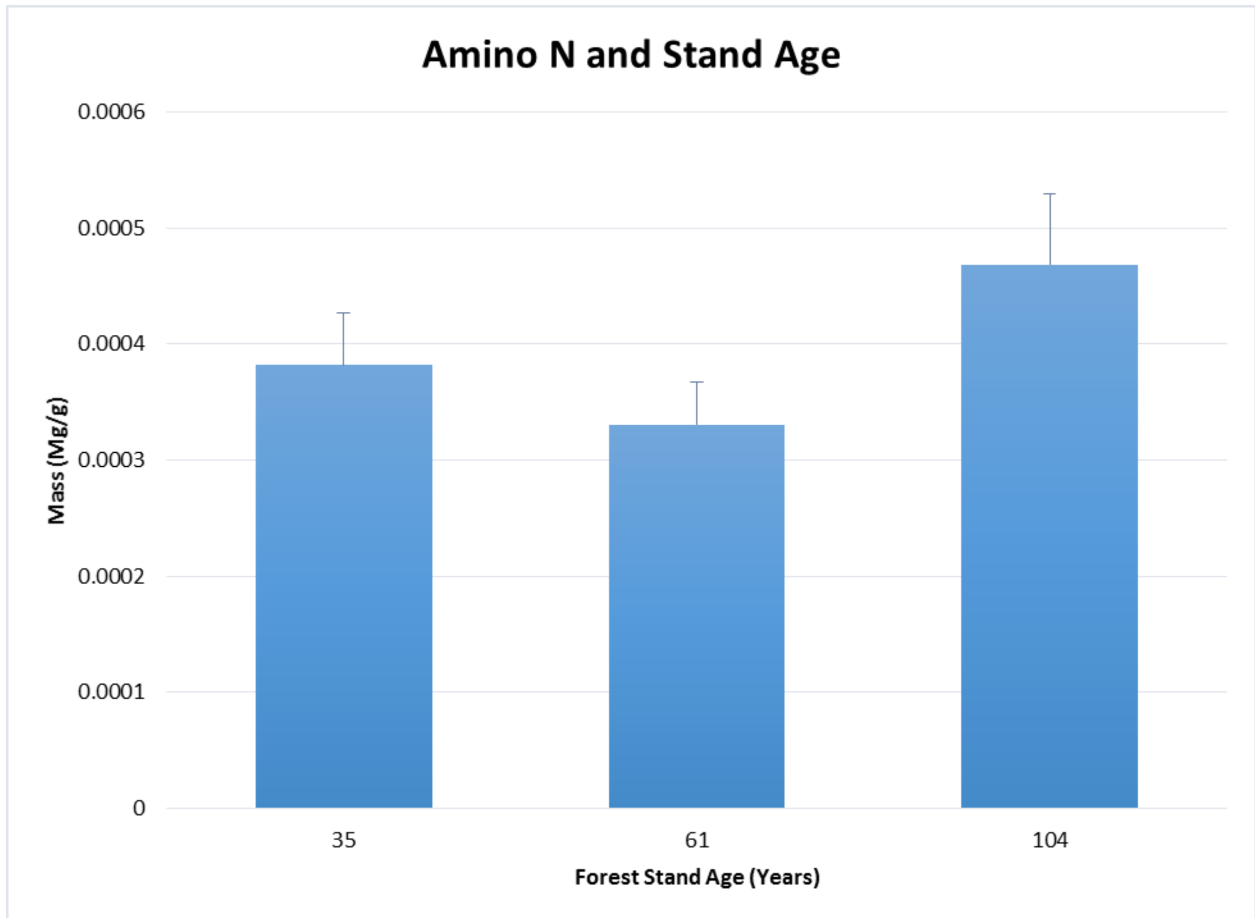

Fig. 8 $F_{2,39}$, p=.273

Overall Comparison of Microbial N, NH_4^+ and NO_3^- in soils:

To make an overall comparison of microbial N, NH_4^+ and NO_3^- to stand age we used an ANOVA test which compared the mass (mg/g) of microbial N, NH_4^+ and NO_3^- to stand age. (Fig 3). Overall we found that Microbial N mass (mg/g) is similar across the three stand ages. We also found that NO_3^- mass (mg/g) is similar across the stand ages as well. For NH_4^+ mass (mg/g) we found that the 104 age stand is significantly higher compared to the 35 and 61 stand ages which are similar to one another. This is consistent with the p values for each because the p values of microbial N (p=.863) and NO_3^- (p<.003) are greater than 0.05 which shows no significant difference. NH_4^+ has a p value of p<0.000 which shows a significant difference in mass compared to stand age because the p value is smaller than 0.05

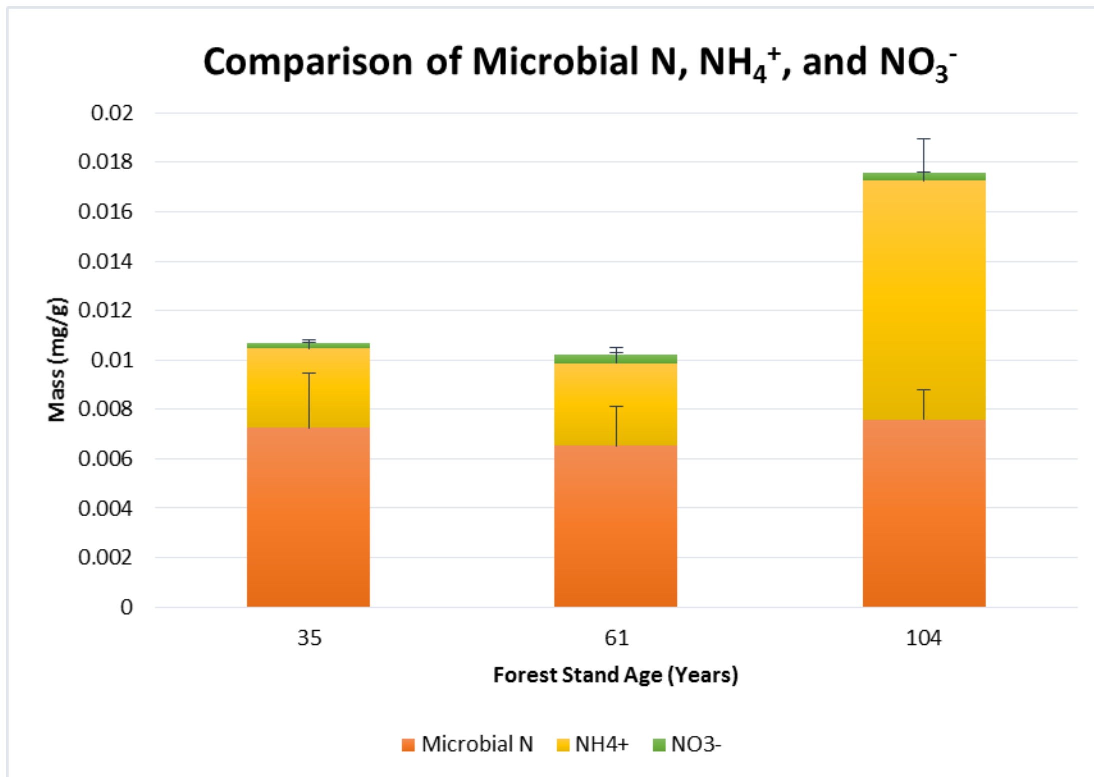

Fig. 9 Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003)

Discussion:

In the burn plots at the University of Michigan Biological Station (UMBS), we wanted to look at how nitrite, ammonium, amino-N, or total microbial N varied between forest stand ages. We also wanted to see if there was a relationship between amino-N and ammonium content in soil as well as a relationship between amino-N and nitrite content in soil. We came up with three hypotheses to answer our questions. The first hypothesis was that nitrite, ammonium, amino-N or total microbial N would be greater in older forests because microbial communities in older stands will have more time to develop. We found that there was no significant difference so we rejected this hypothesis. Our second hypothesis was that there would be a positive relationship between amino-N and ammonium content in the soil because ammonium indicates decomposition by microbes. We found that there was a significant difference in amino-N and ammonium content so we accept this hypothesis. Our third hypothesis was that there is a positive relationship between amino-N and nitrate content in soil across stand age because microbes participate in nitrification. We found that there was no significant difference so we rejected this hypothesis.

Similar studies have been conducted relating to differences in nutrient levels and microbial biomass. In a study done in 2007 by Chris Gough et al, it indicates that leaf litter and aboveground wood levels are higher in older plots. From this information and our results on ammonium levels, we think that a reason for higher ammonium levels in the oldest stand are because the microbes that are present may be more effective at producing ammonium. In our results we saw the lowest levels of nitrate in the youngest burn plot. A reason for this is that early succession species such as bigtooth aspen, are fast growing and as they grow they take up nitrates quickly as they attempt to fill in the canopy. Because of this, maybe at younger successional stages, bacteria are more involved in mineralization and ammonification. In a study done in 2003 by Laura White et al, it states that rates of N mineralization and nitrification were initially rapid and then they declined for the first 20 years of stand development and then began to increase toward a maximum value.

In conclusion, we rejected all of our hypotheses except one. We accepted the hypothesis that there was a positive relationship between amino-N and ammonium levels. To gain a better understanding of why there is a relationship between amino-N and ammonium levels as well as why there is not a positive relationship between amino-N and nitrate levels and no significant difference in nitrite, ammonium, amino-N or total microbial N in older forests, studies in the future should look at the factors that are causing the nutrient levels to vary or not vary as well as the types of bacteria or microbes present in the forests that are producing the nutrients. Future studies should also look at the types of bacteria and microbes that are present directly after disturbances such as burns and those that come in later.

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Appendix

1. Figure 1: soils being collected using a cylinder and mallet
2. Figure 2: sieving of soils
3. Figure 3: soils being homogenized
4. Figure 4: Chloroform testing
5. Figure 5: KCL extractions
6. Figure 6: regression of amino-N and NH_4^+
7. Figure 7: regression of amino-N and NO_3^-
8. Figure 8: ANOVA of amino-N and stand age
9. Figure 9: ANOVA of microbial N, NH_4^+ and NO_3^-