Microbial Biomass, Soil Ammonium and Nitrate in a 69-year Secondary Successional *Populus grandidentata* Chronosequence

Sarah Perry

University of Michigan Biological Station
EEB 381 – General Ecology
8/17/2015
Dr. Shannon Pelini

Abstract

Over the last two hundred years, logging and fire have caused significant changes in the landscape of northern Lower Michigan. Nutrient cycling is impacted as these disturbances affect the forest ecosystem, causing secondary succession. Soil microbes perform the important function of nitrogen nitrification and mineralization, which causes variation in the amount of ammonium and nitrate available in the soil. The trends in microbial and amino N biomass, nitrate, and ammonium levels across secondary successional chronosequences are currently not well understood. We collected "A" horizon soil samples from three *Populus grandidentata* forests of varying secondary successional ages. We performed a potassium sulfate (K_2SO_4) extraction on chloroform-fumigated and non-fumigated soil samples. Then, we ran a ninhydrin assay on the extractions to determine soil ammonium, nitrate, microbial N biomass, and amino N biomass levels in the soil. Total microbial N, NH_4+, and NO_3- are significantly larger in the 104-year-old burn plot (Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003)). There is not a significant difference in amino N mass between the three stands ($F_{2,39}$, p=.273). There is a positive relationship between amino N mass and NH4+ mass (R^2=0.365, P<0.000, 95% Confidence Level). Lastly, there is not a significant relationship between Amino N mass and nitrate mass (R^2=0.081, P<0.061, 95% Confidence Level). Total nitrogen content in the soil is higher in later-successional forest stands, and microbial biomass does not account for large changes in soil nitrate. Higher microbial biomass indicates higher ammonium content in the soil. These results are significant because nitrogen in the soil affects forest productivity, an ecosystem service we need to perform carbon sequestration and emitting of oxygen.

I grant the Regents of the University of Michigan the non-exclusive right to retain, reproduce, and distribute my paper, titled in electronic formats and at no cost throughout the world.

The University of Michigan may make and keep more than one copy of the Paper for purposes of security, backup, preservation and access, and may migrate the Paper to any medium or format for the purpose of preservation and access in the future.

Signed,

Sarah Pang

Sarah Perry
August 16th, 2015
Group Project Manuscript

Microbial Biomass, Soil Ammonium and Nitrate in a 69-year Secondary Successional *Populus grandidentata* Chronosequence

Abstract

Over the last two hundred years, logging and fire have caused significant changes in the landscape of northern Lower Michigan. Nutrient cycling is impacted as these disturbances affect the forest ecosystem, causing secondary succession. Soil microbes perform the important function of nitrogen nitrification and mineralization, which causes variation in the amount of ammonium and nitrate available in the soil. The trends in microbial and amino N biomass, nitrate, and ammonium levels across secondary successional chronosequences are currently not well understood. We collected "A" horizon soil samples from three *Populus grandidentata* forests of varying secondary successional ages. We performed a potassium sulfate (K_2SO_4) extraction on chloroform-fumigated and non-fumigated soil samples. Then, we ran a ninhydrin assay on the extractions to determine soil ammonium, nitrate, microbial N biomass, and amino N biomass levels in the soil. Total microbial N, NH_4+, and NO_3- are significantly larger in the 104-year-old burn plot (Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003)). There is not a significant difference in amino N mass between the three stands ($F_{2,39}$, p=.273). There is a positive relationship between amino N mass and NH4+ mass (R^2=0.365, P<0.000, 95% Confidence Level). Lastly, there is not a significant relationship between Amino N mass and nitrate mass (R^2=0.081, P<0.061, 95% Confidence Level). Total nitrogen content in the soil is higher in later-successional forest stands, and microbial biomass does not account for large changes in soil nitrate. Higher microbial biomass indicates higher ammonium content in the soil. These results are significant because nitrogen in the soil affects forest productivity, an ecosystem service we need to perform carbon sequestration and emitting of oxygen.

Introduction

The temperate deciduous forests of Northern Lower Michigan vary highly in successional age due to disturbance frequently and heterogeneously affecting the landscape. Commercial logging was prevalent in the Great Lakes Region from the late 19th to early 20th centuries[1]. Historically, fire has also been a frequent disturbance in the forests of northern Lower Michigan. In Pellston, MI, The University of Michigan Biological Station (UMBS) property contains 10,000 acres of forested property on Douglas and Burt Lakes. In 1911, a section of the property known as the "Burn Plots" was cut down and burned. Forest ecologists at the Biological Station, interested in understanding secondary succession following a fire, began experimentally burning

---

[1] Gough, Christopher, Christoph Vogel, Katherine Harrold, Kristen George, and Peter Curtis. "The Legacy of Harvest and Fire on Ecosystem Carbon Storage in a North Temperate Forest." *Global Change Biology* 13 (2007): 1935-949. Blackwell Publishing Ltd, 2007. Web.

segments of the "Burn Plots" roughly 2.5-3 acres in size. Experimental burns were conducted in the "Burn Plots" in 1936, 1948, 1954, 1980, and 1998[2].

Disturbances such as fire and logging have a major impact on nutrient cycling, particularly that of nitrogen. White et al (2003) performed research on nitrogen nitrification and mineralization in the "Burn Plots" in 2003. Net nitrogen mineralization, i.e. the conversion of atmospheric nitrogen gas (N_2) to ammonium (NH_4+), is highest in the most recently disturbed plot. N mineralization then decreases during the first 18 years of succession, and then increases at a linear rate as succession progresses. An identical trend occurs with N nitrification, or the oxidation of ammonium to nitrate, and then to nitrite.[3] Knute et al (1984) concluded that net nitrogen mineralization is an important factor limiting production in unfertilized forest ecosystems[4]. Soil nitrogen availability is often a limiting factor for forest growth and productivity, which makes our understanding of the nitrogen cycle essential.[5] The human population depends on highly productive forests for the sequestration of carbon dioxide and the production of oxygen.

Microbes, single-celled bacteria in the soil, play an important role in the nitrogen cycle and thus forest productivity by recycling nutrients from dead organic matter back into the soil. Microbes consume dead organic matter for energy and for the assimilation of nitrogen into their cells for the production of proteins[6]. Microbes require energy to perform this important ecosystem service, which causes them to respire. Microbial respiration is the largest source of atmospheric carbon dioxide[7], thus understanding microbial activity in forest soils is important to reducing atmospheric greenhouse gas levels and therefore climate change. Do nitrate, ammonium, amino-N, or total microbial N content in soils vary with forest stand age? Is there a relationship between amino N and ammonium content in soil? Is there a relationship between amino N and nitrate content in soil?

To answer these questions, we developed a number of hypotheses. (i) Nitrate, ammonium, amino-N, and total microbial N content will be greater in older forest stands because

---

[2] "UMBS Burn Plots." *UMBS Burn Plots*. U-M School of Literature, Science, and the Arts, n.d. Web. 03 Aug. 2015. <http://umbs.lsa.umich.edu/research/researchsite/umbs-burn-plots.htm>.

[3] White, Laura L., Donald R. Zak, and Burton V. Barnes. "Biomass Accumulation and Soil Nitrogen Availability in an 87-year-old Populus Grandidentata Chronosequence." *Forest Ecology and Management* 191 (n.d.): 121-27. Web. 30 July 2015.

[4] Nadelhoffer, Knute J., John D. Aber, and Jerry M. Melillo. "Seasonal Patterns of Ammonium and Nitrate Uptake in Nine Temperate Forest Ecosystems." *Plant and Soil* 80 (1984): 321-35. Web.

[5] White et al (2003)

[6] Meade-Callahan, Maura. "Microbes: What They Do & How Antibiotics Change Them." *Actionbioscience*. AIBS, 2015. Web. 16 Aug. 2015.

[7] Pelini, Shannon. "Lecture." Lecture. University of Michigan Biological Station, Pellston, MI. 29 June 2015. Web. 29 June 2015.

microbial communities in older stands will have had more time to develop. (ii) There will be a positive relationship between amino N and ammonium content in soil because ammonium indicates decomposition by microbes. (iii) There will be a positive relationship between amino N and nitrate content in soil across stand age because microbes participate in nitrification.

To test these hypotheses, we gathered 15 samples from three "Burn Plots" and performed a potassium sulfate (K_2SO_4) extraction on non-fumigated and chloroform-fumigated soils. We then performed a ninhydrin assay on the extractions to test for soil ammonium (NH_4+), nitrate (NO_3-), and soil microbial biomass.

Methods

Soil Sample Collection

We took a total of forty-five soil samples from three burn plots of varying successional stages; we sampled plots burned in 1911, 1954, and 1980. Within each burn plot, we set up three randomly-selected subplots, then took five soil samples from each subplot (See Figure 1). To determine from where within each subplot to remove the soil samples, we measured 5 meters due north of the randomly selected site and then took a soil sample every 72 degrees along the circumference of a 5-meter radius circle. We used a soil core to remove the soil from the "A" horizon, then separated the leaf litter into a labeled paper bag and the soil into a labeled plastic bag. We sampled soil from the "A" layer because microbial activity is highest in this layer[8].

To prevent non-microbial organic matter in the soil samples from skewing our data, we separated out plant roots and particulate organic matter (POM) from each soil sample, and then sieved each of the samples through a 2 millimeter sieve. Following sieving, we placed each sample into a vial and then into a refrigerator to prevent mold from growing inside the sample until we were able to prepare the samples for chloroform fumigation and potassium sulfate (K_2SO_4) extraction.

Homogenization and Weighing

We homogenized each of our samples before placing ~4 grams from each sample into two vials: one labeled "Control" and the other labeled "Fumigated." In a soil sample, the larger, heavier sediments tend to settle at the bottom of the vial and the lighter sediments settle on top. Homogenization ensures that when taking subsamples from each soil sample, we place an evenly mixed subsample into the vials. We placed each of the samples to be fumigated into a glass vial labeled with tape and pencil, as ink is not legible following a chloroform fumigation.

---

[8] Agnelli, Alberto, Judith Ascher, Giuseppe Corti, Maria Ceccherini, Paolo Nannipieri, and Giacomo Pietramellara. "Distribution of Microbial Communities in a Forest Soil Profile Investigated by Microbial Biomass, Soil Respiration and DGGE of Total Extracellular DNA." *Soil Biology & Biochemistry* 36 (2004): 859-68. Elsevier, 2004. Web. 3 Aug. 2015.

Potassium Sulfate (K_2SO_4) Extraction and Chloroform Fumigation

The forty-five "Control" samples are not fumigated, so we performed a K_2SO_4 extraction on them following homogenization and weighing. We added 40 milliliters of 0.1 M K_2SO_4 to each of the soil subsamples, then placed the subsamples in the "shaker" at a rate of 180 strokes per minute for an hour. We then poured the subsamples through a Number 42 filter paper using a funnel. This process allows us to extract total nitrogen from the soil sample. The "Control" samples will allow all soil nitrogen to seep through the filter, however microbes are too large to seep through the filter so the extraction gives us an idea of soil nitrogen available to microbes, but not within microbes.

We performed a chloroform fumigation on the "Fumigated" samples to break open the microbial cells. The purpose of the fumigation is so that during the potassium sulfate extraction, microbial and amino nitrogen will seep through the Number 42 filter paper, and the results of said extraction will provide us with total soil nitrogen, from which we can subtract nitrogen available to microbes to determine microbial biomass. To fumigate, we placed each glass vial into a dessicator (See Figure 2) with an Erlenmeyer flask containing 75 ml of chloroform and ~20 boiling chips. We allowed the desiccator to sit for 48 hours, boiling the chloroform twice a day. We then ran the "Fumigated" samples through the same potassium sulfate extraction which we performed on the "Control" samples.

Lastly, we performed a ninhydrin assay to determine the content of soil nitrogen in each of the extracted samples.

Results

Total microbial N, NH_4+, and NO_3 are significantly larger in the 104-year-old burn plot (Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003)). This difference is mainly due to a large increase in NH_4+ between the 61-year-old plot and the 104 year old plot (See Figure 3). There is no significant difference in microbial N mass across the chronosequence ($F_{2,39}$, p=.863) (See Figure 4). There is a significant difference in the mass of NH_4+ between the 104-year-old plot and the 35- and 61-year old plots ($F_{2,39}$, p=<.000) (See Figure 5). There is a significant difference in the mass of NO_3- between the 61-year-old stand and the 35-year-old stand, and the 104 year old stand ($F_{2,39}$, p=.003) (See Figure 6). There is not a significant difference in amino N mass between the three stands ($F_{2,39}$, p=.273) (See Figure 7).

There is a positive relationship between amino N mass and NH_4+ mass (R^2=0.365, P<0.000, 95% Confidence Level) (See Figure 8). There is not a significant relationship between Amino N mass and nitrate mass (R^2=0.081, P<0.061, 95% Confidence Level) (See Figure 9).

Discussion

The sum of microbial N, ammonium, and nitrate is significantly higher in the 104-year-old forest stand than in the 35- and 61-year-old stands. This difference is largely associated with a substantial increase in ammonium from the 61-year-old to the 104-year-old stand. It is possible that the soil in the oldest stand contains a higher proportion of certain microbe genera or species which participate in N mineralization more frequently or more efficiently than nitrification. It is also possible that ammonium levels increase so significantly in the oldest burn plot because of higher availability of leaf litter in older burn plots, providing the microbes with a substantially higher amount of material to consume and convert into ammonium. The increase in leaf litter with forest stand age has been confirmed by Chris Gough et al (2007)[9]. The difference in the sum of microbial N, ammonium, and nitrate is not due to differences in microbial N because of the lack of significant difference in microbial N across the chronosequence. The small mass of nitrate in comparison to microbial N and ammonium cannot account for differences in the total soil Nitrogen value either.

Though the size of the microbial nitrate pool is small, there are significant differences in the mass of nitrate in the soil between the forest stands. Nitrate mass is highest in the intermediate (61-year-old) plot, slightly smaller in the 104-year-old plot, and then smallest in the 35-year-old plot. Nitrate levels are lowest in the youngest stand, and this is likely due to the quick rate of growth occurring above ground as early successional species, mainly *Populus grandidentata*, attempt to fill the canopy. Nadelhoffer et al (1984) can confirm that plant uptake has a large impact on soil nitrate levels as the study concluded that nitrate supplied most of the N taken up annually by vegetation (this result occurred at 8 out of 9 study sites)[10]. It is possible that the oldest plot, the one experiencing an intermediate amount of nitrate in the soil, has reached a climax rate of nitrate uptake by plants, and the reason for the highest nitrate content in the intermediate plot is that the rate of nitrate uptake by plants varies widely before reaching a constant rate. Additionally, Kronzucker et al (1997) concluded that there is a clear, significantly positive relationship between nitrate concentration in the soil and nitrate uptake by plants (See Figure 10)[11]. This trend helps us to further explain the trend in nitrate levels across our successional chronosequence. Nitrate uptake by plants will be largest in the forest stand in which the most nitrate is being produced. It is possible that the most nitrate is being produced in the youngest stand as microbes rapidly attempt to decompose leaf litter still left over from the fire, and the fast-growing early-successional tree species quickly take up the nitrate. The trend we see in the Kronzucker et al (1997) study also confirms that nitrates are a limiting nutrient for plants, because the higher the concentration in the soil, the more they intake, and this trend does not

---

[9] Gough, Christopher, Christoph Vogel, Katherine Harrold, Kristen George, and Peter Curtis. "The Legacy of Harvest and Fire on Ecosystem Carbon Storage in a North Temperate Forest." *Global Change Biology*13 (2007): 1935-949. Blackwell Publishing Ltd, 2007. Web.

[10] Nadelhoffer, Knute J., John D. Aber, and Jerry M. Melillo. "Seasonal Patterns of Ammonium and Nitrate Uptake in Nine Temperate Forest Ecosystems." *Plant and Soil* 80 (1984): 321-35. Web.

[11] Kronzucker, Herbert J., M. Yaeesh Siddiqi, and Anthony DM Glass. "Conifer root discrimination against soil nitrate and the ecology of forest succession."*Nature* 385.6611 (1997): 59-61.

seem to be leveling off at any certain concentration of nitrates in the soil. Lastly, it is likely that the amount of nitrate in the soil is significantly smaller than the amount of ammonium in the soil is due to this uptake by plants and also because nitrates are more susceptible to loss by leaching.

There is no significant difference in microbial biomass between forest stands. This is likely due to a number of factors including leaf litter content in the stands, and species composition and growth rates. There is no significant difference in amino N across stand age, likely because there is no significant difference in microbial N across stand age. There is a significant, positive relationship between amino N mass and NH_4+. this is because microbes produce ammonium during mineralization. There is no significant relationship between amino N mass and nitrate mass. This is likely because NO_3- uptake by tree species likely has a larger impact on NO_3- pools than microbial nitrification does.

Forest secondary succession causes changes in the nitrogen cycle over time. Forest stand age does not necessarily indicate soil microbial biomass, and forest growth rates correlate with NO_3- and NH_4+ levels in soil. These trends impact a forest's productivity and therefore its ability to sequester carbon, an important ecosystem service to the human population.

Appendix

Figure 1: Soil Sampling Set-up

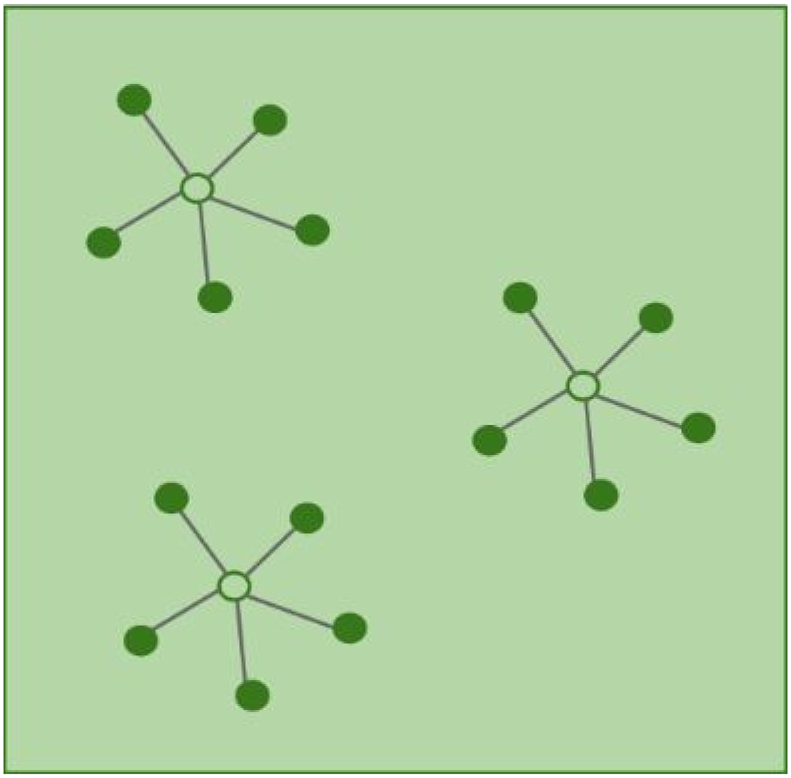

Figure 1 shows the set-up, within one sample “Burn Plot,” of our soil sample collections. Within one “Burn Plot,” we selected three subplots, or the green, unfilled circles in the figure. From each subplot, we took five soil samples from the “A” horizon.

Figure 2: Visual of Dessicator

Figure 2 shows a visual of the dessicator containing our chloroform-fumigated samples.

Figure 3: Comparison of Microbial N, NH4+, and NO3-

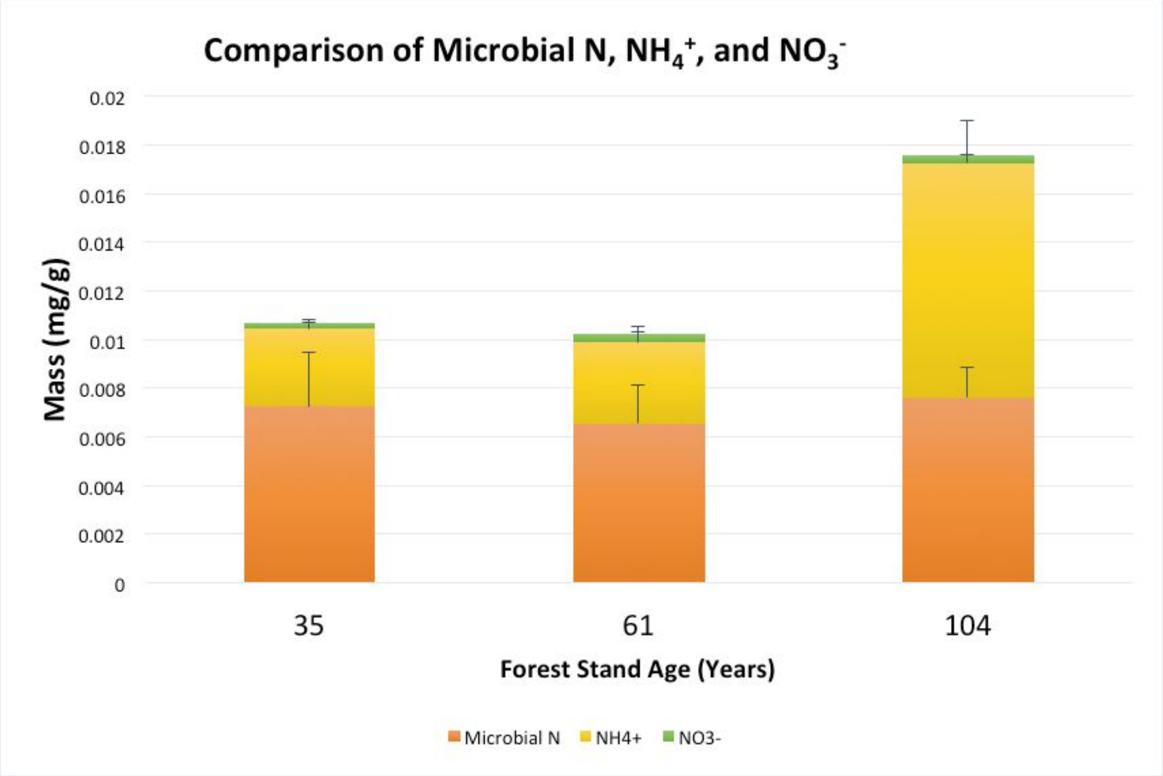

Figure 3 shows the total mass of soil nitrogen, broken down into Microbial N, NH4+, and NO3- present in the soil samples at each forest stand. Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003).

Figure 4: Comparison of Microbial N and Stand Age

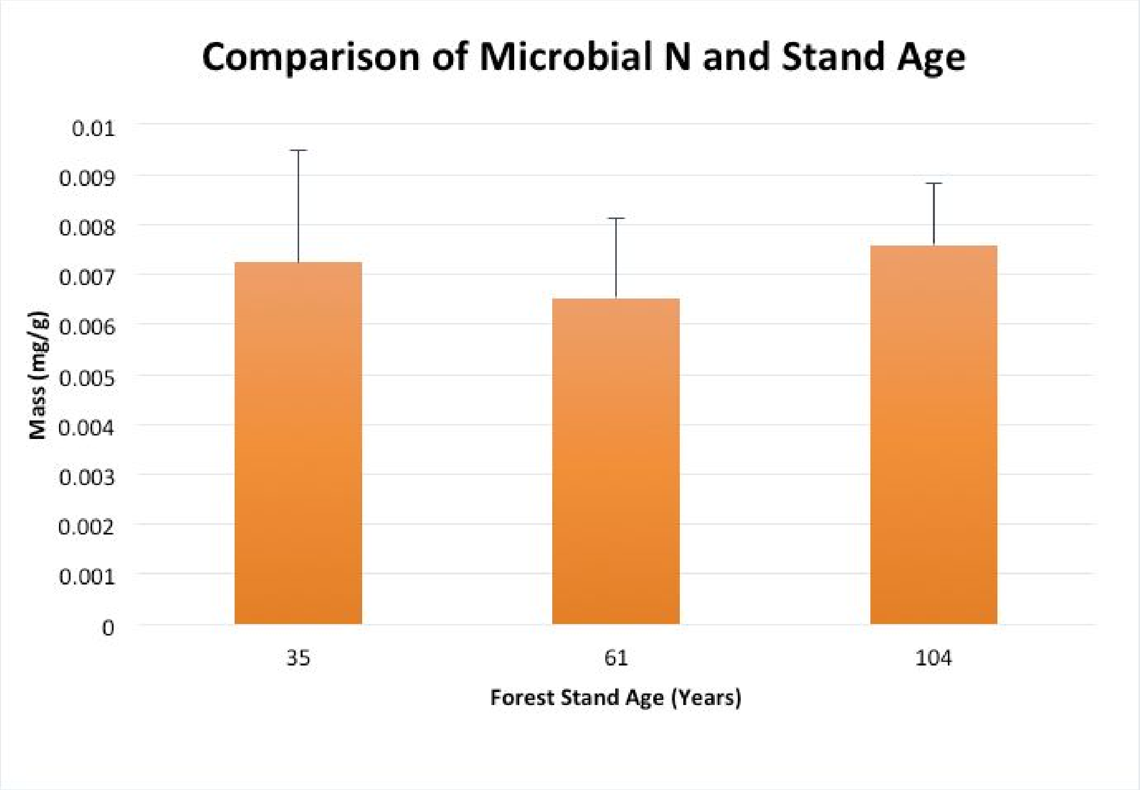

Figure 4 shows microbial N mass present in our soil samples at each forest stand. $F_{2,39}$, p=.863.

Figure 5: Comparison of NH4+ and Stand Age

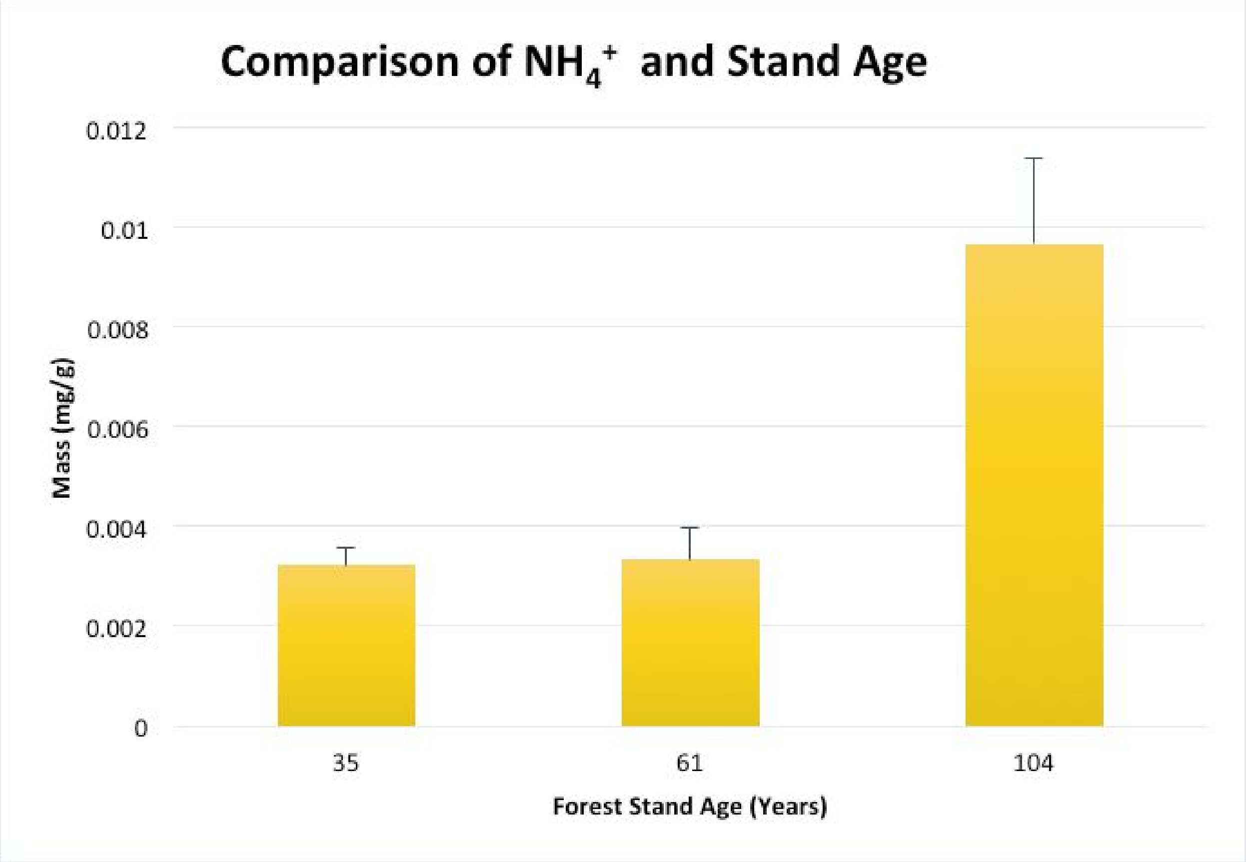

Figure 5 shows the mass of ammonium (NH4+) for the three forest stands. $F_{2,39}$, p=<.000.

Figure 6: Comparison of NO3- and Stand Age

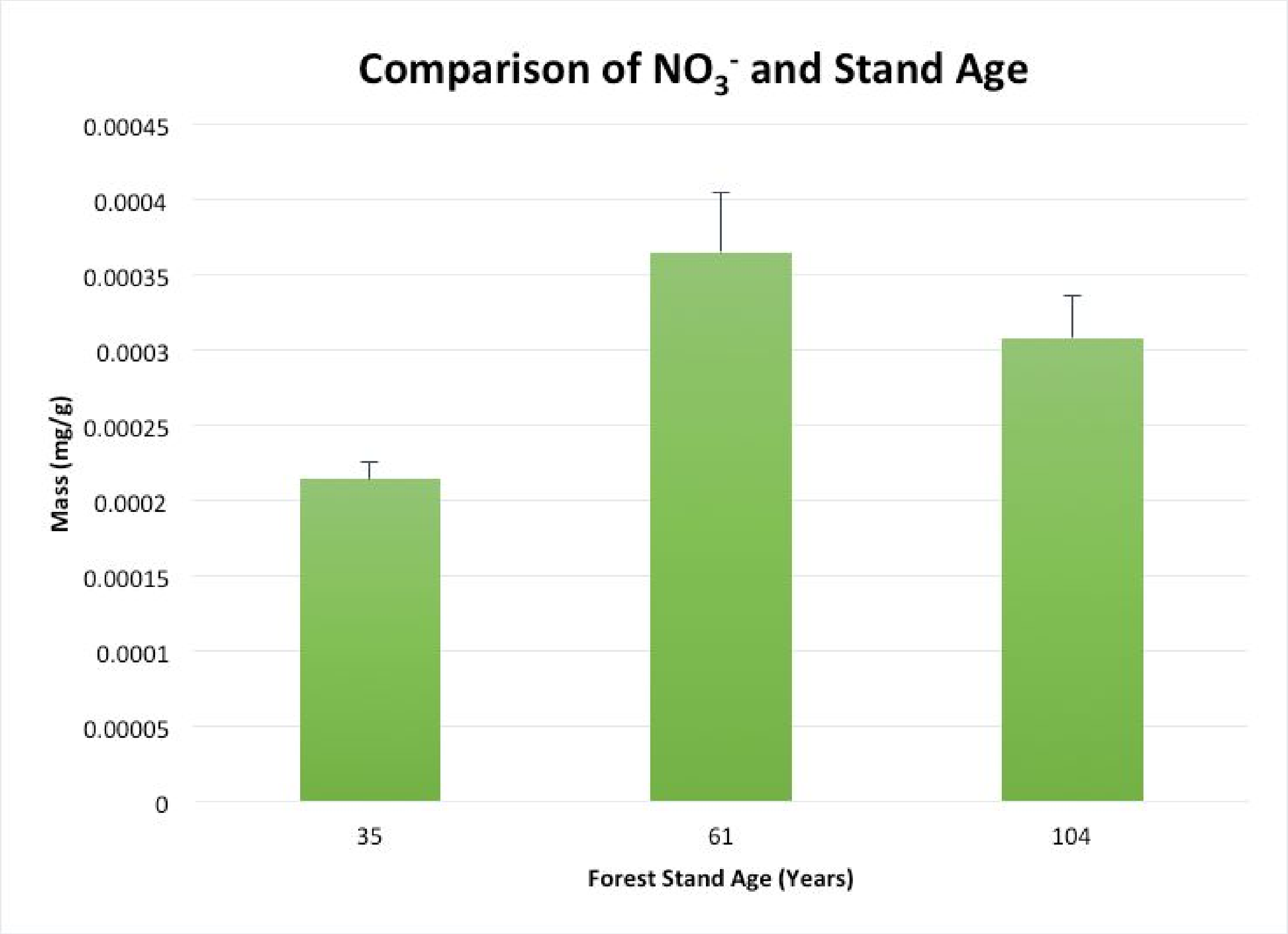

Figure 6 shows the mass of nitrate (NO3-) as it varies with stand age. $F_{2,39}$, p=.003.

Figure 7: Comparison of Amino N and Stand Age

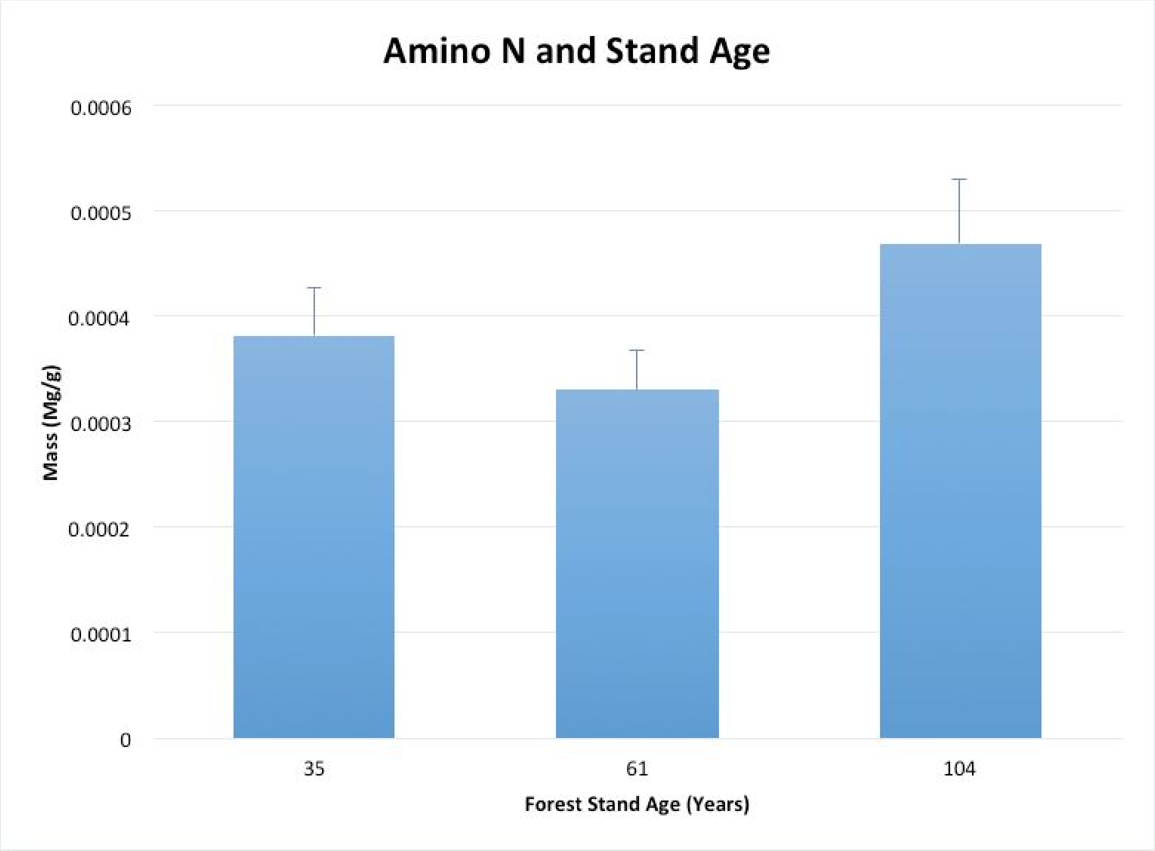

Figure 7 shows the mass of Amino N from our soil samples in the three forest stands. $F_{2,39}$, p=.273.

Figure 8: Relationship between Amino N and NH4+

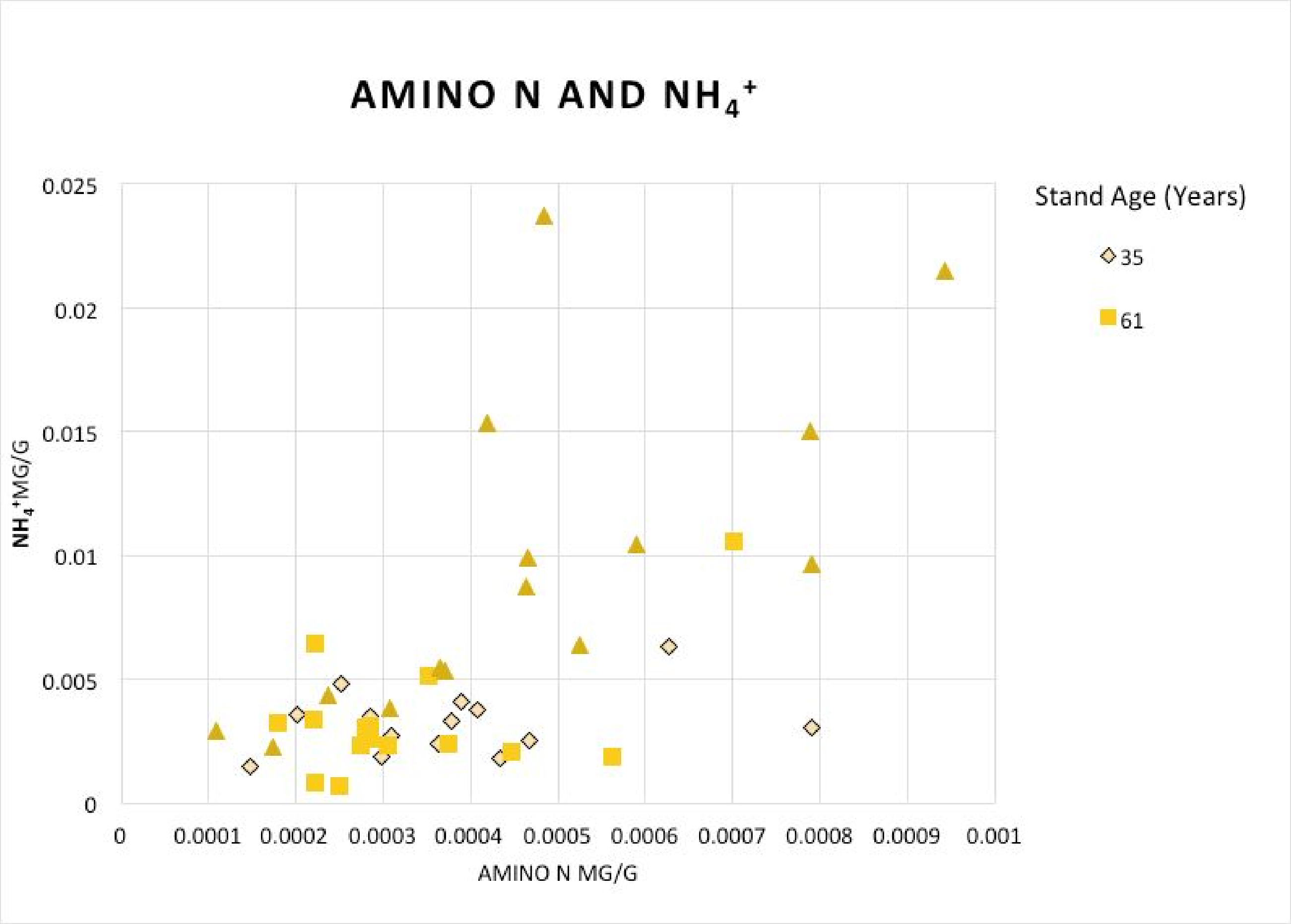

Figure 8 shows a significant relationship between soil amino N and NH4+. R^2=0.365, P<0.000, 95% Confidence Level.

Figure 9: Relationship between Amino N and NO3-

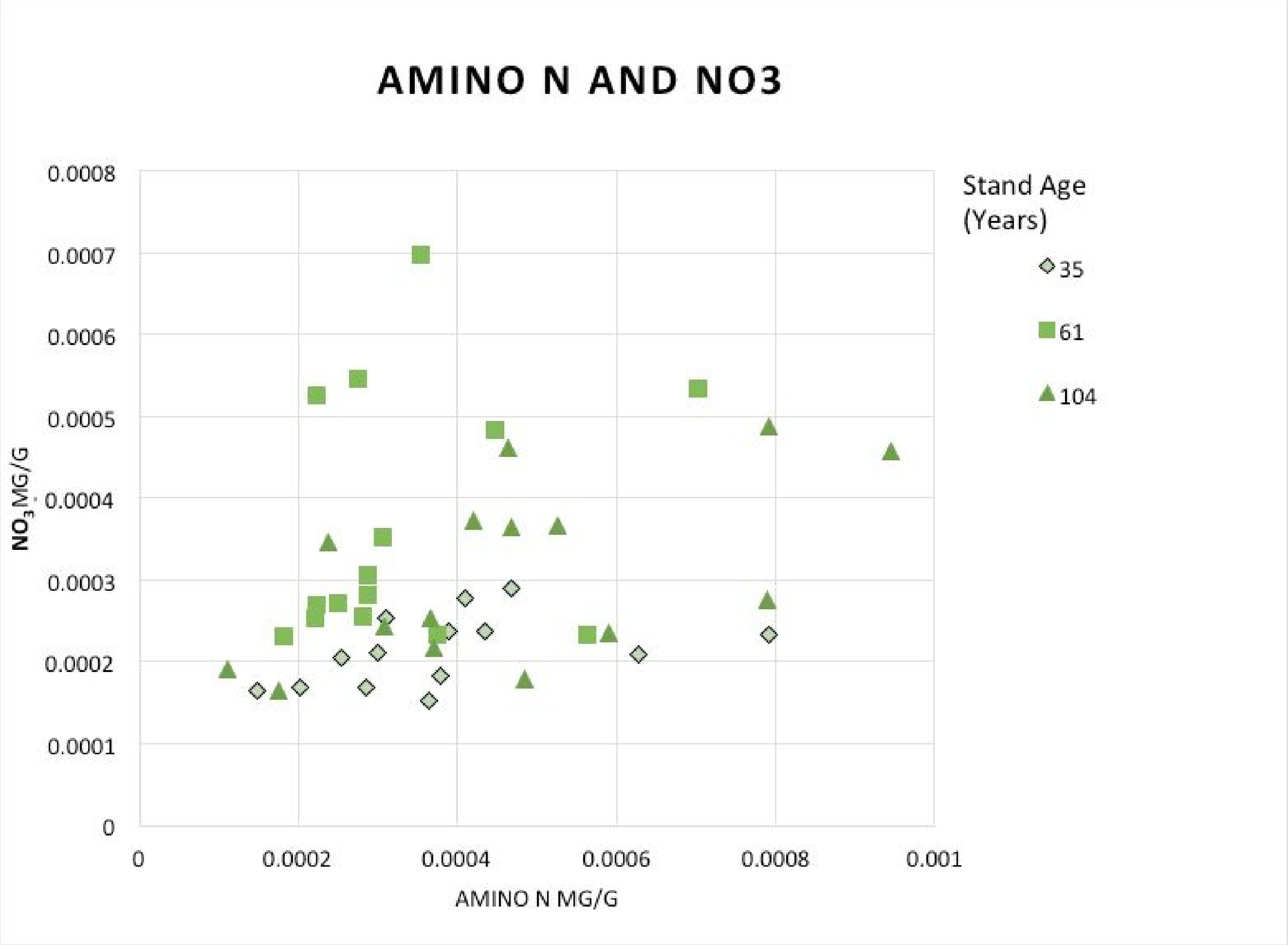

Figure 9 shows the relationship between Amino N and NO3-. R^2=0.081, P<0.061, 95% Confidence Level.

Figure 10: Relationship between Ammonium and Nitrate Concentration and Influx

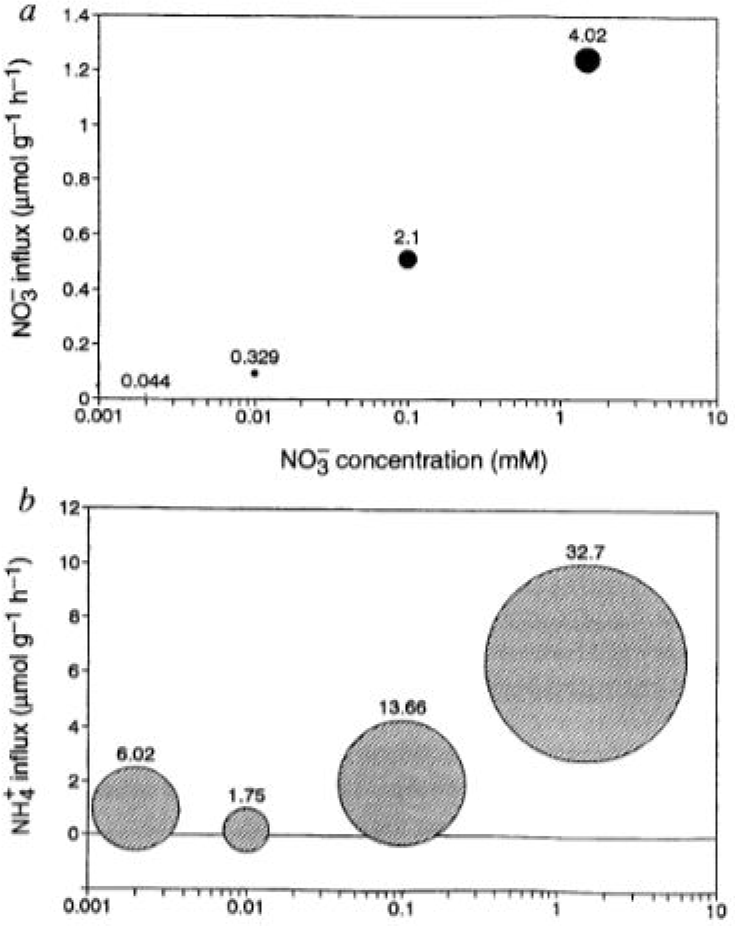

Figure 10 shows that NH4+ influx increases as NH4+ concentration increases (NH4+ concentration is the x-axis on graph b). The same trend occurs with nitrate concentration in the soil, however the trend is even clearer and with less variation in nitrate.

Literature Cited

Agnelli, Alberto, Judith Ascher, Giuseppe Corti, Maria Ceccherini, Paolo Nannipieri, and Giacomo Pietramellara. "Distribution of Microbial Communities in a Forest Soil Profile Investigated by Microbial Biomass, Soil Respiration and DGGE of Total Extracellular DNA." *Soil Biology & Biochemistry* 36 (2004): 859-68. Elsevier, 2004. Web. 3 Aug. 2015.

Gough, Christopher, Christoph Vogel, Katherine Harrold, Kristen George, and Peter Curtis. "The Legacy of Harvest and Fire on Ecosystem Carbon Storage in a North Temperate Forest." *Global Change Biology*13 (2007): 1935-949. Blackwell Publishing Ltd, 2007. Web.

Kronzucker, Herbert J., M. Yaeesh Siddiqi, and Anthony DM Glass. "Conifer root discrimination against soil nitrate and the ecology of forest succession."*Nature* 385.6611 (1997): 59-61.

Nadelhoffer, Knute J., John D. Aber, and Jerry M. Melillo. "Seasonal Patterns of Ammonium and Nitrate Uptake in Nine Temperate Forest Ecosystems." *Plant and Soil* 80 (1984): 321-35. Web.

Pelini, Shannon. "Lecture." Lecture. University of Michigan Biological Station, Pellston, MI. 29 June 2015. Web. 29 June 2015.

"UMBS Burn Plots." *UMBS Burn Plots*. U-M School of Literature, Science, and the Arts, n.d. Web. 03 Aug. 2015. <http://umbs.lsa.umich.edu/research/researchsite/umbs-burn-plots.htm>.

White, Laura L., Donald R. Zak, and Burton V. Barnes. "Biomass Accumulation and Soil Nitrogen Availability in an 87-year-old Populus Grandidentata Chronosequence." *Forest Ecology and Management* 191 (n.d.): 121-27. Web. 30 July 2015.