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

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EEB 381 – General Ecology
8/17/2015
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Abstract
Both ammonium (NH4) and nitrate (NO3) 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 NH4, NO3, total microbial mass and amino N content between sites. We found significant differences between NH4 and NO3 content as well as a significant relationship between amino N and NH4 across stand age. In addition to NO3 and NH4 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'té, 1998).

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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 (Knute 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’té, 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’té, 1998). Both ammonium (NH4) and nitrate (NO3) 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 CO2 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 CHCl₃ for 48 hours to kill microbes and to extract amino nitrogen, then transferred to 40mL of 3M K₂SO₄ and shook for 60 minutes for homogenization in a benchtop shaker. We then filtered the solution through #42 GF filter paper into an NH₄ and NO₃ ninhydrin solution. We followed the same procedure for the non-fumigated samples with the exception of storing the soil in CHCl₃ 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 NO₃, NH₄, Amino N and total microbial N amounts using ANOVA across the different forest stand ages and also regressed NO₃ and NH₄ with amino N to assess relationships between these components of the nitrogen cycle.

Results

One-way ANOVA yielded significant results in NH₄⁺ (F₂,39, p=<.000), NO₃ (F₂,39, p=.003) across stand ages, but not total microbial N (F₂,39, p=.863) or amino N (F₂,39, p=.273). Regression of amino N and NH₄ returned a significant relationship between the variables (R²=0.365, P<0.000, α=.05) while amino N and NO₃ did not share statistically significant (R²=0.081, P<0.06, α=.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.
NH4 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 NH$_4$ in all pooled stand ages. Amino N is a good predictor of NH$_4$ throughout stand age.
Fig. 2.
R²=0.081, P<0.061, 95% Confidence Level. There is not a significant relationship between amino N and NO₃ in all pooled stand ages. Amino N is not a good predictor of NH₄.
Fig. 3. An overall comparison of Microbial N (F_{2,39}, p=.863), NH$_4^+$ (F_{2,39}, p=<.000), NO$_3^-$ (F_{2,39}, p=.003). Although stand 104 had the highest amount of microbial N and NH$_4^+$, it had the second highest amount of NO$_3^-$ (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. $F_{2,39}, p<.000$. NH4 changes throughout time in stand age.
Fig. 6. \( F_{2,39}, p = .003 \). NO\(_3\) fluctuates over stand age.
Fig. 7. $F_{2,39}, p=.273$. There is no significant difference between amino N mass and stand age.


