ABSTRACT

NITROGEN AND PHOSPHORUS AVAILABILITY IN FORESTS OF VARYING AGES IN THE BARTLETT EXPERIMENTAL FOREST WHITE MOUNTAINS, NEW HAMPSHIRE

by Tera Jean Ratliff

Human-induced changes such as nitrogen deposition and forest harvest can alter biogeochemical cycling in temperate forests. However, it is still unclear what impacts increased N availability and successional stage have on productivity. Nitrogen (N) and phosphorus (P) availability were examined along an age gradient in northern hardwoods in the Bartlett Experimental Forest, New Hampshire, USA. Net N mineralization decreased with age, but no patterns were discovered for available P. However, there was strong evidence for N and P coupling in these sites with results suggesting that N availability influenced P availability through the production of phosphatase enzymes. The apparent interaction of N and P, via phosphatase, is good evidence that resource optimization processes balance nutrient availability across a wide gradient in fertility. This could be viewed as contributing to colimitation, but it could also be that N is the primary limiting nutrient because of its underlying effects on P availability.

NITROGEN AND PHOSPHORUS AVAILABILITY IN FORESTS OF VARYING AGES IN THE BARTLETT EXPERIMENTAL FOREST WHITE MOUNTAINS, NEW HAMPSHIRE

A Thesis

Submitted to the

Faculty of Miami University

In partial fulfillment of

The requirements for the degree of

Master of Arts and Science

Department of Zoology

By

Tera Jean Ratliff

Miami University

Oxford, Ohio

2012

Advisor _____

Dr. Melany C. Fisk

Reader _____

Dr. Michael J. Vanni

Reader _____

Dr. Thomas O. Crist

TABLE OF CONTENTS

List of Tables

List of Figures

Acknowledgements

I would like to thank Kevin Minick, Stephanie Bailey, and many other past and present members of the Fisk Lab for their assistance in the field and laboratory. Thanks also to the US Forest Service for the use of field sites in the Bartlett Experimental Forest. This study was supported by the NSF Long-Term Ecological Research Program.

Introduction

Nitrogen (N) has historically been regarded as the nutrient most limiting to productivity in temperate forest ecosystems. This paradigm has largely been the result of generalizations about the lack of mineral N (Vitousek and Howarth, 1991) and observations indicating the importance of low N availability to growth on young soils. Atmospheric deposition and within-system recycling are the predominant pathways by which N is obtained. Therefore, productivity on geologically young soils, such as are found in temperate forests of the northeast, is expected to be N limited (Walker and Syers, 1976). Phosphorus (P), on the other hand, is primarily obtained through the weathering of mineral parent material in soils, and external sources are believed to be small compared to internal sources (Okin et al, 2004). As a result, forest productivity on older substrates, such as those found in many tropical regions, is expected to be limited by P (Walker and Syers, 1976).

The general patterns of N and P limitation hypothesized by the Walker and Syers model does not consider the various short-term disturbances that may result in proximal limitation by another nutrient. The theory behind N limitation in geologically young forests is based on the assumption that newly formed soils are deficient in N but have sufficient amounts of P from parent material to support growth. Nitrogen availability will increase as the system ages due to continued inputs from the atmosphere, organisms that can fix N, and internal recycling; while P availability will decrease due to sequestration in the standing biomass, mineral sorption, and the lack of significant inputs to the system. Within this context of geologic time, any process that upsets this balance, such as increased N inputs and/or increased sorption of P to minerals, may at least temporarily lead to a shift in the limiting nutrient or to simultaneous limitation by more than one nutrient (co-limitation) (Elser et al, 2007). Therefore, while the Walker and Syers model provides us with a good starting point for understanding nutrient limitation over geologic time scales, the role of disturbance still needs to be examined at shorter time scales. To understand anthropogenic impacts on biogeochemical cycling in the northern hardwood forest, I studied nine stands of three different age groups in New Hampshire, USA to test the

general hypothesis that forest harvest and nitrogen deposition reduce availability of P relative to that of N.

Settlement and industrialization have brought about changes in the N and P cycles in northeastern hardwood forests. Fertilizer production, the planting of N-fixing crops, deforestation, and fossil fuel combustion are just some of the anthropogenic practices that have resulted in increased amounts of reactive nitrogen in terrestrial and aquatic systems and greater fluxes to and from the atmosphere (Galloway et al, 2004). Atmospheric nitrogen deposition from the combustion of fossil fuels has a long documented history of effects on forested systems.

Acid rain was first attributed to air pollution by Robert Angus Smith in 1872 (Smith, 1872) but did not emerge as a widespread ecological issue until the late 1960's and early 1970's (Likens et al, 1972; Likens and Bormann, 1974; Oden, 1968). Still, the importance of the N deposition component from this anthropogenic input was initially overlooked, and we are still trying to understand the long-term impacts of these changes (Aber et al, 1989; McLauchlan et al, 2007). What is known is that human activities have doubled the amount of N being transported from the atmosphere to active pools on land (Vitousek et al, 1997).

In addition to the effects of acid rain, forests in the eastern parts of the United States have a well-documented history of deforestation. From the early 1700's until the 1980's, large tracts of US forest lands were clear-cut or selectively harvested (Dale et al, 2000). During this time period, most of the forests in the northeastern part of the country were cleared for agricultural land or harvested for wood products (Dale et al, 2000). This resulted in large amounts of nutrient exports from the ecosystem in harvested biomass and leachate from the soil due to decreased uptake by vegetation (Dale et al, 2000; Likens et al, 1970; Pierce et al, 1972). The subsequent early successional forest has been shown to have reduced P mineralization, increased adsorption of P in the mineral soil (Wood et al, 1984; Yanai, 1998) and higher allocation of nutrients to aboveground biomass (Gleeson and Tilman, 1990).

Combined effects of disturbance such as acid deposition and harvest may alter the relative balance among recycled nutrients if, for example, N recovers to pre-disturbance levels

more rapidly than does P. P is readily adsorbed to soil minerals especially at conditions we may expect to predominate with human disturbance (low pH from acid rain and recent forest harvest) (Hsu and Rennie, 1962), and it is unclear how long it takes for P cycling to recover (Yanai, 1998). Previous work in these forests suggested that P was limiting to productivity, especially in young, aggrading sites (Naples and Fisk, 2010). Furthermore, models (multiple element limitation, MEL) of nutrient cycling in northeastern hardwood forests predicted that young forest stands that have passed the initial secondary succession phase would be P-limited and mature forest stands would be N or P limited, depending on the ability of the system to retain excess P during the very early succession phase when N is limiting (5-30 years) (Rastetter et al, in review).

These observations, along with data from recent studies, have led to a general questioning of the paradigm of N limitation in temperate forests. Understanding the processes underlying limitation in these systems, is important for the prediction of future effects of continued N deposition on aquatic systems as well as feedbacks to the atmosphere and ecosystem carbon storage under increased CO_2 (Aber et al, 1989; Elser et al, 2009; ; Johnson, 2006; Luo et al, 2004) Liebig's law of the minimum is the classical view of nutrient limitation, stating that growth is always limited, at any one time, by the nutrient in shortest supply relative to demand (Liebig, 1842). However, more recent views suggest Liebig's Law is an oversimplification of nutrient limitation, especially when dealing with multiple species within a whole ecosystem (Danger et al, 2008; Harpole et al, 2011). Resource optimization theory, on the other hand, suggests that organisms should allocate their assets to avoid limitation by any one nutrient (Bloom et al, 1985). Under resource optimization theory, an organism will continually reallocate effort to obtain nutrients in scarcest demand and decrease allocation to other resources. Over time, all of the resources should be closely in balance with each other, and all resources should be equally limiting to growth (Bloom et al, 1985, Chapin et al, 1987). Under resource allocation, nutrients in plentiful supply can be used (allocated) to obtain other nutrients. Therefore, nutrients may be expected to have a tendency to co-vary together, since the ability to obtain one may be dependent upon the presence of another. Recent studies have indicated that, on a global scale, ecosystem productivity is often co-limited by multiple

resources (Elser et al, 2007;Harpole et al, 2011; Rastetter et al, 1997). If co-limitation is as common in terrestrial systems as recent studies suggest (Elser et al, 2007; Harpole et al, 2011), this would support the idea of resource optimization (Bloom et al, 1985; Chapin et al, 1987).

The nutrient requirements of microbial communities and feedbacks between above and belowground processes may facilitate the development of co-limitation. Through decomposition, soil microbes provide most of the soluble nutrients available to forest plants on an annual basis (Schlesinger, 1991), and plants provide the major source of carbon for decomposers. At the same time, plants and microbes directly compete for soil nutrients making their association both mutualistic and competitive (Hartz and Kinzig, 1993; Hodge et al, 2000). The end result of this competition should be a tight linkage between above and below-ground processes which can result in either positive or negative feedback loops (Wardle, 2004). Bacteria and fungi play an important role in the recycling of nutrients in forest ecosystems and, as with plants, can allocate resources (enzyme production) to obtain the nutrient that are most limiting to growth. Fungi and bacteria are responsible for up to 80% of all N and 75% of all P acquired by terrestrial plants through direct uptake by mycorrhizae and mineralization of organic matter (van der Heijden et al, 2008), but there are still many gaps in understanding the effects of microbes on overall nutrient cycling in the northeastern forests.

Homeostasis in microbial nutrient use suggests that their mechanism of nutrient acquisition should give insight into the availability of N and P in relation to each other. The N:P content of soil microbes has been shown to vary independently of soil N:P, indicating that soil microbes are homeostatic (not flexible in their resource use) (Cleveland and Liptzin, 2007) and should put effort into maintaining their nutrient resources within a relatively narrow range. Therefore, the effort by microbes to obtain nutrients may give insight into the nature of nutrient limitation in forest ecosystems. In particular, microbes exert disproportional effects on the nutrient supply in forested ecosystems due to the recycling of nutrients at a different ratio than their uptake (Makino et al, 2003). Microbial productivity is C limited in most terrestrial systems (Alde´n et al, 2001; Ekblad and Nordgren, 2002; Ilstedt and Singh, 2005; Joergensen and Scheu, 1999; Nordgren, 1992; Smith and Paul, 1990) but can be secondarily limited by N or

P (Demoling et al, 2007; Göransson et al, 2011; Reed et al, 2011) leading to constraints on enzyme production, especially when N is in low supply (Allison and Vitousek, 2005). Under an N-limited scenario, there may be a rise in the number of N-fixing organisms with a high carbon requirement. This would be expected to lead to an increase in mineralization of organic matter in an effort to meet that carbon requirement (Fontaine and Barot, 2005; Moorhead and Sinsabaugh, 2006). In a P-limited scenario, demand for P should increase phosphatase enzyme production (Houlton et al, 2008; Olander and Vitousek, 2000; Treseder and Vitousek, 2001). Allocation to enzymes has been recognized as the primary pathway by which microbes break down complex organic matter (Allison and Vitousek 2005; Asmar et al, 1994; Sinsabaugh, 1994). Consequently, enzyme production acts as an important facilitator in the process of decomposition and nutrient mineralization (Asmar et al, 1994; Sinsabaugh et al, 1994), and their energetic costs should result in their production only when the resource they acquire is limiting (Allison and Vitousek, 2005; Koch, 1985).

There is sufficient evidence that enzyme activity responds to N fertilization. However, it is still unknown if enzymatic activity varies with N availability in natural systems. Numerous studies have found that N fertilization alters the activity of extra-cellular enzymes in the soil (Ajwa et al, 1999; Carreiro et al, 2000; Currey et al, 2010; DeForest et al, 2004; Gallo et al, 2004; Johnson et al, 1998; Michel and Matzner, 2003; Ramirez et al, 2012; Saiya-Cork et al, 2002; Treseder and Vitousek, 2001), generally leading to an increase in C mineralizing enzymes and phosphatase activity (Ajwa, 1999; Allison and Vitousek, 2005; Olander and Vitousek, 2000; Saiya-Cork et al, 2002; Sinsabaugh and Foreman, 2003; Sinsabaugh et al, 2009; Treseder and Vitousek, 2001) and a suppression of lignin-degrading enzymes (Carreiro et al, 2000; DeForest et al, 2004; Gallo et al, 2004; Michel and Matzner 2003; Saiya-Cork et al, 2002). Organisms exude extra-cellular phosphatase enzymes into the soil where they break down the ester-bonded organic P into bioavailable phosphate ions which can then be used for growth (Sinsabaugh et al, 2008). Both plants and microbes can produce phosphatase enzymes, and their production comes at a high N cost to the organism (Houlton et al, 2008; Olander and Vitousek, 2000; Treseder and Vitousek, 2001). This coupling between N and P, through the production of these enzymes, would result in high phosphatse activity when N is plentiful and P

is deficient and low phosphatase activity when P is plentiful in relation to N. A recent meta-analysis by Marklein and Houlton (2011) also found that N-fertilization enhanced and P-fertilization suppressed phosphatase enzyme activity. Despite these studies, we do not know whether these transient effects of excess N and P availability translate to longer-term patterns that develop in nutrient cycles among ecosystems that inherently differ in N and P cycling.

Understanding long term effects of anthropogenic disturbance on northern hardwood forests requires consideration of the relationships and potential feedbacks between N and P in this system. The overall goal of this research is to examine potential for feedbacks by testing the relationships between N and P availability in forests with a history of deforestation and increased N deposition. This project will lay the groundwork for future work examining nutrient limitation in these forest sites. To accomplish this goal, I examined soil parameters related to N and P availability and microbial biomass in forest sites in the White Mountains in New Hampshire.

Hypothesis 1: N availability will change relative to P with forest age in managed northern hardwood ecosystems. I predict that N availability will be high in young secondary successional forests and decline with age, while P availability will show the opposite trend consistent with model predictions (Rastetter, in review) and fine root foraging patterns (Naples and Fisk, 2010) in these forest ecosystems. This should result in a greater P deficiency relative to N in young sites than mature sites in managed northern hardwood systems.

If this is the case, then effort by organisms to maintain balance in nutrient resources should result in increased investment in phosphatase enzymes when P availability is low and demand is high (deficiency in P relative to N in young forests) and higher allocation to phenol oxidase when N is deficient relative to P (mature forests). Additionally, C mineralizing enzyme activity is expected to correspond to high relative N availability (young forests), which alleviates secondary N limitation of C mineralization.

Alternative Hypothesis 1: On the other hand, single limitation by any one nutrient in these systems may be an overly simplistic view. Consequently, an important alternative to this hypothesis is that N and P availability co-vary across forests of different ages. This alternative

would be supported if available N and P are similar among forests and are correlated with each other even if total availability varies. If this is the case, I predict that allocation to enzyme production drives this coupling as a mechanism of promoting balance. More specifically, I expect that higher N availability alleviates limitation of enzyme production and that production of phosphatase, glucosidase, and cellobiohydrolase activity will increase with higher N availability. Conversely, I predict that polyphenol oxidase activity will decrease with higher N availability where its N mining effects confer less benefit**.**

Hypothesis 2: Given the importance of microbial processes to nutrient availability in forested ecosystems (Schlesinger, 1991) and the known associations between microbial and enzymatic activity in forest soils, I also explored the relationship between microbial stoichiometry and nutrient availability Base on previous research done on homeostasis in soil microbes (Cleveland and Liptzin, 2007), I predict that the soil microbial biomass in the sites is homeostatic, and microbial N:P will not vary with soil N:P. Alternatively, microbial N:P will vary with soil N:P indicating that the microbial community is not homeostatic. This prediction further leads to the following research question. 1) If evidence for homeostasis is found, does allocation by microbes to extra-cellular enzyme activity provide insight into the effects of altered nutrient cycling in these sites?

Methods

Study Site

This study was implemented at the Bartlett Experimental Forest (44°2’39”N, 71°9’56”W) (nrs.fs.fed.us/ef/) in the White Mountains of Southeast New Hampshire, USA. The climate is humid continental with warm summers and cold winters. Mean air temperature is -9°C in January and 18°C in July. Total annual precipitation is 127 cm and snowpack often forms each winter to a depth of 1.5 to 2 m (Gamal-Eldin, 1998). Soils are moist spodosols (typic and aquic haplorthods) that developed on granite-and gneiss-derived glacial tills.

To examine the relationship between N and P availability in the northern hardwoods, I used a chronosequence approach with nine forest stands; three each of young (20-30 years), mid-aged (33-50 years), and mature (>120 years) varying in elevation from 250 to 400m. Forest age was determined as time since clear cut. In each stand, four 50x50 m plots were established with at least a 20 m buffer between each plot, and the inner 30x30 m was sampled in late June or early July in the 2008 to 2010 growing seasons. Forest composition is typical of northern hardwoods, with an overstory dominated by sugar maple (*Acer saccharum*), American beech (*Fagus grandifolia*), yellow birch (*Betula alleghaniensis*), and white ash (*Fraxinus americana*) in mature forests and by pin cherry (*Prunus pensylvanica)*, white birch (*Betula papyrifera*), American beech, and red maple (*Acer rubrum*) in young forests. Mid-aged forests are transitional between the two (Figure 1). In addition to logging, these sites were affected by the 1938 hurricane. Additionally, the White Mountain region is in an area of the country with a history of acid deposition resulting in increased sulfur and nitrogen inputs to these sites.

Sample Collection

We collected an average of 30 cores from the surface organic layer of each plot in each stand in June or July of 2008 and 2009. During the 2008 sampling season, only three plots were sampled in each of the mid- and mature-aged stands. Young sites were not sampled in 2008. All plots and sites were sampled in 2009. The cores were divided into Oe and Oa horizons based on visual standards and were composited by horizon within each subplot before transporting back to the lab. Samples were kept cold prior to analyses.

Soil Properties

In the lab, samples were homogenized and subsamples were weighed for soil moisture/organic matter, nitrogen, phosphorus, and enzyme analyses. We also incubated resin bags in situ in 2010, to quantify P availability. To account for differences in field moisture that affect subsample dry mass, soil moisture was measured by taking a subsample at time of analysis and drying it at 60°C to a constant mass. Water loss from this subsample was used to convert fresh mass of all other subsamples to dry mass.

Nitrogen

Available nitrogen was quantified using laboratory incubations. We weighed two soil subsamples of about six grams fresh weight per subplot and immediately extracted NH_4^+ and NO_3^- from one subsample (t_i) by shaking in 40 mL of 2M KCl for one hour on a reciprocal shaker. The second subsample (t_f) was placed in a canning jar and incubated in the lab for 21-28 days and then extracted in the same way as the first. Sample extracts were filtered through Whatman #1 qualitative grade paper and stored at 4°C until analysis. We used the indophenol method (method 351.2, US EPA 1983) to determine NH_4^+ concentration, and a cadmium reduction method (method 353.2, US EPA 1983) to determine NO_3^- concentrations in the subsamples. Net nitrogen mineralization was calculated as follows:

$$Nmin = \{(NO_{3tf} + NH_{4tf}) - (NO_{3ti} + NH_{4ti})\}/Time_{days}$$

Microbial nitrogen was quantified using the chloroform fumigation technique (Brookes et al, 1985; Vance et al, 1987). We weighed two soil subsamples of about six grams fresh weight per subplot and immediately extracted total dissolved nitrogen (TDN = NO_3^- + NH_4^+ + Dissolved organic nitrogen "DON") from one subsample (u = unfumigated) by shaking in 40 mL of 0.5M K_2SO_4 for thirty minutes on a reciprocal shaker. To lyse the microbial cells, we fumigated the other subsample (f = fumigated) in an air-tight desiccator with a beaker of chloroform for three days and then extracted in the same way as the unfumigated. The samples were then digested for total dissolved nitrogen using a persulfate digestion method (D'Elia et al, 1977). Microbial nitrogen (MBN) is assumed to be the difference between the unfumigated and fumigated samples and was calculated as follows:

$$MBN = (TDN_f - TDN_u)/0.54 \text{ (extraction coefficient, Vance et al, 1987)}$$

Phosphorus

Available phosphorus was quantified using an in-situ resin-extractable phosphorus technique (Lathja, 1988). Nylon bags of uniform size containing 10 g of bicarbonate-form anion-exchange resins (JT Baker) were incubated beneath the forest floor in each stand beginning in

May of 2010. Six bags per subplot were deployed in the lower boundary of the Oa horizon by inserting into slices (approximately 5 cm wide) made at a 30 - 45 degree angle. The resins have a high affinity for P and are intended to simulate uptake by plant roots by sorbing labile P that is available for plant uptake.

Resin bags were removed from the field in August of 2010 and kept on ice during transport back to the lab where they were stored at 4°C until further analysis. Prior to analysis, the bags were rinsed well to remove residual soil and allowed to air dry. Resin P was extracted by shaking air-dried beads in 200mL of 0.5M HCl for one hour on a reciprocal shaker. We pH adjusted the extracts by titrating 20mL of sample with 0.25M H_2SO_4 and 4M NaOH using phenolpthalien as an indicator, brought them to a consistent volume of 25mL, and analyzed using the Murphy and Riley procedure (1962).

Microbial phosphorus was quantified using the same procedure used for microbial biomass N, with the exception of the extractant which was 0.5M $NaHCO_3$ instead of 0.5M K_2SO_4. The samples were then digested for total dissolved phosphorus using a persulfate digestion method (EPA/600/R-93/100, Method 365.1). Microbial phosphorus (MBP) is assumed to be the difference between the unfumigated and fumigated samples and was calculated as follows:

$$MBP = (TDP_f - TDP_u)/0.40 \text{ (extraction coefficient, Vance et al, 1987)}$$

Bicarbonate P was calculated using the unfumigated MBP sample.

N:P Ratio

Lab measurements of the N mineralization rate during the 2009 season were compared to in-situ resin P availability from the 2010 field season. Ratios were calculated using the total transformation in N availability over the 21 day incubation to the total P in solute from resin bags incubated for 3 months in the field with both measures standardized to a daily rate. Estimating available N:P in these sites was complicated by differences in the way we measured N and P. Measuring P in-situ provides a more accurate snapshot of availability than in-lab measures, but this means that our units were not directly comparable. Nevertheless, the ratios acquired may still be useful for comparing relative availability among forest ages.

Enzymes

Phosphatase, glucosidase, cellobiohydrolase, and polyphenol oxidase activity were quantified ($\mu mol \cdot h^{-1} \cdot g^{-1}$ dry mass) in the laboratory by extracting a subsample weighing about 7 grams (fresh weight) in 100 mL of acetate buffer (50 mM, pH 5) on a reciprocal shaker for one hour. We blended samples prior to analysis to ensure equal distribution of soil particles and divided them into six replicate tubes containing 200µl of sample and 200µl of appropriate substrate (p-nitrophenyl-phosphate, p-nitrophenyl beta-glucopyranoside, p-nitrophenyl beta-D cellobioside, or $_L$3,4-Dihydroxyl-L-phenyl-alanine, respectively) (Tabatabai, 1982). We ran controls containing just sample and buffer and just substrate and buffer to account for any interference that might occur. Phosphatase, glucosidase, and cellobiohydrolase were shaken at room temperature (25°C) for one hour, two hours, and four hours, respectively. We centrifuged the samples, stopped the reaction using 1M NaOH, and analyzed optical density at 410nm. Polyphenol oxidase was shaken at room temperature for four hours, centrifuged, and the supernatant was analyzed for optical density at 450nm. Enzyme activity was calculated for each substrate type individually as follows:

$$OD/(EC*T_h* DM)$$

Where, OD = Average Optical Density – Control, EC = Extinction Coefficient, T_h = Incubation Time (hours), and DM = Dry Matter.

Statistical Analyses

Statistical analyses were done using the nlme (Pinheiro et al, 2012) and multcomp (Torsten et al, 2008) packages in R v. 2.14.1 statistical programming language (R Development Core 2011). Age-related patterns in nutrient availability were tested using random-variable nested ANOVA with plots nested within sites nested within age. When an effect of age was found, significant differences were determined using Tukey's honestly significant difference test. Relationships between enzyme activity and nutrient availability and relationships between microbial biomass and nutrient availability were examined using linear regression and ANOVA in R for 2009 data. Weighted least squares regression lines were added to plots using Igor Pro

(Wavemetrics, Lake Oswego, OR, USA) software. Nutrients, enzymes, and microbial biomass were averaged within each plot for all analyses. Tests were run separately for each soil horizon to minimize the effects of natural differences between Oe and Oa horizons. With the exception of net N mineralization, analyses were only done on samples from the 2009 sampling season due to unequal sampling sizes in 2008. The means for 2008 are also presented to show relative patterns over time.

Results

Nitrogen availability differed with forest age in the Oe horizon in 2008 with higher N availability in mid-aged than in mature forests (Figure 2). N availability did not significantly differ with forest age in 2009 but general trends showed a decrease in N mineralization as forests mature. Phosphorus availability did not differ by forest age (Figure 3). The Ratio of available N to P (N mineralization: Resin-available P) did not differ with age (73.93 +/- 7.00 in young, 84.76 +/- 12.09 in mid-aged, and 65.72 +/- 13.49 in mature). Variation among stands within ages was high (Table 2) for N and both forms of P, with overlapping values for all three variables. Activity of enzymes involved in decomposition and P cycling did not differ with forest age.

Spatial patterns of N and P availability were stronger than the age-related patterns. Resin P availability and N mineralization were significantly related in the Oe horizon ($P = 0.02$; Figure 4). Nitrogen mineralization and resin P were both positively related ($P<0.05$ and $P<0.01$, respectively) to phosphatase activity in the Oe horizon of these forests (Figure 4), but there was no relationship between bicarbonate P and phosphatase activity (Figure 4) or N mineralization and bicarbonate P (not shown). There were also no significant relationships between glucosidase, cellobiohydrolase, and polyphenol oxidase and N mineralization or resin P across stands.

Nitrogen and phosphorus measured in the microbial biomass varied independently of each other and did not differ with stand age. Microbial N:P averaged 5.6 +/- 0.65 in the Oe horizon and 9.2 +/- 2.19 in the Oa horizon, but was not related to available N or P across stands (Figure 5). Microbial N:P was positively correlated with phosphatase activity in the Oe horizon ($P<0.05$, Figure 6), but was not correlated with any of the C mineralizing enzymes. Resin P and nitrogen mineralization tended to increase with higher microbial P and microbial N respectively, but the relationships were not significant ($P\sim0.10$).

Discussion

While I found weak support for the hypothesis that N availability changes relative to P availability as forests age, the far more striking result was covarying N and P availability, independent of age, across the forest stands. Strong relationships between N mineralization and resin P availability demonstrate that N and P availability are tightly coupled in these sites and suggest that mechanisms exist to balance availability over relatively short time scales. Based on model findings (Rastetter et al, in review) and previous field results (Naples and Fisk, 2010), I predicted a shift from P to N limitation as forests matured, and age-related trends in 2008 were consistent with my predictions that N availability would decline but not that P availability would increase with stand age. However, variation among stands in this study was high, suggesting that a chronosequence approach is not ideal for detecting change over successional time in either N or P availability. Ratios of N and P availability factor out spatial variation and do not show any strong age-related pattern. This suggests that even with decreasing N availability as forests mature, the differences are not large enough to create marked age-related differences in the relative availability of these nutrients, and provides more direct evidence that N availability did not decline relative to P over succession.

Co-limitation and Resource Optimization Theory

While age-related adjustments may be taking place in the nutrient status of these northern hardwood sites, I found strong support for the alternative hypothesis that nutrients covary in these forest stands. Coupled with phosphatase results, this suggests that resource optimization by organisms works to maintain balance in the nutrient supply rate even when the overall availability of nutrients is high. This effort to maintain stoichiometric balance could, as the Rastetter et al (in review) model and resource optimization theory suggest (Bloom et al, 1985), eventually result in a situation where growth is simultaneously limited by more than one element. Co-limitation of forest stands would be expected to result in N and P cycles that are tightly linked, and this study supports this idea at least for the biologically active organic pool. High nitrogen availability in these sites resulted in high phosphorus availability, and the likely mechanism for this coupling was driven by the availability of nitrogen.

The findings in this study are especially significant in that they show that the mechanism of using excess N to obtain P operates across varying time scales and levels of nutrient availability and not just in response to manipulation. Unlike in previous studies by other authors (Allison and Vitousek, 2005; Clarholm, 1993; Marklein and Houlton, 2011; Treseder and Vitousek, 2001) high phosphorus availability did not result in reduced phosphatase enzyme activity, but both high phosphatase activity and high available P were tied to high nitrogen availability (Figure 4A and 4B) again indicating that N availability is the driver behind these patterns. It is important to note that the studies cited all looked at the effects of P fertilization on extra-cellular phosphatase production. This would be expected to decrease P demand and therefore nullify the benefits of producing phosphatase enzymes. This contrasts with my study, where I assumed that high N availability would result in high P demand and indicates that fertilization studies may over-simplify the relationship between N and P availability and phosphatase demand. High N availability did increase investment in phosphatase enzyme production in these study sites. These results are consistent with previous N fertilization studies (Ajwa et al, 1999; Carreiro et al, 2000; Currey et al, 2010; DeForest et al, 2004; Gallo et al, 2004; Johnson et al, 1998; Michel and Matzner, 2003; Ramirez et al, 2012; Saiya-Cork et al,

2002; Treseder and Vitousek, 2001). When making these observations, it should not be overlooked that higher enzyme activity can only work within the bounds of potentially available organic P. Beyond this, the amounts of biotically recyclable P have to increase. So, at one end of the spectrum, there are limits to the effectiveness of phosphatase activity that are difficult to overcome. At the other end of the spectrum, when N and P availability are balanced or N is solely limiting, phosphatase is no longer needed. My results fall in the middle of this spectrum indicating that even across northern hardwood forest stands with a wide range of N availability, N and P demand are closely linked and there is a large enough pool of potentially mineralizable P to overcome potential P limitation.

While N limitation could underlie the apparent coupling in N and P availability in these forests, these results could also reflect co-limitation by N and P. Resource optimization theory proposes that organisms should constantly modify their effort to acquire nutrients that are in highest demand (Bloom et al, 1985). This should eventually result in a scenario where all resources are in close balance with each other and are all equally limiting to growth (Bloom et al, 1985). This idea lends itself well to the concepts behind co-limitation of nutrients at the ecosystem scale. In this study, resource optimization likely contributes to tight coupling of nutrient cycling, and could lead to a situation in which N and P are both limiting to growth and the system is independently co-limited (Harpole et al, 2011). This would be the case if adding P increases productivity by alleviating the need to produce phosphatase enzymes (Marklein and Houlton, 2011) and freeing up N for further growth. At the same time, adding N results in further allocation to phosphatase enzymes keeping P availability in relative balance with N. However, it is also possible that N limitation underlies the patterns seen here, as the availability of P appears to depend on the availability of N to produce phosphatase (Figure 4A, 4B, and 4C). In fact, in this study low nitrogen availability in the mature sites may have constrained the availability of phosphorus through reduced allocation to phosphatase enzymes, thereby promoting potential storage in the organic pool. The pool of potentially mineralizable P is still in excess in these sites indicating that the effectiveness of producing phosphatase has not yet reached its capacity in these forests. N and P fertilization studies would help to resolve the question of whether forest sites in the northeastern hardwood forest are co-limited by N and P

simultaneously or are primarily limited by N with P becoming limiting only on very fine spatial scales.

Age-related Patterns in Nutrient Availability

N deposition and the imbalance of N and P associated with forest harvest (Wood et al, 1984; Yanai, 1998) should increase the likelihood that P will become limiting, at least on shorter time scales. P availability can decline with stand age in boreal forests (Brais, 1995). While such changes have not been reported in northern hardwoods, the immediate loss of P to mineral sorption after forest harvest (Yanai, 1998) suggests that P availability should be lowest in sites recovering from a recent disturbance. Models have also suggested that forest harvest will be followed by preferential loss of P and retention of N in northern hardwoods (Rastetter et al, in review) and that, in the absence of a mechanism to retain P, this loss will lead to a prolonged period of P limitation after the initial regrowth stage (about 30 years). However, I was unable to detect differences among the young, mid-aged, and mature stands at Bartlett Experimental Forest. Higher (but not significantly) labile organic P pools in mature forests suggest that potentially available P recovers over successional time, consistent with my predictions. Because of high variation among stands, it may be necessary to follow individual stands over time to reach conclusive results regarding changes in P cycling over secondary succession. According to the MEL model, after a century of succession, the system should have reset itself so that N and P are once again tightly coupled and the system is co-limited (Rastetter et al, in review). The significant relationship between N and P in this study suggests that the tight coupling between these nutrients is regained much earlier than the 100 years suggested, consistent with the idea that, when in high availability, N can be allocated to alleviate short-term P limitation.

However, the question still remains as to how P is retained in the system after succession. P should be readily lost from the biotically active pools to more occluded forms in soil after forest harvest (Wood et al, 1984; Yanai, 1998), but we found no such pattern of reduced P availability in the stands of younger age classes. Phosphorus availability was high when N availability was also high regardless of stand age indicating that P was either retained in the rapidly cycling pools after forest harvest or recovered from the more recalcitrant pools after

the initial period of succession. Recovery of P from pools traditionally considered to be unavailable can happen in a couple of ways. Acidification of forest soils under atmospheric nitrogen and sulfur deposition can release P that is bound to aluminum and iron oxides (Sherman et al, 2006) and/or biotic demand can drive P to be redistributed from the recalcitrant pools into the labile pools (Block et al, 2012; Richter et al, 2006; Turner et al, 2002). This can happen by mining recalcitrant pools through the production of organic acids and complex enzymes (Block et al, 2012; Richter et al, 2006; Turner et al, 2002) or by weathering of primary minerals such as apatite (Walker and Syers, 1976) and may lead to a decrease in slowly-available pools over time (Block et al, 2012; Richter et al, 2006). In order to fully resolve questions about changes in P cycling in successional northern hardwoods, changes in the distribution of P among slowly recycling and labile pools needs to be addressed.

Previous studies have not found conclusive patterns of N availability with forest age (Ryan et al, 1997). Many studies demonstrated a lack of consistent patterns in N availability over succession (Brais et al, 1995; Covington, 1981; Fisk et al, 2002; Vitousek et al, 1989). However, other studies found similar results to those found in this study and demonstrated that N availability declines with forest age during secondary succession (Binkley et al, 1995; Brais et al, 1995; Frazer et al, 1990; Matson and Boone, 1984). In many cases, patterns that emerge are reliant upon the nutrient in question (Brais et al, 1995; Covington, 1981), the history of the site (Vitousek et al, 1982; Vitousek et al, 1989), and whether the overall status of the site is nutrient rich or nutrient poor (Vitousek et al, 1982). Microbial N immobilization and accumulation of N in detritus may increase with stand age (Davidson et al, 1992; Fisk et al, 2002; Gorham et al, 1979; Vitousek et al, 1988) and contribute to lower net N mineralization in mature sites in this study. Furthermore, increases in atmospheric CO_2 since industrial times provide a potential source of increased N storage capacity in that growth of the microbial and plant communities may be stimulated which will, in turn, lead to the increased immobilization of N (Huntington, 2005; Luo et al, 2004). Taking atmospheric N deposition into account, there is a convincing argument for increased microbial activity in mature forests, since secondary N limitation to microbial growth is less likely to occur under high nitrogen availability and competition with plants should be reduced.

Microbial Drivers of Nutrient Availability

Despite the importance of microbial organisms to nutrient cycling in terrestrial systems and the assumption that a large portion of the nitrogen in these forested systems is immobilized by microbes as forests age, we still lack understanding of the relationships between above and below-ground nutrient processes in hardwood forests. Part of the problem arises from the fact that microbial biomass estimates are often a poor predictor of microbial activity and decomposition rates (Grey and Williams, 1971; McGill et al, 1986). In spite of this, studies have shown that there are relationships between microbial biomass and soil nutrient pools (Cleveland and Liptzin, 2007; Zak et al, 1990). Additionally, the higher turnover rates associated with the microbial biomass (Fisk and Fahey, 2001; Holmes and Zak, 1999; Lipson et al, 2001; Schmidt et al, 2007; Stark and Hart, 1997) should contribute to tight coupling between the microbial community and the available nutrient pool.

Homeostasis in microbial communities should lead to large effects of the microbial community on nutrient supplies due to constraints on the organisms to keep their nutrient supply in balance (Makino et al, 2003; Sterner and Elser, 2002). The N:P of microbes in these forest stands tends to vary within a range of 4:1 to 7:1. There are a few exceptions in the data, but they appear to be independent of net N mineralization and resin available P, our best estimates of readily available nutrients in these sites. Therefore, our data do not contradict the findings of Cleveland and Liptzin (2007). The soil microbes in the northeastern hardwoods appear to be vary but do so irrespectively of their resource N:P indicating potential constraint in their nutrient cycling rates. Cleveland and Liptzin used total soil N:P in their observations rather than estimates of available N and P. This measurement would include forms of N and P that are unavailable to microbes and may not be a true estimate of consumer and resource stoichiometric constraints. However, our methods of estimating microbial N availability likely underestimated the pool of N potentially utilizable by microbes (Schimel and Bennett, 2004). Additionally, higher microbial nutrient pools measured separately as microbial N and microbial P did tend to correspond to higher available N and P pools, but these results were not significant. Known relationships regarding shifts in microbial composition and differences in the

N:P of fungus compared to bacteria could contribute to apparent variability (Cleveland and Liptzin, 2007; Reiners, 1986; Sterner and Elser, 2002). So, while our data are in keeping with the idea that microbial nutrient uptake is not predictable based on nutrient availability, there is still a need to understand how nutrient availability shifts microbial community composition and the implications of potential feedbacks associated with those shifts (Strickland and Rousk, 2010).

It has been hypothesized that bacteria (low N:P) should dominate the soil microbial community under high nutrient availability and fungi (high N:P) should dominate at low availability of nutrients (van der Heijden et al, 2008) and that the dominance of fungi should result in high retention of nutrients in the organic pool and a negative feedback with plants (Wardle et al, 2004) . In practice, fertilization with N, which may be expected to shift microbial composition to a bacteria dominated community, has also resulted in negative feedbacks in some terrestrial ecosystems (Aber et al, 1998; Fisk and Fahey, 2001; Magill et al, 1996; Magill et al, 1997; McNulty et al, 1996). The processes surrounding this pattern are not completely understood but could be the result of microbial C, N, and P demand and induced limitation by a secondary nutrient.

The homeostasis of the microbial biomass determines the ratio of nutrients returned to the soil (Sterner and Elser, 2002). Whatever is left over after growth and enzyme allocation is returned to the available pool for further uptake by plants and microbes. Consequently, microbes are able to influence nutrient availability in several ways. In addition to the production of phosphatase enzymes, discussed previously, microbes can allocate to the "mining" of N by decomposing recalcitrant organic matter through polyphenol oxidase production (Craine et al, 2007; Moorehead and Sinsabaugh, 2006; Sinsaubaugh et al, 2010). One potential explanation for decreased decomposition with N fertilization lies behind the idea that microbes are often secondarily limited by N availability (Demoling et al, 2007; Göransson et al, 2011; Reed et al, 2011; Schimel and Weintraub, 2003). Therefore, when N is available in high enough amounts, microbes no longer need to invest in costly lignin-degrading enzymes for the purpose of obtaining recalcitrant N (Berg, 2000; Carreiro et al, 2000; Fog, 1988; Hagedorna et al, 2003; Magill and Aber, 1998; Swanston et al, 2004) and have the flexibility to invest

allocation to C acquiring enzymes, like glucosidase and cellobiohydrolase, that more directly translate to growth potential (Moorehead and Sinsabaugh, 2006; Schimel and Weintraub, 2003; Sinsaubaugh et al, 2009). Contrary to previous studies (Ajwa et al, 1999; Allison and Vitousek, 2005; Carreiro et al, 2000; Currey et al, 2010; Saiya-Cork et al, 2002) in terrestrial systems, we found no evidence of a relationship between C mineralizing or lignin-degrading enzymes and N or P availability.

The lack of a relationship between C mineralizing enzyme activity and N availability is surprising. The idea that microbes are primarily C limited and enzymes N costly lends itself to the conclusion that high N availability should result in preferential metabolism of C-rich compounds by microbes (Ajwa et al, 1999; Berg, 2000; Carreiro er al, 2000). However, high N availability in a natural system is not equivalent to excess N added in fertilizations; and it is possible, that I am unable to detect any patterns given continued demand for N even at the high end of N availability found in the forest stands. Phenol oxidase activity can be an indicator of N demand (Craine et al, 2007; Moorehead and Sinsabaugh, 2006), but there was also no evidence of increased allocation to production of this enzyme at low N availability. Due to the high costs associated with producing enzymes, it is possible that the benefits of producing C and N mineralizing enzymes are not as clear as those of producing P mineralizing enzymes making patterns harder to detect (Olander and Vitousek, 2000). In conclusion, N availability in these systems may not directly relate to N demand.

Microbial biomass did relate to the activity of phosphatase enzymes in the organic horizon of these forest stands with a trend towards increasing investment in phosphatase production with increasing N:P. Microbes have higher P requirement than plants as evidenced by their relatively low N:P ratio indicating that they should allocate more toward P acquisition than plants do (Sterner and Elser, 2002). As previously mentioned, one possible way organisms can do this is through increasing the production of extracellular phosphatase (Sinsabaugh et al, 2008). It is true that these enzymes can be the result of allocation by plants, microbes, or a combination of the two, but phosphatase activity is often used as an indicator of microbial nutrient demand (DeForest et al, 2012; Hill et al, 2012; Sinsabaugh et al, 2009). Unfortunately,

it is not possible for us to distinguish between plant and microbial sources of phosphatase. Inferences made on the importance of root-derived versus microbial phosphatase activity tend to rely on observations and lack of correlation between microbial biomass and phosphatase activity (Colvan et al, 2001). However, the higher demand for P by microbes compared to plants combined with the relationship we found between microbial N:P, which is a better predictor of resource needs than total microbial biomass P, and phosphatase activity in the biotically active Oe horizon of the forested sites, suggests that the microbial community is playing a large role in the production of this enzyme and the coupling of N and P in the northern hardwood forest.

Conclusion

Age-related processes have received a lot of attention for their importance to long-term changes in nutrient cycling. However, human disturbance alters these roles in ways that may not always be easy to predict. Our data indicate that despite historical incidences of forest harvest and recent increases in N deposition, the N and P cycles in these forests seem fairly resilient and remain tightly coupled over the time period sampled across forests of all ages. Hence, feedbacks between N and P recycling processes play a larger role in availability than previously considered, and more attention needs to be given to the idea of co-limitation in situations where there is interdependence between nutrients. Furthermore, the enzymatic responses of the microbial community to changes in nutrient availability in these systems suggest that P limitation induced by changes in N cycling in the northeastern hardwoods is more readily overcome than N limitation as long as there is a recyclable pool of P. While fertilization studies are designed to test potential future effects of N deposition, the effects of deposition in the northeastern US have not yet pushed N availability to the point that labile organic P has been depleted, suggesting that N remains the fundamental limiting nutrient and appears to regulate the loss of P in these systems after disturbance. I propose that maintaining excess labile P pools under chronic N enrichment and across sites of varying fertility is possible because of redistribution of slowly-recycling P pools. Long-term alterations in the cycling of

nutrients in this system still remain to be studied, and it is important to test this hypothesis if we are to predict the timing and magnitude of N enrichment necessary to produce long-term P limitation.

References

Aber, JD, Nadelhoffer KJ, Steudler P, Melillo JM. 1989. Nitrogen saturation in northern forest ecosystems. BioScience. 39: 378-386.

Aber JD, McDowell W, Nadelhoffer K, Magill A, Bernston G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I. 1998. Nitrogen saturation in temperate forest ecosystems: hypotheses revisited. Bioscience 48: 921-933.

Ajwa HA, Dell CJ, Rice CW. 1999. Changes in enzyme activities and microbial biomass of tallgrass prairie soil as related to burning and nitrogen fertilization. Soil Biology and Biochemistry 31: 769-777.

Alde´n L, Demoling F, Bääth E. 2001. Rapid method of determining factors limiting bacterial growth in soil. Applied and Environmental Microbiology 67: 1830–1838.

Allison SD, Vitousek PM. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biology and Biogeochemistry 37: 937-944.

Asmar F, Eiland F, Nielsen NE. 1994. Effect of extracellular-enzyme activities on solubilization rate of soil organic nitrogen. Biology and Fertility of Soils 17: 32-38.

Berg B. 2000. Litter decomposition and organic matter turnover in northern forest soils. Forest Ecology and Management 133: 1322.

Binkley D, Smith FW, Son Y. 1995. Nutrient supply and declines in leaf area and production in lodgepole pine. Canadian Journal of Forest Research 25: 621-628.

Block CE, Knoepp JD, Fraterrigo JM. 2012. Interactive effects of disturbance and nitrogen availability on phosphorus dynamics of southern Appalachian forests. Biogeochemistry. Online content. http://dx.doi.org/10.1007/s10533-012-9727-y.

Bloom AF, Chapin III FS, Mooney HA. 1985. Resource limitation in plants – an economic analogy. Annual Review of Ecology and Systematics 16:363-392.

Brais S, Camiré C, Bergeron Y, Paré D. 1995. Changes in nutrient availability and forest floor characteristics in relation to stand age and forest composition in the southern part of the boreal forest of northwestern Quebec. Forest Ecology and Management 76: 181-189.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method for measuring microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17: 837-842

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF. 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81(9): 2359-2365.

Chapin III FS, Bloom AJ, Field CB, Waring RH. 1987. Plant responses to multiple environmental factors. BioScience 37:49-57.

Clarholm M. 1993. Microbial biomass P, labile P, and acid phosphatase activity in the humus layer of a spruce forest, after repeated additions of fertilizers. Biology and Fertility of Soils 16(4): 287-292.

Cleveland CC, Liptzin D. 2007. C:N:P stoichiometry: Is there a "Redfield Ratio" for the microbial biomass? Biogeochemistry 8(3):235-252.

Colvan S, Syers J, O'Donnell A. 2001. Effect of long-term fertilizer use on acid and alkaline phosphomonoesterase and phosphodiesterase activities in managed grassland. Biology and Fertility of Soils 34(4): 258-263.

Covington WW. 1981. Changes in forest floor organic matter and nutrient content following clear cutting in northern hardwoods. Ecology 56:715-720.

Craine JM, Morrow C, Fierer N. 2007. Microbial nitrogen limitation increases decomposition. Ecology 88(8): 2105-2113.

Currey PM, Johnson D, Sheppard LJ, Leith ID, Toberman H, Van Der Wal R, Dawson LA, Artz RRE. 2010. Turnover of labile and recalcitrant soil carbon differ in response to nitrate and ammonium deposition in an ombrotrophic peatland. Global Change Biology 16: 2307-2321.

Dale VH, Brown S, Haeuber RA, Hobbs NT, Huntly N, Naiman RJ, Riebsame WE, Turner MG, Valone TJ. 2000. Ecological principles and guidelines for managing the use of land. Ecological Applications 10(3): 639-670.

Danger M, Daufresne T, Lucas F, Pissard S, Lacroix G. 2008. Does Leibig's law of the minimum scale up from species to communities? Oikos 117: 1741-1751.

Davidson EA, Hart SC, Firestone MK. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73: 1148-1156.

D'Elia CF, Steudler Pa, Corwin N. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. Limnology and Oceanography 22(4): 760-764.

DeForest JL, Zak DR, Pregitzer KS. 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology and Biochemistry 36: 965-971.

DeForest JL, Smemo KA, Burke DJ, Elliott HL, Becker JC. 2012. Soil microbial responses to elevated phosphorus and pH in acidic temperate deciduous forests. Biogeochemistry 109: 189-202.

Demoling F, Figueroa D, Bääth E. 2007.Comparison of factors limiting bacterial growth in different soils. Soil Biology and Biochemistry 39: 2485-2495.

Ekblad A, Nordgren A. 2002. Is growth of soil microorganisms in boreal forests limited by carbon or nitrogen availability? Plant and Soil 242: 115–122.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters 10: 1135–1142.

Elser JJ, Anderson T, Baron JS, Bergström AK, Jansson M, Kyle M, Nydick KR, Steger L, Hessesn DO. 2009. Shifts in lake N:P stoichiometry and nutrient limitation driven by atmospheric nitrogen deposition. Science 326(5954): 835-837.

Fisk MC, Schmidt SK, Seastedt T. 1998. Topographic patterns of above- and belowground production and nitrogen cycling in alpine tundra. Ecology 79: 2253-2266.

Fisk MC, Fahey TJ. 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53: 201-223.

Fisk MC, Zak DR, Crow TR. 2002. Nitrogen storage and cycling in old- and second-growth northern hardwood forests. Ecology 83(1):73-87.

Fog K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biological Reviews of the Cambridge Philosophical Society 63: 433-462.

Fontaine S, Barot S. 2005. Size and functional diversity of microbe populations control plant persistence and longterm soil carbon accumulation. Ecology Letters 8:1075–1087.

Frazer DW, McColl JG, Powers RF. 1990. Soil nitrogen mineralization in a clearcutting chronosequence in a northern California conifer forest. Soil Science Society of America Journal 54: 1145-1152.

Gallo ME, Amonette R, Lauber C, Sinsabaugh RL, Zak DR. 2004. Short-term changes in oxidative enzyme activity and microbial community structure in nitrogen-amended north temperate forest soils. Microbial Ecology 47.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153-226.

Gamal-Eldin, Jane 1998. Bartlett Experimental Forest.

Gleeson SK, Tilman D. 1990. Allocation and the transient dynamics of sucession on poor soils. Ecology 71(3): 1144-1155.

Göransson H, Venterink HO, Bääth E. 2011. Soil bacterial growth and nutrient limitation along a chronosequence from a glacier forefield. Soil Biology and Biochemistry 43: 1333-1340.

Gorham E, Vitousek PM, Reiners WA. 1979. The regulation of chemical budgets over the course of terrestrial ecosystem succession. Annual Review of Ecology and Systematics 10: 53-84.

Grey TRG, Williams ST. 1971. Microbial productivity in the soil. Symposia of the Society for General Microbiology 21: 255-286.

Hagedorn F, Spinnler D, Siegwolf R. 2003. Increased N deposition retards mineralization of old soil organic matter. Soil Biology and Biochemistry 35: 1683-1692.

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters 14(9): 852-862.

Harte J, Kinzig AP. 1993. Mutualism and competition between plants and decomposers: implications for nutrient allocation in ecosystems. The American Naturalist 141(6): 829-846.

Hill BH, Elonen CM, Seifert LR, May AA, Tarquinio E. 2012. Microbial enzyme stoichiometry and nutrient limitation in US streams and rivers. Ecological Indicators 18: 540-551.

Hodge A, Robinson D, Fitter A. 2000. Are microorganism more effective than plants at competing for nitrogen? Trends in Plant Science 5(7): 304-308.

Holmes WE, Zak DR. 1999. Soil microbial control of nitrogen loss following clear-cut harvest in northern hardwood ecosystems. Ecological Applications 9: 202-215.

Hothorn T, Bretz F, Westfall P. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346—363.

Houlton BZ, Wang YP, Vitousek P, Field C. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454: 327–330.

Hsu PH, Rennie DA. 1962. Reactions of phosphate in aluminum systems: adsorption of phosphate by x-ray amorphous “aluminum hydroxide”. Canadian Journal of Soil Science 42:197-209.

Huntington, TG. 2005. Can nitrogen sequestration explain the unexpected nitrate decline in New Hampshire streams? Ecosystems 8:3 pp 331-333.

Ilstedt U, Singh S. 2005. Nitrogen and phosphorous limitations of respiration in a tropical phosphorous-fixing acrisol (ultisol) compared with organic compost. Soil Biology and Biochemistry 37: 1407–1410.

Joergensen RG, Scheu S. 1999. Response of soil microorganisms to the addition of carbon, nitrogen and phosphorous in a forest Rendzina. Soil Biology and Biochemistry 31: 859–866

Johnson D, Leake JR, Lee JA, Campbell CD. 1998. Changes in soil microbial biomass and microbial activities in response to 7 years simulated pollutant nitrogen deposition on a heathland and two grasslands. Environmental Pollution 103: 239-250.

Johnson DW. 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO2. Ecology 87: 64-75.

Koch AL. 1985. The macroeconomics of bacterial growth. *In*: Fletcher, M., Floodgate, G.D. (Eds.), Bacteria in their Natural Environments. Academic Press, London, pp. 1–42.

Lajtha, K. 1988. The use of ion exchange resin bags for measuring nutrient availability in an arid ecosystem. Plant and Soil 105: 105-111.

Liebig, J. 1842. Animal Chemistry, or Organic Chemistry in its Application to Physiology and Pathology. Johnson Reprint Corporation, New York, USA.

Likens GE, Bormann FH, Johnson NM, Fisher DW, Pierce RS. 1970. Effects of forest cutting and herbicide treatment on nutrient budgets in the Hubbard Brook watershed ecosystem. Ecological Monographs 40(1): 23-47.

Likens GE, Bormann FH, Johnson NM. 1972. Acid rain. Environment 14(2): 33–40.

Likens GE, Bormann FH. 1974. Acid rain: a serious regional environmental problem. Science 184(4142): 1176–79.

Lipson DA, Raab TK, Schmidt SK, Monson RK. 2001. An empirical model of amino acid transformations in alpine soil. Soil Biology and Biochemistry 33: 189-198.

Luo Y, Su B, Currie WS, Dukes JS, Finzi A, Hartwig U, Hungate B, McMurtrie RE, Oren R, Parton WJ, Pataki DE, Shaw MR, Zak DR, Field CB. 2004. Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54: 731-739.

Magill AH, Downs MR, Nadelhoffer KJ, Hallet RA, Aber JD. 1996. Forest ecosystem response to four years of chronic nitrate and sulfate additions at Bear Brooks Watershed, Maine, U.S.A. Forest Ecology Management 84: 29-37.

Magill AH, Aber JD, Hendricks JJ, Bowden RD, Melillo JM, Stuedler PA. 1997. Biogeochemical response of forest ecosystems to simulated chronic nitrogen deposition. Ecological Applications 7: 402-415.

Makino W, Cotner JB, Sterner RW, Elser JJ. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Functional Ecology 17: 121-130.

Marklein AR, Houlton BZ. 2011. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial eosystems. New Phytologist 193(3): 696-704.

Matson PA, Boone RD. 1984. Nitrogen mineralization and natural disturbance: wave-form dieback of mountain hemlock in the Oregon Cascades. Ecology 65: 1511-1516.

McGill WB, Cannon KR, Robertson JA, Cook FD. 1986. Dynamics of soil microbial biomass and water soluble organic C in Breton L after 50 years of cropping two rotations. Canadian Journal of Soil Science 66: 1-19.

McLauchlan KK, Craine JM, Oswald WW, Leavitt PR, Likens GE. 2007. Changes in nitrogen cycling during the past century in a northern hardwood forest. PNAS 104: 7466-7470.

McNulty SG, Aber JD, Newman SD. 1996. Nitrogen saturation in a high elevation spruce-fir stand. Forest Ecology Management 84: 109-121.

Michel K, Matzner E. 2003. Response of enzyme activities to nitrogen addition in forest floors of different C-to-N ratios. Biology and Fertility of Soils 38: 102-109.

Moorhead DL, Sinsabaugh RL. 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76:151–174.

Murphy J, Riley JP. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36.

Naples BK, Fisk MC. 2010. Belowground insights into nutrient limitation in northern hardwood forests. Biogeochemistry 97:109-121.

Nordgren A. 1992. A method for determining microbially available N and P in an organic soil. Biology and Fertility of Soils 13: 195-199.

Odén S. 1968. Acidification of air and precipitation and its consequences on the natural environment (Swedish National Science Research council, Bulletin 1, Stockholm) *In:* JH Gibson, Acid Deposition: Long-Term Trends. National Academy Press, Washington, DC.

Okin GS, Mahowald N, Chadwick OA, Artaxo P. 2004. Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. Global Biogeochemical Cycles 18(2).

Olander L, Vitousek P. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49: 175–191.

Park BB, Yanai RD, Vadeboncoeur MA, Hamburg SP. 2007. Estimating root biomass in rocky soils using pits, cores, and allometric equations. Soil Science Society of America Journal 71:206-213.

Pierce RS, Martin CW, Reeves CC, Likens GE, Bormann FH. 1972. Nutrient loss from clearcuttings in New Hampshire. pp. 285-295. *In*: Symp. on Watersheds in Trans., Ft. Collins, Colorado.

Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team. 2012. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-103.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Ramirez KS, Craine JM, Fierer N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biology. doi: 10.1111/j.1365-2486.2012.02639.x.

Rastetter EB, Ågren GI, Shaver GR. 1997. Responses of N-limited ecosystems to increased CO2: a balanced-nutrition, coupled-element cycles model. Ecological Applications 7:444-460.

Rastetter EB, Yanai RD, Thomas RQ, Vadeboncoeur MA, Fahey TJ, Fisk MC, Kwiatkowski BL, Hamburg SP. 2012. Recovery from disturbance requires resynchronization of ecosystem nutrient cycles. In review.

Reed SC, Vitousek PM, Cleveland CC. 2011. Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. Biogeochemistry 106: 323-336.

Richter DD, Allen HL, Li J, Markewitz D, Raikes J. 2006. Bioavailability of slowly cycling soil phosphorus: major restructuring of soil P fractions over four decades in an aggrading forest. Oecologia 150:259-271.

Ryan MG, Binkley D, Fownes JH. 1997. Age-related decline in forest productivity: pattern and process. Advances in Ecological Research 27: 213-262.

Saiya-Cork KR, Sinsabaugh RL, Zak DR. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. Soil Biology and Biochemistry 34: 1309-1315.

Schimel JP, Weintraub MN. 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology and Biochemistry 35: 549-563.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85(3): 591-602.

Schlesinger WH. 1991. Biogeochemistry: An Analysis of Global Change. Academic Press, New York.

Schmidt SK, Costello EK, Nemergut DR, Cleveland CC, Reed SC, Weintraub MN, Meyer AF, Martin AM. 2007. Biogeochemical consequences of rapid microbial turnover and seasonal succession in soil. Ecology 88(6): 1379-1385.

Sherman J, Fernandez IJ, Norton SA, Ohno T, Rustad LE. 2006. Soil aluminum, iron, and phosphorus dynamics in response to long-term experimental nitrogen and sulfur additions at the Bear Brook watershed in Maine, USA. Environmental Monitoring and Assessment 121: 419-427.

Sinsabaugh RL. 1994. Enzymic analysis of microbial pattern and process. Biology and Fertility of Soils 17: 69-74.

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison SD, Crenshaw C, Contosta AR, Cusack D, Frey S, Gallo ME, Gartner TB, Hobbie SE, Holland K, Keeler BL, Powers JS, Stursova M, Takacs-Vesbach C, Waldrop MP, Wallenstein MD, Zak DR, Zeglin LH. 2008. Stoichiometry of soil enzyme activity at a global scale. Ecology Letters 11(11): 1252-1264.

Sinsabaugh RL, Hill BH, Follstad Shah JJ. 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462: 795-798.

Sinsabaugh RL. 2010. Phenol oxidase, peroxidase, and organic matter dynamics of soil. Soil Biology and Biochemistry 42: 391-404.

Smith, RA. 1872. *Air and Rain: The Beginnings of a Chemical Climatology*, Longmans, Green and Co., London, 600 pp.

Smith JL, Paul EA. 1990. The significance of soil microbial biomass estimations. p. 357-396. *In*: GM Bollag and G Stotzkey (eds). Soil biochemistry. Vol. 6. Marcel Dekker, New York, NY.

Stark JM, Hart SC. 1997. High rates of nitrification and nitrate turnover in undisturbed conifer forests. Nature 385: 61-64.

Sterner, RW, Elser JJ. 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, NJ.

Strickland MS, Rousk J. 2010. Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42: 1385-1395.

Swanston C, Homann PS, Caldwell BA, Myrold DD, Ganio L, Sollins P. 2004. Long-term effects of elevated nitrogen on forest soil organic matter stability. Biogeochemistry 70(2): 229-252.

Odèn S. 1968. The acidification of air and precipitation and its consequences on the natural environment. Bulletin 1, Swedish National Science Research Council, Ecology Committee. 68 pp.

Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR (eds) Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, Wis., pp 903–948.

Tiessen H, Moir JO. 1993. Characterization of available P by sequential exctraction. In:Carter MR, editor. Soil sampling and methods of analysis. Boca Raton (FL): Lewis.

Treseder K, Vitousek P. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 82:946–954.

Turner BL, Baxter R, Whitton BA. 2002. Seasonal phosphatase activity in three characteristic soils of the English uplands polluted by long-term atmospheric nitrogen deposition. Environmental Pollution 120: 313-317.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19: 703-708.

van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11: 296-310.

Vitousek PM, Gosz JR, Grier CC, Melillo JM, Reiners WA. 1982. A comparative analysis of potential nitrification and nitrate mobility in forest eosystems. Ecological Monographs 52: 155-177.

Vitousek PM, Fahey TJ, Johnson DW. 1988. Element interactions in forest ecosystems: succession, allometry, and input-output budgets. Biogeochemistry 5: 7-34.

Vitousek PM, VanCleve K, Matson PA. 1989. Nitrogen availability and nitrification during succession: primary, secondary, and old-field seres. Plant and Soil 115: 229-239.

Vitousek PM, Howarth RW. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13(2): 87-115.

Vitousek PM, Aber J, Howarth R, Likens G, Matson P, Schindler D, Schlesinger W, Tilman G. 1997. Human alteration of the global nitrogen cycle: source sand consequences. Ecological Applications 7:737–750.

Vitousek PM, Porder S, Houlton BZ, Chadwick OA. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecological Applications 20(1): 5-15.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. Geoderma 15:1–19.

Wardle DA, Bardgett RD, Klirnomos JN, Setälä H, van der Putten WH, Wall DH. 2004. Eological linkages bewtween aboveground and belowground biota. Science 304: 1629-1633.

Wood T, Bormann FH, Voigt GK. 1984. Phosphorus cycling in an northern hardwood forest: biological and chemical control. Science 223(4634): 391-393.

Yanai RD. 1998. The effect of whole-tree harvest on phosphorus cycling in a northern hardwood forest. Forest Ecology and Management 104:281-295.

Yanai RD, Blum JD, Hamburg SD. 2005. New insights into calcium depletion in northeastern forests. Journal of Forestry 103(1):14-20.

Zak DR, Grigal DF, Gleeson S, Tilman D. 1990. Carbon and nitrogen cycling during old-field succession: constraints on plant and microbial biomass.

Table 1.

	Numerator DF	Denominator DF	F-value	p-value
Oe				
Enzymes				
Glucosidase	2	6	5.12	0.0504
Cellobiohydrolase	2	6	1.43	0.3104
Phosphatase	2	6	0.54	0.6092
Polyphenol Oxidase	2	6	1.28	0.3455
Nutrients				
Bicarbonate P	2	6	1.99	0.2168
N mineralization	2	6	2.41	0.1705
Oa				
Enzymes				
Glucosidase	2	6	0.91	0.4523
Cellobiohydrolase	2	6	2.75	0.1418
Phosphatase	2	6	1.89	0.2326
Polyphenol Oxidase	2	6	1.29	0.3412
Nutrients				
Bicarbonate P	2	6	1.07	0.3993
N mineralization	2	6	2.20	0.1921

Table 2.

Source	Gluc	Cell	PPO	Phos	Bic P	Microbial P	Microbial N	Microbial N:P
2008								
Oe								
Mid	2.0 (0.26)	0.6 (0.06)	1.8 (0.28)	28.5 (5.11)	151 (10.8)	436 (75.0)	1013 (77.9)	6.2 (0.82)
Mature	2.1 (0.44)	0.6 (0.10)	0.9 (0.24)	6.4 (4.88)	115 (14.5)	360 (27.5)	883 (49.1)	5.3 (0.44)
Oa								
Mid	0.4 (0.03)	0.1 (0.03)	0.8 (0.17)	6.1 (0.63)	53 (5.8)	218 (50.4)	493 (18.9)	5.8 (1.20)
Mature	0.2 (0.04)	0.1 (0.01)	0.6 (0.08)	2.7 (0.77)	77 (3.6)	87 (35.5)	509 (49.7)	13.9 (6.64)
2009								
Oe								
Young	5.9 (0.47)	1.5 (0.19)	1.8 (0.25)	46.2 (1.32)	63 (4.4)	565 (13.5)	1352 (82.2)	5.8 (0.49)
Mid	3.5 (0.57)	0.9 (0.14)	3.0 (0.78)	48.7 (2.81)	71 (6.6)	66 (143.7)	1377 (90.3)	5.7 (1.63)
Mature	3.5 (0.74)	1.1 (0.36)	2.3 (0.41)	43.3 (5.48)	82 (7.6)	491 (82.9)	1100 (113.4)	5.4 (1.45)
Oa								
Young	1.0 (0.18)	0.3 (0.05)	0.8 (0.06)	15.9 (1.80)	53 (6.9)	204 (16.0)	56 (20.2)	9.2 (3.56)
Mid	0.8 (0.09)	0.2 (0.02)	1.2 (0.31)	13.5 (1.18)	54 (0.7)	241 (62.4)	547 (60.6)	7.2 (2.10)
Mature	0.8 (0.11)	0.2 (0.02)	0.8 (013)	11.1 (2.6)	63 (6.5)	183 (60.9)	501 (35.8)	11.1 (6.06)

Figure 1.

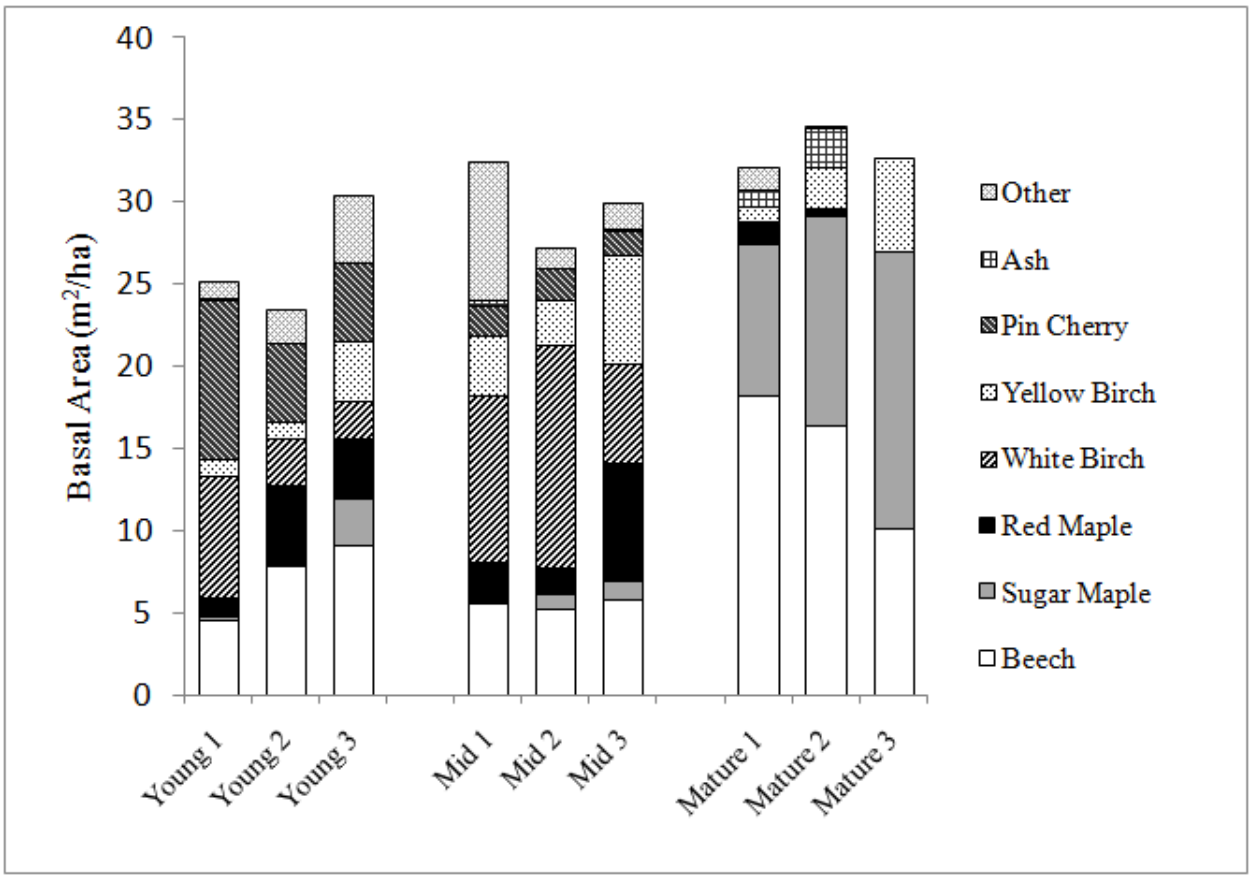
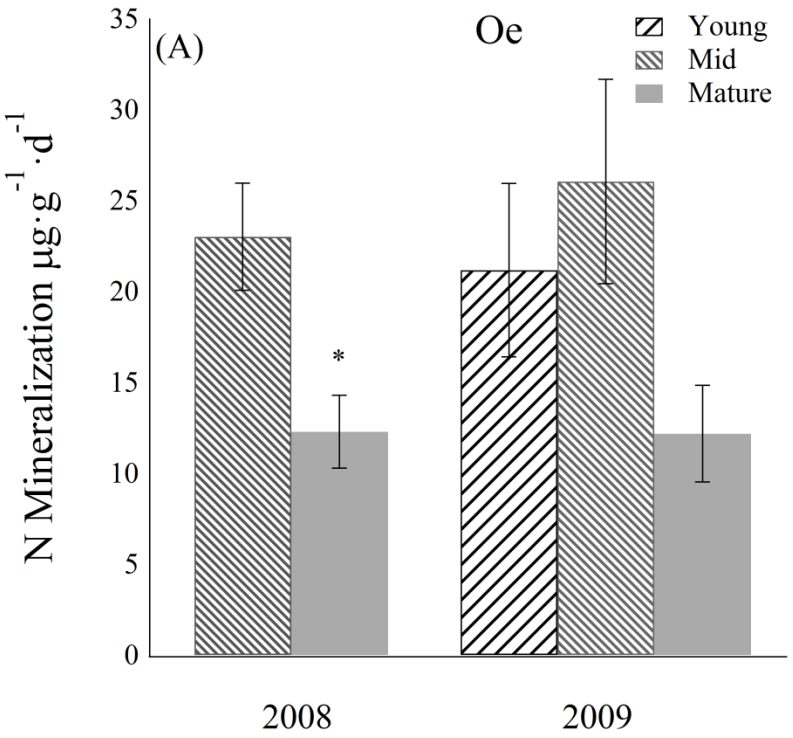

40
35
30
25
20
15
10
5
0
Basal Area (m²/ha)
Young 1
Young 2
Young 3
Mid 1
Mid 2
Mid 3
Mature 1
Mature 2
Mature 3
Other
Ash
Pin Cherry
Yellow Birch
White Birch
Red Maple
Sugar Maple
Beech

Figure 2.


(A)
Oe
Young
Mid
Mature
N Mineralization μg·g-1·d-1
35
30
25
20
15
10
5
0
*
2008
2009

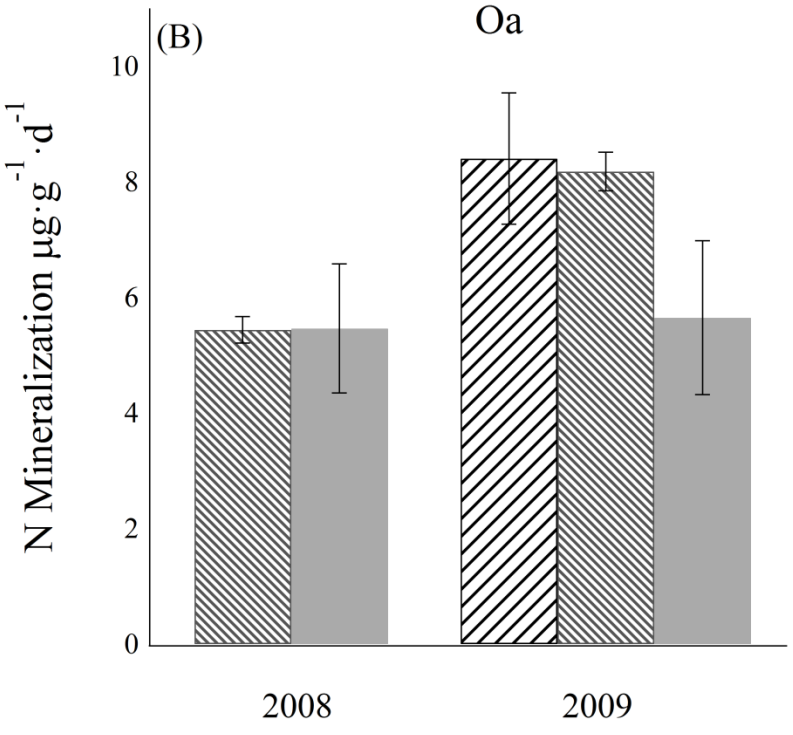

(B)
Oa
N Mineralization μg·g-1·d-1
10
8
6
4
2
0
2008
2009

Figure 3.

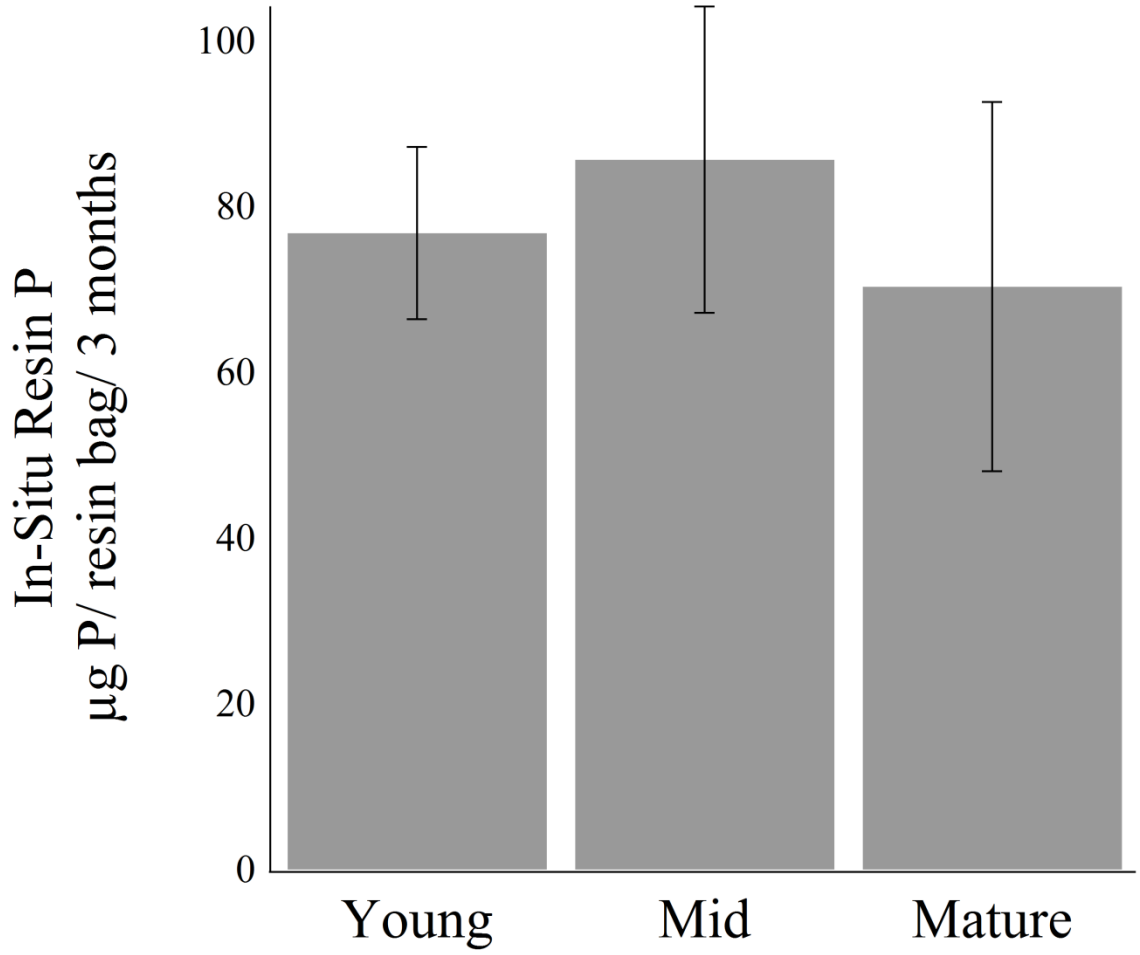
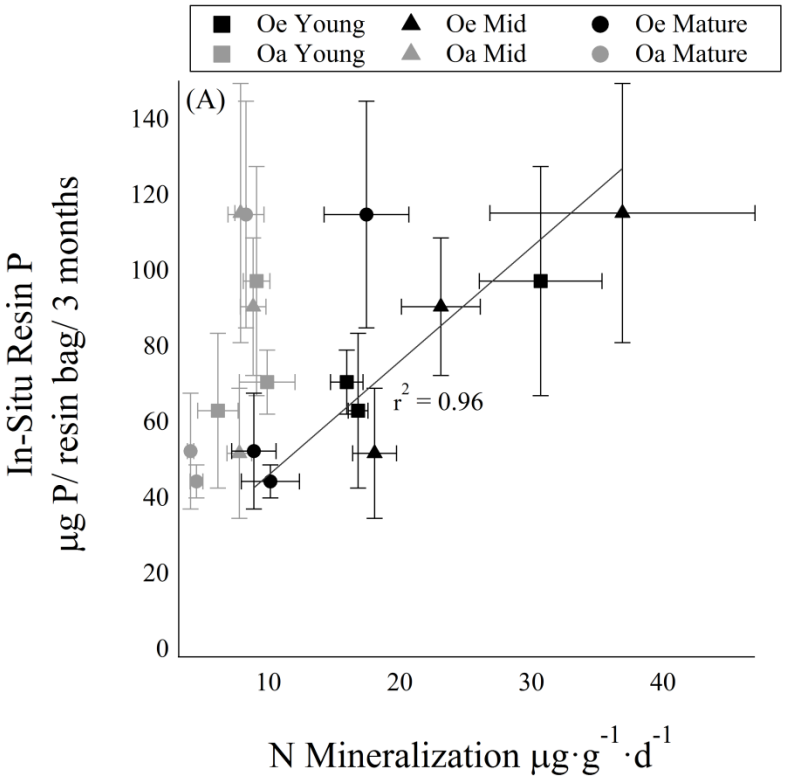
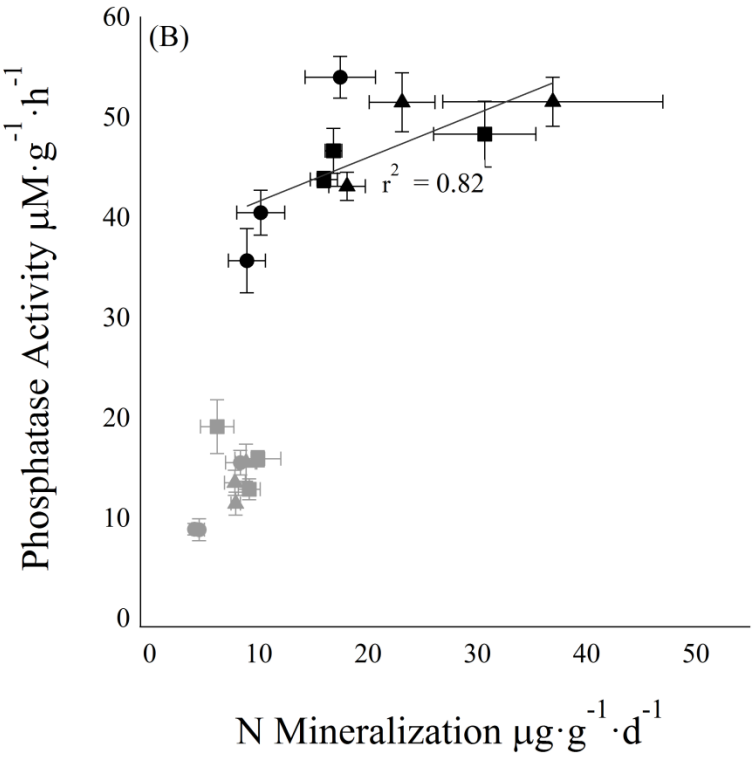
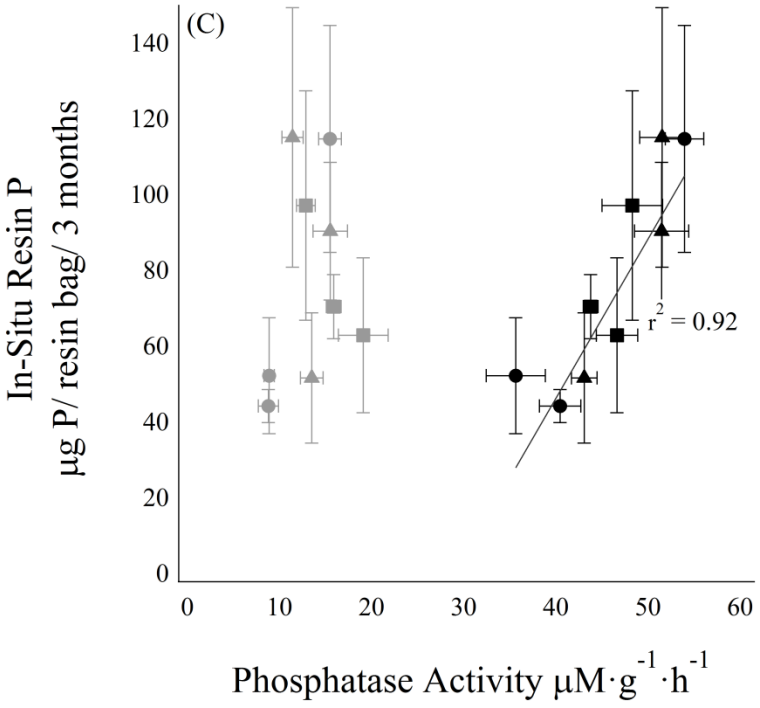

In-Situ Resin P
µg P/ resin bag/ 3 months
100
80
60
40
20
0
Young
Mid
Mature

Figure 4.


Oe Young
Oe Mid
Oe Mature
Oa Young
Oa Mid
Oa Mature
(A)
In-Situ Resin P
µg P/ resin bag/ 3 months
r² = 0.96
N Mineralization µg·g⁻¹·d⁻¹


(B)
Phosphatase Activity µM·g⁻¹·h⁻¹
r² = 0.82
N Mineralization µg·g⁻¹·d⁻¹


(C)
In-Situ Resin P
µg P/ resin bag/ 3 months
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
r² = 0.92
Phosphatase Activity µM·g⁻¹·h⁻¹

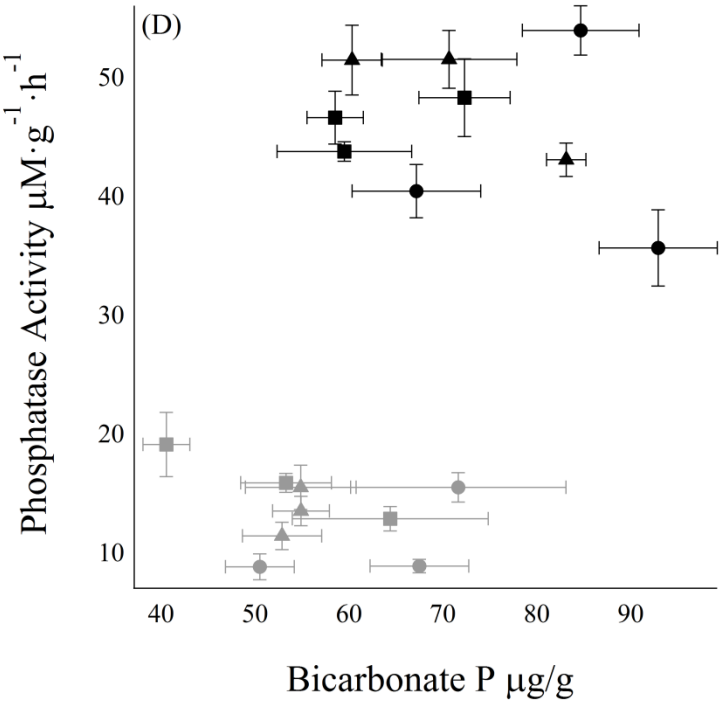

(D)
Phosphatase Activity µM·g⁻¹·h⁻¹
50
40
30
20
10
40
50
60
70
80
90
Bicarbonate P µg/g

Figure 5.

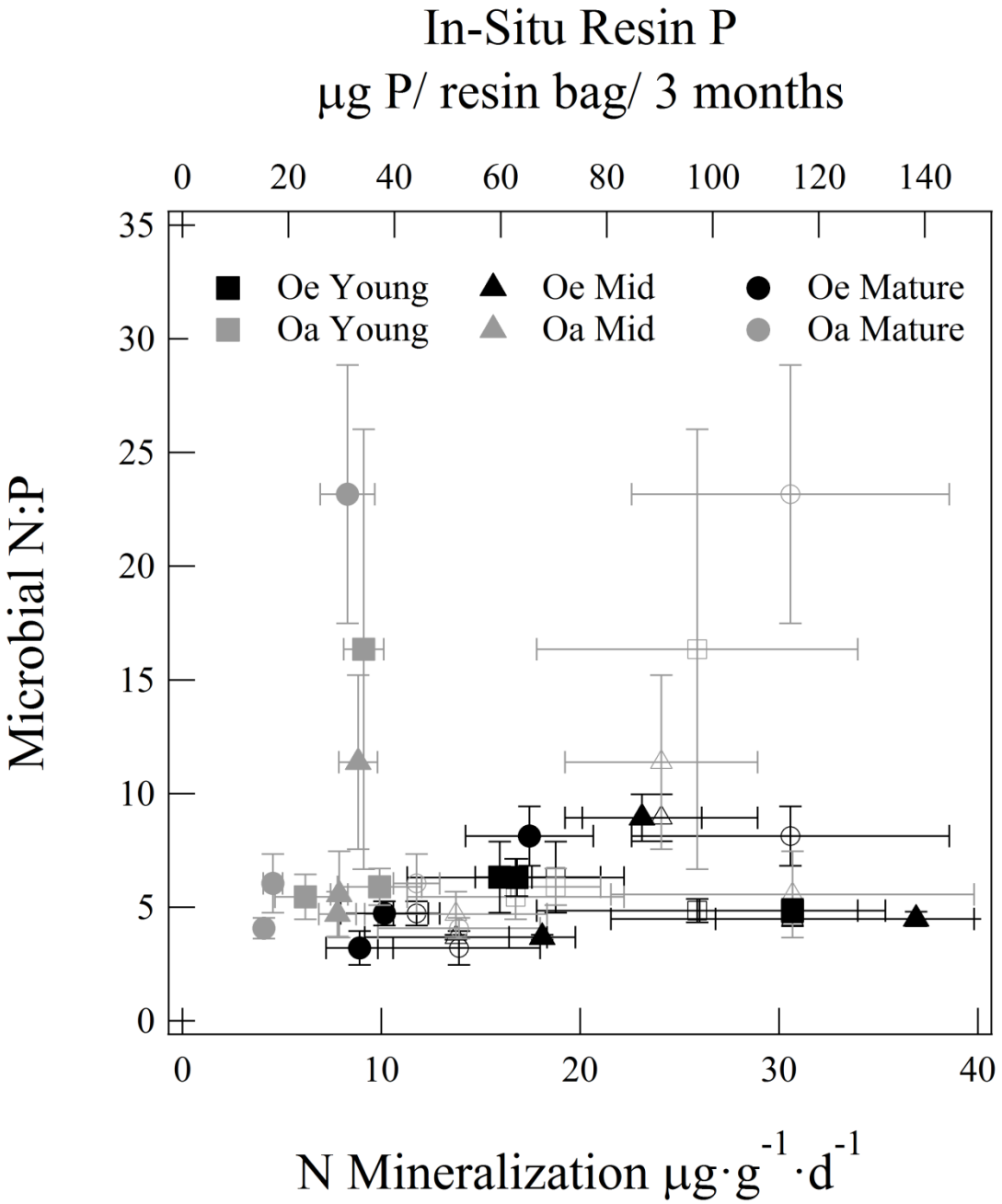
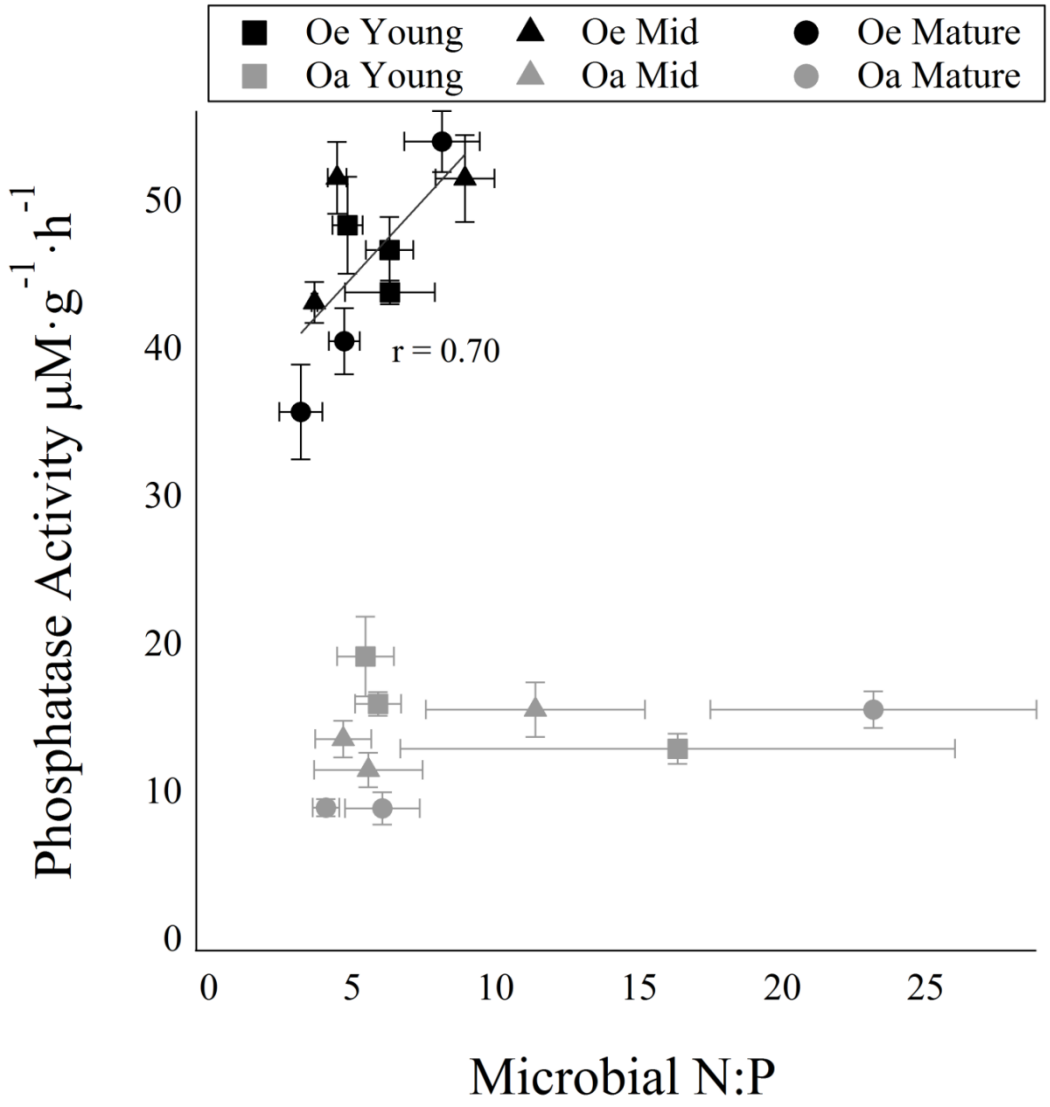

In-Situ Resin P
µg P/ resin bag/ 3 months
Microbial N:P
N Mineralization µg·g-1·d-1
Oe Young
Oa Young
Oe Mid
Oa Mid
Oe Mature
Oa Mature
0
20
40
60
80
100
120
140
35
30
25
20
15
10
5
0
0
10
20
30
40

Figure 6.


Oe Young
Oe Mid
Oe Mature
Oa Young
Oa Mid
Oa Mature
r = 0.70
Phosphatase Activity μM·g-1·h-1
Microbial N:P
0
10
20
30
40
50
0
5
10
15
20
25