Relationship between microbial biomass, ammonium, and nitrate in a secondary successional forest

Aryn Fromm

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

Abstract

Microbes fill an essential role in forest ecosystem by breaking down detritus into available nutrients for plants. Microbes recycle nitrogen, making it possible for nitrogen limited forests to grow. Our study aimed to identify changes in microbial biomass, ammonium, and nitrate, through the secondary successional forests within the University of Michigan Biological Station burn plots. After sieving soil samples collected from the 1911, 1954, and 1980 burn plots, we performed potassium sulfate extractions on control samples and chloroform fumigated samples. Then we ran a ninhydrin assay test on the extractions. From our data we concluded that; 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 explain nitrate and ammonium levels in soil. These conclusions can be used to better characterize the effects of disturbances on forest ecosystems. Disturbances, like logging, can be better managed if there is a greater understanding of how forests interact with microbial communities.

Aryn Fromm
General Ecology
Final Paper
August 17, 2015

Relationships between microbial biomass, ammonium, and nitrate in a secondary successional forest

ABSTRACT

Microbes fill an essential role in forest ecosystem by breaking down detritus into available nutrients for plants. Microbes recycle nitrogen, making it possible for nitrogen limited forests to grow. Our study aimed to identify changes in microbial biomass, ammonium, and nitrate, through the secondary successional forests within the University of Michigan Biological Station burn plots. After sieving soil samples collected from the 1911, 1954, and 1980 burn plots, we performed potassium sulfate extractions on control samples and chloroform fumigated samples. Then we ran a ninhydrin assay test on the extractions. From our data we concluded that; 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 explain nitrate and ammonium levels in soil. These conclusions can be used to better characterize the effects of disturbances on forest ecosystems. Disturbances, like logging, can be better managed if there is a greater understanding of how forests interact with microbial communities.

INTRODUCTION

We aim to study the change in microbial biomass at different stages of secondary succession at the UMBS burn plots. Microbes are microorganisms living in the soil, primarily bacteria and fungi. Microbes play an important role in nutrient cycling and decomposition (Hoorman 2011). One of the most notable contributions of microbes to forest ecosystems is their ability to fix nitrogen and make nutrients available to plants. Microbes fix nitrogen by breaking down leaf litter and other organic matter that builds up on the forest floor. Plants use the nitrogen that microbes transform from NH4 to the more accessible NO3. Microbes are also important to the carbon cycle. Through decomposition, microbes break down organic matter, thus recycling carbon to forest soils.

Quantifying microbial biomass at different stages of forest succession, may improve our understanding of disturbances in Michigan forests, which is helpful in a state where considerable logging occurs. The logging industry creates disturbances when large swathes of forests are cut

down (White et al. 2004). To avoid a collapse in forest ecosystems, there must be sustainable logging methods. Understanding how Michigan forests respond to disturbances is key to establishing more sustainable logging.

White et al. 2004 showed that in big tooth aspen dominated forests, N limits forest growth. This implies that nitrogen fixing microbial communities are highly important in sustaining forest ecosystems. Additional studies completed at the UMBS burn plots by White et al. and Gough et al. show that above ground biomass increases with forest stand age.

We asked the following questions: Do levels of nitrate, ammonium, amino-N, or microbial N vary in soil across forest stand age? Is there a relationship between amino N and ammonium content in soil across stand age? Is there a relationship between amino N and nitrate content in soil across stand age?

We hypothesized that nitrate, ammonium, amino-n, and total microbial N content will be greater in older forest stands because microbial communities will have had more time to develop. We also hypothesized that there will be a positive relationship between amino N and ammonium content in soil because ammonium indicates decomposition by microbes. Lastly, we hypothesized that there is a positive relationship between amino N and nitrate content in soil across stand age because microbes participate in nitrification.

Our study compared the change in nitrogen and below ground microbial biomass through stages of secondary forest succession. Further, we assessed the effect of disturbance on microbial biomass. We analyzed the correlations between forest stand age, Amino N, NH4 and NO3.

Considering the necessary services microbes provide, we must look at how soil and nitrogen change through succession to fully understand the impact of disturbances on Michigan forests.

MATERIALS AND METHODS

Research Site

Located in northern lower Michigan, burn plots on the University of Michigan Biological Station (UMBS) property demonstrate a chronosequence of forest disturbances. The burn plots

originated as a series of three natural burns in 1901, 1911, and 1923, but after 1936 and onward the plots were experimentally burned to study disturbance. Disturbances are events, manmade or not, that disrupt and cause change in an ecosystem (Cain 2011). The controlled disturbances of the burns creates an opportunity to study secondary succession. Secondary succession is the series of changes an ecosystem undergoes after a disturbance—despite the disturbance though, soils remain intact (Cain 2011). Within a chronosequence of nearly 100 years, the different stages of secondary succession for big tooth aspen dominated forests are readily visible in the UMBS burn plots.

Soil

We took soil samples from 6 burn plots (1911, 1936, 1948, 1954, 1980, and 1998). Within each plot, there were 3 randomly selected sub plots. These sub plots were 5m radius circles. We extracted 5 soil samples from each of the 18 sub sites, to get a total of 90 soil samples. After using a soil core to extract the soil, we separated out the leaf litter and then placed the soil and leaf litter into labeled bags.

Keeping the soil samples separate, we emptied the bags into 2 mm sieves. Roots and particulate organic matter was separated from the soil by hand and through use of the sieves. Throughout this process we kept the soil refrigerated in coolers. Lastly, the soil sample were stored in refrigerators before fumigation and chemical analysis.

We then weighed two sub samples of 4 g for each of the 90 samples. Half of the sub samples were fumed and the other half were not (control). We placed the fumed sub samples in desiccators with a 125/250 ml Erlenmeyer containing ~20 boiling chips and 75 ml of ethanol-free chloroform/ desiccator. The desiccators were evacuated until the chloroformed boiled, twice a day for 48 hours. We extracted all 180 subsamples, fumed and non-fumed, using 0.1M K_2SO_4 solution at 5:1 solution to DRY soil rate. Then they shook for 1 hour at 180/ stroked/ minute and were filtered using a No. 42 filter paper. We placed aliquots of the filtrate solution in oscillation vials for analyses; fumigated: extractable OC (15 ml) and persulfate oxidation (15 ml); non-fumigated: NH_4 and NO_3 (10 ml), persulfate oxidation (15 ml) and inorganic N (40+ ml).

Lastly, we re-weighed the masses of the fumed and non-fumed subsamples. By subtracting NinHydrin from NH4 for both fumed and non-fumed samples, we calculated "before" and after" values. Then, after subtracting the "before value from the "after value" and multiplying the difference by the reference number, we calculated microbial biomass. We used

regression analyses to compare microbial biomass, NO3, and NH4 across the different burn plots. We also completed ANOVA tests for NH4 and stand age, NO3 and stand age and Amino N and stand age.

RESULTS

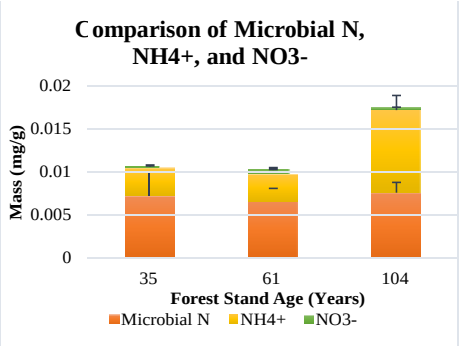

Fig. 1.
Microbial N ($F_{2,39}$, p=.863), NH_4^+ ($F_{2,39}$, p=<.000), NO_3 ($F_{2,39}$, p=.003)

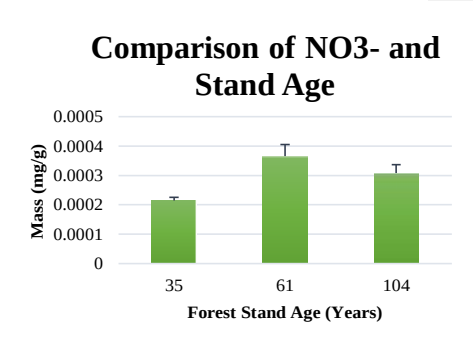

Fig. 2
$F_{2,39}$, p=.003

All three plots had similar levels of microbial N. The 1911 plot had a significantly higher presence of NH4 than the 1954 and 1980 plots, which were at similar levels. Plot 1980 had the lowest level of NO3, though not significant. The 1954 plot had the highest level of NO3 and the 1911 plot had an intermediate level of NO3.

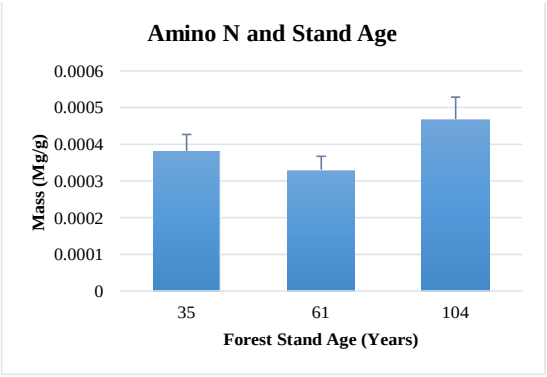

Fig. 3.
$F_{2,39}$, p=.273

Amino N was greatest at the 1911 plot, though the 1954 and 1980 plots were slightly lower. There was no significant difference between levels of Amino N across the burn plots.

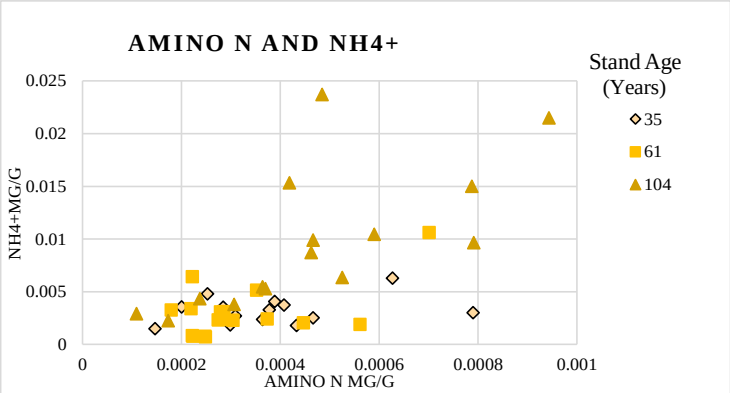

Fig. 4.
R^2=0.365, P<0.000, 95% Confidence Level.

Each age of forest stand had different curves for the relationship between Amino N and NH4. NH4 levels were significantly higher in the 1911 burn plots. NH4 was consistently lower in the 1980 plot and showed less variation in the range of NH4 values than the 1911 and 1954 plot. The 19 54 plot had only slightly higher levels of NH4 than the 1980, and a much greater variation.

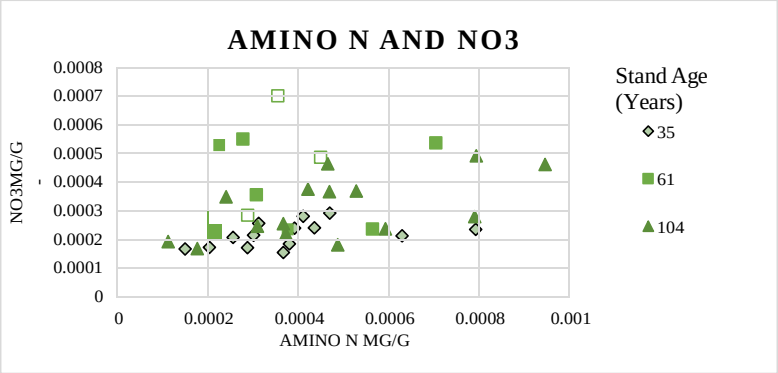

Fig. 5.
R^2=0.081, P<0.061, 95% Confidence Level.

Though it was not statistically significant, the youngest forest stand showed less variation in levels of NO3. The 1911 and 1954 both showed considerable variation in NO3 levels

DISCUSSION

NO3 and forest stand age

In contrast to our hypothesis, we found no trend in NO3 across forest age. However, we did find that NO3 levels were lowest at the youngest burn plot (figure 1). This corresponded with the study completed by Nadelhoffer et al. 1984, which showed that NO3 pools in the soil were smaller than NH4 pools. NO3 mass was so much smaller at the youngest plot due to the higher demand of available N by plants. Younger plants use more NO3 for growth, which result in lower levels of NO3 present in young forests (Nadelhoffer et al. 1984). However, stand age is not a good predictor of NO3 levels. NO3 mass was only a fraction of the recorded NH4 mass (figure 1). Decreased levels of NO3 across all of the burn plots may be caused by the leaching of soluble NO3 out of the soil (Nadelhoffer et al. 1984).

NH4 and forest stand age

High levels of NH4 at the 1911 plot supported our initial prediction, however the reasoning is not the same (figure 1). Rather than the idea that time allowed for a more developed microbial community in the older plot, microbial communities are dependent upon the resources available to them. Older forest plots have greater leaf litter and above ground wood levels, which become resources for microbes to decompose (Gough et al. 2007). We infer from these data and the study completed by Gough et al. in the UMBS burn plots that high NH4 mass in the 1911 plots indicates high levels of decomposition by microbes.

Amino N and forest stand age

Amino N did not vary greatly across forest stand age, though the highest mass was recorded at the 1911 plot. These data allowed us to reject our hypothesis that forest stand age predicts microbial biomass.

NH4 and Amino N

The significant relationship between NH4 and Amino N showed that NH4 increased with microbial biomass (figure 4). These data supported our original hypothesis. Greater microbial biomass allows for faster rates of decomposition and mineralization. The product of mineralization is NH4, so greater microbial activity leads to higher levels of NH4 in the soil.

NO3 and Amino N

In contrast to out hypothesis, there was no statistically significant relationship between NO3 mass and Amino N mass, the 1980 plot had lower levels of NO3 (figure 5). Amino N is the indicator of microbial biomass. From these data we can see that the 1980 burn plot has lower

NO_3 levels as well as lower levels of microbial biomass compared with the 1954 and 1911 plots. This supports the earlier stated relationship between NO_3 and forest stand age. However, for plots 1954 and 1911, there is considerable variation in the data (figure 5). The high usage of NO_3 by plants may contribute to this variation. Another explanation for variation could be that the type of microbes present in the soil are more heavily involved in the mineralization process rather the process of nitrification.

Our study shows that forest secondary succession causes changes in the nitrogen cycle over time by changing inputs and uptakes of N. We also determined that forest age stand does not necessarily indicate soil microbial biomass. Finally, we correlated forest growth rates with NO_3 and NH_4 levels in the soil.

These conclusions show that there is a strong connection between microbial activity and above ground factors, such as leaf litter and primary productivity. Future research pairing above ground data with below ground data would be helpful in further understanding how microbial biomass, nitrate, and ammonium change across different successional stages. Also, analysis of additional burn plots, younger or older, may substantiate the trends we saw in our data or it may provide new information.

REFERENCES

Agnelli, Alberto, et al. "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* 36.5 (2004): 859-868.

Cain, Michael, William Bowman, and Sally Hacker. *Ecology*. 3rd ed. Sunderland: Sinauer Associates, 2014. Print.

Gough, Christopher M., et al. "The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest." *Global Change Biology* 13.9 (2007): 1935-1949.

Harris, Jim. "Soil microbial communities and restoration." *Oikos* 117 (2008): 1833.

Hoorman, James J. "Understanding Soil Microbes and Nutrient Cycling." *The Ohio State University Extension* (2011): 1-4.

Jia, Guo-mei, et al. "Microbial biomass and nutrients in soil at the different stages of secondary forest succession in Ziwulin, northwest China." *Forest Ecology and Management* 217.1 (2005): 117 125.

Kent, Angela D., and Eric W. Triplett. "Microbial communities and their interactions in soil and rhizosphere ecosystems." *Annual Reviews in Microbiology* 56.1 (2002): 211-236.

Kuramae, Eiko E., et al. "Microbial secondary succession in a chronosequence of chalk grasslands." *The ISME journal* 4.5 (2010): 711-715.

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* 80.3 (1984): 321-33

Susyan, Evgeny A., et al. "Forest succession on abandoned arable soils in European Russia Impacts on microbial biomass, fungal-bacterial ratio, and basal CO 2 respiration activity." *European journal of soil biology* 47.3 (2011): 169-174.

Taylor, J. P., et al. "Comparison of microbial numbers and enzymatic activities in surface soils and subsoils using various techniques." *Soil Biology and Biochemistry* 34.3 (2002): 387 401.

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* 191.1 (2004): 121-127.