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ABSTRACT

INVESTIGATING NUTRIENT CO-LIMITATION IN NORTHERN HARDWOOD FORESTS

by

Shinjini Goswami

Availability and recycling of nutrients constrain primary productivity in many ecosystems. Current ecosystem theories have evolved from an earlier paradigm of limitation of ecosystem productivity by a single nutrient to that of co-limitation by multiple nutrients. Nutrient co-limitation is inferred when ecosystem productivity increases more in response to two nutrients added together than to either nutrient added alone. In this dissertation, I present several related studies that explore ecosystem-, community- and species-level nutrient limitation/co-limitation in northern hardwood forests. The goal of my dissertation is to examine various mechanisms that can mediate ecosystem-level nitrogen (N) and phosphorus (P) co-limitation by linking research at three levels of hierarchy: belowground processes, whole plant (seedlings) and ecosystem-level (mature trees).

Chapter 1: Phosphorus limitation of aboveground production in northern hardwood forests. According to theories of long-term ecosystem development, forest productivity on glacially derived soils with weatherable P is expected to be limited by N. Using a full factorial N x P fertilization, this chapter examines aboveground productivity response to N and P additions, and presents evidence for P limitation of aboveground growth. I did not find evidence for N and P co-limitation of tree growth, but increased growth response to P could be a consequence of long-term anthropogenic N deposition in these forests. This chapter is in revision in *Ecology*.

Chapter 2: Phosphorus reduces nitrogen availability in northern hardwood forests. Uptake and recycling by plants and soil microorganisms exert control on the stoichiometry of available nutrients, potentially influencing ecosystem responses to perturbations that alter resource availability. This chapter tested whether an excess of one nutrient influenced the availability of another and found that fertilizing with P decreased the availability of N, especially when P was added in combination with N. Interactions between N and P have implications for mechanisms that could mediate N and P co-limitation over time. This chapter is in preparation for submission to *Biogeochemistry*.

Chapter 3: Seedling survival and allocation responses to nutrient additions in northern hardwood temperate forests. This chapter explores whether seedling growth and survivorship is limited by nutrient availability, and I found clear evidence of N addition suppressing seedling survivorship in American beech and sugar maple. These results further demonstrate that improved nutrition could have potential indirect effects such as herbivory damage which could be related to a negative effect of N on regeneration in these forests. This chapter is in preparation for submission to *Journal of Ecology*.

INVESTIGATING NUTRIENT CO-LIMITATION IN NORTHERN HARDWOOD FORESTS

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DEDICATION

This dissertation is dedicated to my grandfather, Mr. Adwaitya K. Goswami who first taught me to ask questions and be curious about this nature and life in general. I miss you deeply.

To my parents, Dr. Asim Goswami and Mrs. Subhra Goswami, for all their love and support to put me through the best education possible. I sincerely appreciate their lifelong sacrifices and I wouldn’t have been able to get to this stage without them. This is for you- Maa, Baba.

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General Introduction

Terrestrial ecosystem productivity is light, water and nutrient limited, and even small changes in the balance of available soil nutrients could have profound effects on productivity responses to natural and anthropogenic factors. As terrestrial ecosystems age, soil nitrogen (N) is accumulated through biological fixations while the availability of soil phosphorus (P) declines via weathering and iron or aluminum fixation processes (Walker and Syers, 1976; Vitousek and Farrington, 1997). As a consequence of changes in relative N vs. P availability, the age and soil development determines if these ecosystems are N or P limited. For example, temperate forests on relatively younger soils are considered N limited while tropical forests are typically P limited (Vitousek, 2004). The underlying concepts of single nutrient limitation were derived from Liebig's Law of the Minimum (Liebig, 1842), which states that plant growth is limited by the nutrient in shortest supply relative to plant demand. This idea was originally applied to maximizing agricultural production of individual crop plants; however, the concept was extended to complex ecosystems like forests that are comprised of multiple species with a diversity of traits (Harpole et al. 2011). Experimental fertilization studies both in agricultural and natural systems have shown single- nutrient limitation of primary production but the majority of those studies manipulated only a single nutrient at any particular point in time (Menge et al. 2008).

Resource optimization theory (Bloom et al. 1985, Chapin et al. 1987, 2002) has indicated that productivity in terrestrial ecosystems is often co-limited, not only by N and P, but also by other resources such as light and water. Plants can allocate energy and nutrients that they have in abundance toward acquiring more limiting resources. Over time this could create a condition in which plant growth is equally limited by multiple resources (Chapin et al. 1987, 2002). However, if all plant species could effectively optimize resource acquisition, we would expect the whole ecosystem to be perfectly co-limited. In contrast, if none of the species could optimize resource acquisition, we would expect extreme single-nutrient limitation consistent with Leibig's Law. In a situation where some species can optimize while others cannot, aggregate productivity responses are likely to be co-limited. Consistent with this resource optimization theory, a recent meta-analysis reported a greater productivity response to N+P addition than to either nutrient alone, suggesting that terrestrial ecosystem productivity is often co-limited by N and P (Elser et al. 2007). Therefore, both recently developed theory and observation have led to a general questioning of the N limitation paradigm of ecosystem productivity in temperate forests, and have raised new questions about nutrient interactions that could mediate N, and P co-limitation. Examining the impacts of altered N and P availability on growth and activity of plant and soil-microbial processes such as nutrient mineralization and uptake could improve our understanding of the mechanisms underlying nutrient limitation or co-limitation in these ecosystems.

Temperate forest ecosystems are under-represented in the co-limitation results summarized by Elser et al. (2007) and Harpole et al. (2011). What evidence there is from northern hardwood temperate forests does not clearly support limitation solely by N but instead suggests limitation by P or co-limitation by N and P. Forest ecosystems in the northeastern US have been exposed to long-term anthropogenic N deposition, which has altered soil properties and nutrient cycling (Fenn et al. 1998, Aber et al. 2003). Forests that experience nutrient removal associated with biomass harvest coupled with high NO_3^- leaching (Fisk and Fahey 1991), and an increased potential for soil P immobilization when total plant nutrient uptake is low early in regeneration (Yanai 1992), experience decreased N and P availability (Bormann and Likens 1979), which disrupts the N:P balance (Rastetter et al. 2013). The Multiple Element Limitation model (MEL) predicted a shift from N to P limitation as northern hardwood forests recover from harvesting disturbance, until recycling of N and P becomes stoichiometrically balanced (Rastetter et al. 2013). Hence, to better understand N-P co-limitation it is important to study nutrient dynamics at different stages of succession in a managed forest landscape comprised of multiple aged stands. Experimental observations of fine root responses to nutrient additions suggests P limitation in young forests and N limitation in mature forests at the Bartlett Experimental Forest (BEF) in central NH (Naples and Fisk 2010). Furthermore, a recent meta-analysis for the northeastern US showed evidence for both N and P limitation (Vadeboncoeur 2010).

The concept of "co-limitation" lacks a strict definition (Harpole et al. 2011), but can be defined most simply as the condition in which ecosystem productivity increases more in response to two nutrients added together than to either nutrient added alone. To convincingly test whether productivity in northern hardwood forests is co-limited by N and P the Multiple Element Limitation in Northern Hardwood Ecosystems project (MELNHE) established a large-scale ecosystem study on edaphically similar sites in northeastern US to evaluate forest responses to a full factorial N x P fertilization. MELNHE is a long-term collaborative project which combines modeling and field studies, with the general goal of examining nutrient co-limitation in northern hardwood forest ecosystems. Within the context of intermediate- (such as forest harvest followed by succession) and long-term changes (such as N deposition), my dissertation research focuses on understanding short-term processes (such as soil nutrient interactions, resource optimization in plants and soil microbes) that could mediate co-limitation by N and P in typically managed hardwood forest systems. I further investigate whether short-term soil N and P interactions are driven by soil microbial activity and contribute to aboveground growth responses to nutrient fertilization.

In this dissertation, I also argue that it is necessary to judge plant responses to nutrient availability, not only at an ecosystem level, but also at the level of individual species. These forests are composed of a mixture of different tree species that vary in their life history traits and nutrient use and could respond differentially to the addition of N, P and N+P, thereby, influencing ecosystem-level productivity responses to nutrient additions. Furthermore, because of the relative dominance of early- vs late-successional species in young and mid-age forests, and ectomycorrhizal vs arbuscular mycorrhizal tree species irrespective of forest age, species responses could have implications for ecosystem-level responses to nutrients. I take advantage of the long-term fertilization project (MELNHE) to test ecosystem-, community- and species-level nutrient limitation/co-limitation and to test individual nutrient interactions to examine various mechanisms that can mediate ecosystem-level N and P co-limitation by linking research at three levels of hierarchy: belowground processes, whole plant (seedlings) and ecosystem-level (mature trees) (Fig. 1; individually discussed in the following three chapters):

- Chapter 1: Is aboveground productivity co-limited by N and P in northern hardwood ecosystems? (ecosystem-level, mature trees)
- Chapter 2: Can northern hardwood ecosystems maintain balanced nutrient availability in response to shorter-term disruptions? (ecosystem-level, belowground processes)
- Chapter 3: Seedling survival and allocation responses to nutrient additions in northern hardwood temperate forests (individual species-level, whole plants)

The available evidence suggests that I should find co-limitation (Elser et al., 2007; Vadeboncoeur 2010; Rastetter et al., 2013); if I do, then this study can contribute to developing co-limitation theory by elucidating mechanisms that contribute to the additive or synergistic responses to N and P. If I do not find evidence of co-limitation, this study can contribute to developing co-limitation theory by learning about nutrient interactions that can mediate the onset of N and P co-limitation, or the nutrient-use mechanisms that mediate single-nutrient limitation or correspond with a lack of apparent nutrient limitation in the northern hardwood forest ecosystem. Examining the mechanisms underlying co-limitation will also be necessary for predicting the implications for vegetation dynamics during times of anthropogenic change.

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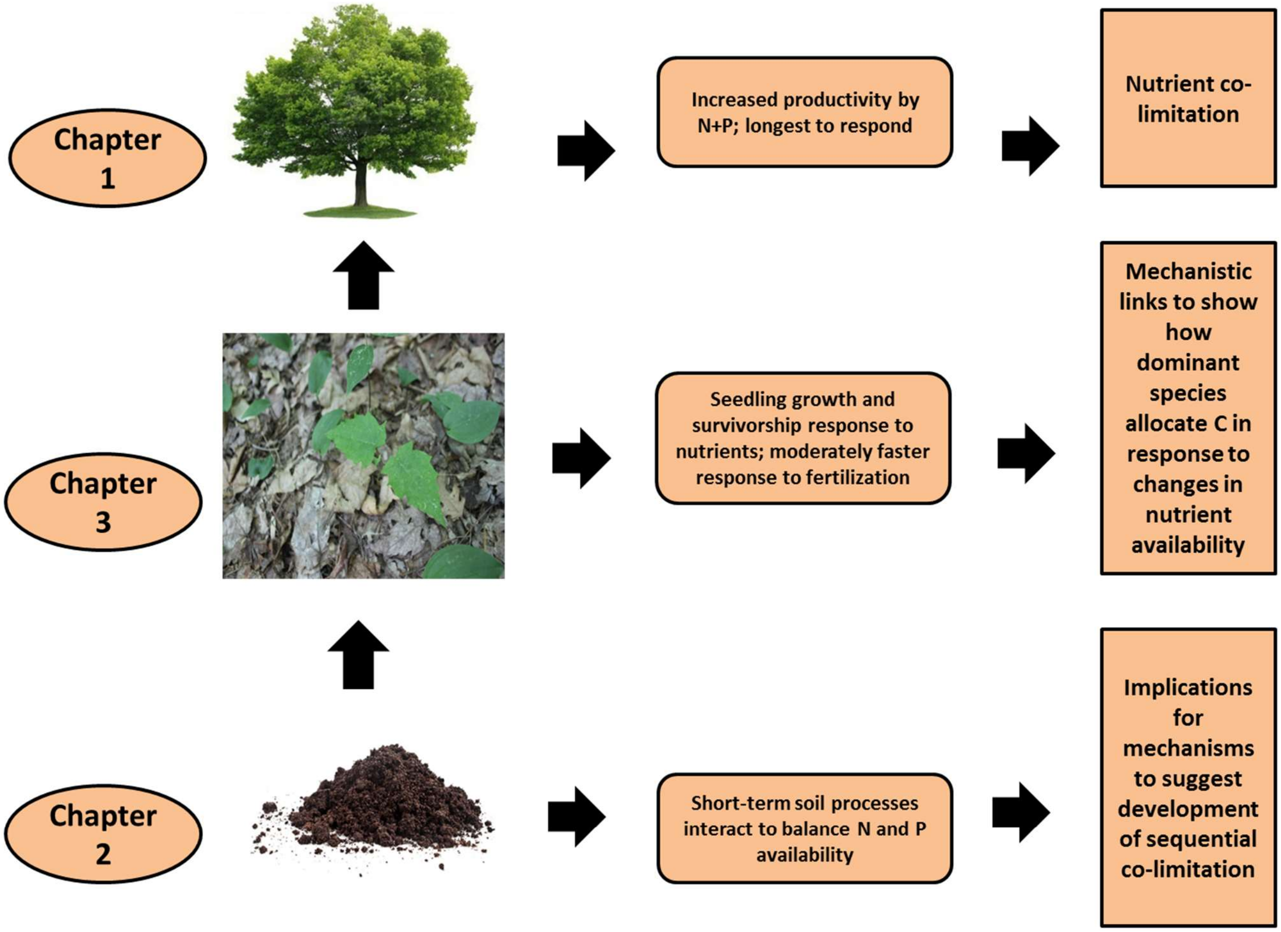

Fig 1. Simplified conceptual model followed in this research, demonstrating links between belowground and aboveground nutrient responses, proposed for understanding mechanisms that can mediate development of ecosystem-level co-limitation of productivity by N and P.

Chapter 1

Phosphorus limitation of aboveground production in northern hardwood forests

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Abstract

Forest productivity on glacially derived soils with weatherable phosphorus (P) is expected to be limited by nitrogen (N), according to theories of long-term ecosystem development. However, recent studies and model simulations based on resource optimization theory indicate that productivity can be co-limited by N and P. We conducted a full factorial N x P fertilization experiment in 13 northern hardwood forest stands of three age classes in central New Hampshire, USA, to test the hypothesis that forest productivity is co-limited by N and P. We also asked whether the response of productivity to N and P addition differs among species and whether differential species responses contribute to community-level co-limitation. Plots in each stand were fertilized with 30 kg N ha^{-1} yr^{-1}, 10 kg P $ha^{-1}yr^{-1}$, N+P together, or neither nutrient (control) for four growing seasons. The productivity response to treatments was assessed using per-tree basal area growth response, or relative growth rate (RGR). RGR responded significantly to P (P=0.02) but not to N (P=0.73). However, evidence for P limitation was not uniform among stands. RGR responded to P fertilization in mid-age (P = 0.02) and mature (P = 0.07) stands, but was greatest in N-fertilized plots of a few stands in these age classes, and there was no effect of P in the young stands. Both white birch (*Betula papyrifera* Marsh.) and beech (*Fagus grandifolia* Ehrh.) responded significantly to P, and no species responded significantly to N. We did not find evidence for N and P co-limitation of tree growth; the response to N+P did not differ from that to P alone, and there was no significant N x P interaction (P=0.68). Our P limitation results support neither the N limitation prediction of ecosystem theory nor the N and P co-limitation prediction of resource optimization theory, but could be a consequence of long-term anthropogenic N deposition in these forests. Inconsistencies in response to P suggest that successional status and variation in site conditions influence patterns of nutrient limitation and recycling across the northern hardwood forest landscape.

Introduction

Availability of mineral nutrients constrains primary productivity in many ecosystems. Because P is obtained from weathering and N is fixed by biota, ecosystem theory has suggested that productivity should be limited by nitrogen (N) on young soils and phosphorus (P) on older soils (Walker and Syers 1976, Vitousek 2004); thus, forest productivity on young, glacially derived soils is expected to be N limited. However, evidence of a greater response to N and P together than to either alone across terrestrial and aquatic ecosystems (Elser et al. 2007) raises questions about controls of ecosystem productivity. Resource optimization theory suggests that plants adjust their physiology to minimize limitation by any single resource, allocating effort to acquire the more limiting resources such that eventually plant growth is co-limited by multiple resources (Bloom et al. 1985, Chapin et al. 1987, 2002). However, there are limits to what plants can do to obtain limiting resources, and even when they are capable of achieving co-limitation, it may take time to adjust the allocation of effort when resource availability changes, as with forest succession or atmospheric deposition.

Some previous fertilization studies conducted in the northeastern US support the idea that productivity in these forests is primarily N limited (Safford 1973, Magill et al. 2000, 2004, Finzi 2009), whereas others indicate P limitation or N and P co-limitation (Lea et al. 1979; Auchmoody 1982, Safford and Czapowskyj 1986). Meta-analyses suggest that productivity in most ecosystems is N and P co-limited (Elser et al. 2007, Harpole et al. 2011), but temperate forests were not well represented (4 out of ~100 terrestrial studies) in those meta-analyses. The Multiple Element Limitation model (MEL) (Rastetter et al. 2013) suggests from a theoretical perspective that N-P co-limitation should eventually prevail as forests develop after large-scale disturbance. A recent meta-analysis for the northeastern US showed evidence for both N and P limitation (Vadeboncoeur 2010), though few previous studies in this region have had the factorial design necessary to test co-limitation.

Co-limitation can arise from several different mechanisms such as those outlined in the framework by Harpole et al. (2011). Co-limitation by N and P could be "synergistic" when the response to N+P added together is greater than the sum of the responses to each nutrient added alone, “additive” when the response to N+P is equal to the sum of the individual responses, or “sub-additive” when the N+P response is less than the sum of the single nutrient responses. In contrast, when the response to the secondary limiting nutrient occurs only after alleviating limitation by the primary limiting nutrient, “sequential” limitation (Davidson and Howarth 2007, Craine 2009) can also give rise to apparent N-P co-limitation.

One instance of additive co-limitation is “community-level” co-limitation that can occur when some species respond primarily to N and others to P (Arrigo 2005). Species traits can influence ecosystem productivity by altering the availability or use of limiting resources (Chapin and Shaver 1985, Chapin et al. 1986, 1987). Tree species can modify their local environment through particular life history and physiological traits, such as shade tolerance, mycorrhizal associations, litter chemistry, and canopy interception (Augusto et al. 2002, Reich et al. 2005, Lang and Polle 2011). Different species in a forest ecosystem can also be limited by different resources as a consequence of traits influencing nutrient acquisition, conservation, and use efficiency. For example, northern hardwood forests have mixtures of tree species that form either ectomycorrhizal (ECM) or arbuscular mycorrhizal (AM) associations, and changes in N or P availability may have differential effects on the growth of AM vs ECM trees. Therefore, it is important to determine whether individual species that differ in their life history traits and resource use respond differently to the addition of N, P and N+P, thereby influencing nutrient co-limitation at the ecosystem level, when responses of all species are averaged.

Understanding the dynamics of nutrient limitation is especially important in managed and anthropogenically altered ecosystems because nutrient inputs and outputs differ from the conditions under which these systems developed. Managed forests have been subject to nutrient removal due to biomass removal and post-disturbance nutrient export (Federer et al. 1989). Additionally, forest ecosystems in the northeastern US have been exposed to long-term anthropogenic N deposition, which has altered soil properties and nutrient cycling processes (Fenn et al. 1998, Aber et al. 2003). Simultaneously, acid deposition has accelerated leaching loss of base cations (Likens et al. 1996, 1998, Lawrence et al. 1997, Bailey et al. 1996), promoting soil acidification and possibly altering soil P availability (Fiorentino et al. 2003). Biomass removal coupled with high NO_3^- leaching and increased potential for soil P immobilization when total plant nutrient uptake is low during forest regeneration (Fisk and Fahey 1991, Yanai 1992) can decrease N and P availability in these forests (Bormann and Likens 1979) and disrupt the N:P balance (Rastetter et al. 2013). The MEL model predicted a shift from N to P limitation as northern hardwood forests recover from harvesting disturbance, until recycling of N and P become stoichiometrically balanced (Rastetter et al. 2013). Hence, to better understand N-P co-limitation it is important to study nutrient dynamics at different stages of succession in a managed forest landscape comprised of multiple stand ages. Furthermore, management effects and changes with succession should be considered in the broader context of anthropogenic effects. The relatively recent history of anthropogenic N deposition coupled with slow mineral weathering of P could cause N enrichment relative to P and thereby induce limitation of productivity by P (Mohren et al. 1986, Aber et al. 1989, Stevens et al. 1993). In these forests, it appears that anthropogenic enrichment of N could lead to "transactional limitation" by P (Vitousek et al. 2010), wherein the slow transfer of P from primary mineral form into biotically recycling pools is insufficient to balance high N inputs.

A conclusive test of whether productivity in northern hardwood forests is limited by a single nutrient or is co-limited by N and P requires a large-scale, long-term ecosystem study evaluating forest response to a full factorial N x P treatment. We initiated a fertilization experiment in 13 northern hardwood forest stands in three sites in central New Hampshire in 2011. Our main goal was to test the hypothesis that northern hardwood forest productivity is co-limited by N and P, in which case we would expect relative growth rates (RGR) to increase in response to addition of N and P together more than to either nutrient alone. We asked the following questions:

(i) is tree growth co-limited by N and P or does single nutrient limitation by N or P prevail?

(ii) does the limiting nutrient differ with time since large-scale disturbance (forest harvest)?

(iii) does the RGR of different species respond to different nutrients?

Methods

We studied 13 northern hardwood forest stands at three different sites located on soils formed in glacial drift in central New Hampshire, USA (Table 1): nine at the Bartlett Experimental Forest (BEF) and two each at Hubbard Brook Experimental Forest (HBEF) and Jeffers Brook (JB). Climate in the study region is humid continental with mean temperatures of -9 ºC in January and 19 ºC in July (at 450 m elevation). The mean annual precipitation is approximately 140 cm evenly distributed throughout the year. During the study period mean July-August temperature was approximately 1 ºC higher than the long-term mean (1901-2000; NOAA) and mean July-August precipitation was 10 cm higher than the long-term mean (1901-2000; NOAA), consistent with long-term climate change in the region (Hamburg et al. 2013). The maximum inter-annual variability in climate during the study duration was 0.8 degrees C in temperature and approximately 6 cm in precipitation. Also, the summers in the years 2012 and 2014 were relatively dry.

Soils are Typic and Aquic Haplorthods overlying glacial drift (deposited approximately 14,000 years ago) originating primarily from granitic rock at BEF, granodiorite and schist at HBEF, and amphibolite at JB. The soils have thick surface organic horizons (~ 5 cm average depth; Vadeboncoeur et al. 2012a) and low pH, varying from 4.2 – 4.9 in the top 10 cm of the mineral soil depending on the stand (Ratliff and Fisk 2016). More detailed descriptions of soil chemistry from quantitative pits can be found in Vadeboncoeur et al. (2012a, 2014).

These 13 forests stands included three each of young (21-25 yr), mid-age (30-40 yr) and mature (>100 yr) forests at BEF and one mid-age and one mature each in HBEF and JB (Table 1). Forest age is given as the time between clear-cut harvest and the year 2011 when treatments began. Young, mid-age and mature forest ages were chosen to represent different stages of stand development in which nutrient demand might differ (Rastetter et al. 2013). Forest composition is typical of northern hardwood stands in each age class (Fig. 1). Mature forests were dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghniensis* Britton), with occasional white ash (*Fraxinus americana* L.), white birch (*Betula papyrifera* Marsh.) and red maple (*Acer rubrum* L.). Mid-age forests were generally dominated by white birch, yellow birch and American beech, followed by pin cherry (*Prunus pensylvanica* L.f.), red maple and bigtooth aspen (*Populus grandidentata* Michx.). Pin cherry, white birch and red maple dominated the young forests. Species composition of northern hardwood forests in this region varies owing to differences in native soil fertility associated with mineralogy and texture of glacial tills and variation in hydrology and soil development (Leak 1991), as well as to past management (Vadeboncoeur et al. 2012b). Stand basal areas and stem size distributions show typical successional changes during stand development (Fig. 2).

Four 50 x 50 m plots were established in each stand (except for two stands, HB-mid and JB-mid, where plots were 30 x 30 m, limited by the extent of the even-aged stand), and randomly assigned to control, N, P, and N+P fertilizer treatments. Fertilizer was applied to the entire 50 x 50 m plot; measurements were made in the inner 30 x 30 m area (20 x 20 m in the smaller plots). Nutrient additions began in spring 2011. N was added at a rate of 30 kg ha^{-1} year^{-1} as pelletized NH_4NO_3 and P was added at a rate of 10 kg ha^{-1} year^{-1} as powdered or granular NaH_2PO_4. N and P were applied twice (early June and mid-July) in the first three years and once (early June) annually thereafter. Fertilizer was pre-weighed for 2.5 x 10 m sub-plots and spread evenly by hand.

Fertilization successfully elevated *in-situ* resin-available N and P in 2011, the first year of treatment (Fisk et al. 2014), and we repeated this assay of soil nutrient availability in 2015. We incubated ion-exchange resin strips (2x6 cm) *in situ* during the last two weeks of July, 2015, approximately one month after fertilization, following the same method as Fisk et al. (2014). We used cation exchange resin strips (Ionics CR67-HMR; Maltz Sales) to quantify NH_4^+ and anion exchange resin strips (Ionics AR-204-SZRA; Maltz Sales) to quantify PO_4^{3-} and NO_3^-. Cation strips and anion strips for NO_3^- were prepared by rinsing in HCl and deionized (DI) H_2O followed by soaking in 1 M NaCl. Anion strips for PO_4^{3-} were prepared by alternating rinses in DI H_2O and 0.5 M $NaHCO_3$. All strips were rinsed with DI H_2O immediately prior to placement in the field. Eight strips per plot were deployed for each nutrient by inserting under the blade of a knife into the organic horizon at a 30°-45° angle from horizontal. Strips were retrieved after 14 days and rinsed in DI H_2O prior to extraction for nutrient analyses. Absorbed NO_3^- and NH_4^+ were extracted with 1 M KCl and PO_4^{3-} was extracted with 0.5 M HCl. Concentrations of NO_3^- and NH_4^+ in the extracts were quantified using an autoanalyzer (Quikchem 8500, Lachat Instrument). Concentrations of PO_4^{3-} were analyzed with the ammonium-molybdate-ascorbic acid method (Murphy and Riley 1962).

Leaf litterfall mass and nutrient concentrations were quantified in 2012 in all but one young and one mid-age stand and litterfall mass was also quantified in 2014. Leaf litter was collected in five litter traps (each with a collection area of 0.23 m^2) per plot, approximately weekly throughout the autumn. Litter mass was quantified after oven-drying to constant mass at 60°C. Litter was finely ground, ashed at 470°C, digested in 6M HNO_3, and P concentrations were analyzed using ICP-OES. Nitrogen concentrations were analyzed on a Flash 2000 NC soil analyzer (ThermoScientific).

Forest composition and basal area (Table 1) were quantified in all 52 plots in August of 2011 (in the first year of treatment) and in August 2015 four growing seasons later. All trees $\geq$10 cm diameter at breast height (DBH) were measured in each plot. Stems <10 cm DBH accounted for the majority of total basal area in young stands but the absence of repeated measurements on individually identified trees, combined with mortality during the study period, precluded estimation of relative growth rates in young trees belonging to this size class. We calculated the four-year stem basal area response (SBAR) of each live tree ($\geq$10.0 cm DBH) as $(SBA_P - SBA_I)/SBA_I$, where SBA_P = 2015 stem basal area and SBA_I = 2011 stem basal area. We annualized the relative growth rate (hereafter, RGR) of each $\geq$10.0 cm DBH stem using $((1+ SBAR)^{(1/n)} -1)$, where n = number of growing seasons of growth observed (4). We also estimated relative density of our stands, using species-specific equations developed by Ducey and Knapp (2010) for northeastern US mixed-species forests. Relative density of a stand is estimated based on stem numbers, diameters, and species-specific wood density, which is a functional trait that can affect forest live tree production (Ducey and Knapp 2010; Woodall et al. 2015). Hence, this estimate of relative density is intended to indicate the potential for further biomass accumulation in a forest stand.

Data analysis

For ecosystem-level analyses, treatment effects on plot-average RGR (for all trees) were tested using a linear mixed-effects model with treatment (N or P addition) and forest age as fixed effects and forest site (BEF, HBEF or JB) and stand (nested within forest site) as random effects, using the nlme package in R (Pinheiro et al. 2016). Forest stand was used as a unit of replication (n=13). We initially fit a full model that included forest age, N, P and their interactions as fixed effects but we did not detect age interactions with N or P; therefore, we excluded the age interaction terms in the final analysis. We used the Akaike Information Criterion (AIC; Bozdogan, 1987) to select the most appropriate model. We considered P values < 0.05 to be significant but also report trends for which P values were between 0.05-0.10. Treatment effects on RGR were qualitatively the same when tree data were analyzed using individual trees as the unit of replication (n= 2905). Nutrient limitation was inferred if RGR in treated plots exceeded that in control plots following N and P fertilization. A higher response to N+P added together than to either nutrient alone would indicate N and P co-limitation of aboveground growth. A statistically significant N x P interaction would indicate synergistic co-limitation, whereas a significantly higher response to N+P together than to either nutrient alone would indicate additive co-limitation. Additive co-limitation could be interpreted as sequential if one nutrient, but not the other, elicited a response when added separately.

We tested for ecosystem-level responses by using a two-way ANOVA to examine treatment effects on resin-available N and P, litter N and P, and average per-plot RGR. This factorial approach compares response variables in plots with N addition (i.e., N and N+P plots) to those with no N addition (i.e., control and P plots) and plots with P addition to those with no P addition, and also tests the interaction between N and P additions. We used post-hoc Tukey comparisons of least-squares means to test the differences between addition of N+P and either N or P alone.

Species-level analyses were conducted for species that occurred in more than 60% of the total 52 study plots, using the same approach as for the ecosystem-level analysis. Community-level co-limitation would be inferred if co-limitation was detected at the ecosystem (plot) level and species were limited by different nutrients. Species were also segregated by mycorrhizal association (AM: maples, cherries and ash or ECM: beech, birches, oak, basswood and conifers) to test for differences in growth rates between these two groups and in group response to treatment.

Results

Ecosystem-level responses

Treatments successfully elevated nutrient availability. Resin-available N in the organic soil horizon (forest floor) was higher in plots receiving N compared with those receiving no N (P=0.001) and resin-available P was higher in plots receiving P vs no P (P=0.02). There was no N x P interaction on resin-available N (P=0. 29) or P (P=0.88). Litter N concentrations were higher in plots receiving N compared with those receiving no N in 2012, after two years of treatment (P=0.01) and litter P concentrations were higher in plots with P vs no P (P<0.0001). There were no N x P or forest age interactions on litter nutrient concentrations.

Litterfall mass differed by forest age in 2012 (P=0.03) but not in 2014, and did not respond to treatments in either year ($P \geq 0.65$ in 2012; $P \geq 0.26$ in 2014) (Table 2). There were no N x P or forest age interactions for litter mass.

Across all 13 stands, RGR of trees ≥10.0 cm DBH responded to P but not to N fertilization (Table 3), with 7% greater RGR, on average, in plots receiving P vs no P (Table 4). The aboveground growth response to N+P was greater than that to N or P alone in six out of 13 stands, but was lower than that in response to N or P alone in four stands and was similar in the remaining three stands. We did not detect N-P co-limitation; there was not a significant N x P interaction (Table 3). The RGR in the N+P plots was not greater than that in P plots (*P*=0.80 for the comparison of least-square means), and the RGR was marginally greater in plots receiving N+P compared with those receiving N (*P*=0.08), consistent with a P effect.

RGR was highest in young, intermediate in mid-age, and lowest in mature forest stands (Fig. 3), consistent with the pattern of relative density, which averaged 0.27 in young, 0.71 in mid-age, and 0.88 in mature stands. Although there was not a significant interaction between forest age and N or P addition, responses to P differed among forest ages (Fig. 3A). RGR was higher in plots receiving P vs no P, by 13% in mid-age stands (*P*=0.02) and by 15% in mature stands (*P*=0.07), but was not higher in plots receiving P vs no P in young stands (-2%; *P*=0.53; Table 4). Addition of N did not affect RGR of trees in any age class ($P \geq 0.63$). In mid-age and mature stands, comparisons of the mean responses at BEF with those including all three sites indicate a slightly stronger productivity response to P at BEF than at HB or JB, and a stronger productivity response to N at HB and JB compared with BEF (Table 4).

The response of RGR to P addition was not universal, and although the response of RGR to N was not significant across all stands, there were stands in which N plots had higher RGR, especially in mature forest (Table 4). The lack of uniform responses within forest age classes (Table 4) suggests variation in the nutrient to which RGR responded.

Species-level responses

White birch, which is an ECM species, was the only species for which we detected significant responses to treatment (*P*= 0.02 for P and *P*= 0.91 for N, Table 3). Growth rates of white birch were greater, by 13%, in plots receiving P vs no P, but did not respond to N. The RGR of white birch differed among forest age classes (Table 3), and the nutrient to which growth responded changed with forest age. The RGR of white birch was 26% greater in plots receiving P vs no P in mid-age stands (Fig. 4A, *P*<0.001), but 17% greater in those receiving N vs no N in young stands (Fig. 4B, *P*=0.02). The RGR of white birch did not respond significantly to P in young stands.

For beech, another ECM species, we did not detect a RGR response to either nutrient across all stands (*P*= 0.14 for P and *P*= 0.57 for N, Table 3). However, RGR of beech was lower by 18% in plots receiving P compared to no P in young stands (*P*=0.06), and higher in the mid-age (15%; *P*=0.09), and mature stands (27%, *P*=0.03; Fig. 5A). In contrast, N fertilization did not affect beech RGR in any forest age class (Fig. 5B). Growth responses to treatments were not detected in any other species. Yellow birch and sugar maple, the two other dominant species in these forests, did not respond to either nutrient (Table 3).

The RGR differed among species (*P*< 0.001; Table 5), and between arbuscular mycorrhizal tree species and ectomycorrhizal tree species. The RGR of ECM tree species was slightly but significantly greater than those of the AM species across all of our stands (7%; *P* < 0.001) and the pattern of higher RGR by ECM species was consistent among all three age classes (Table 5). Growth rates of ECM species were 13% higher in plots receiving P compared to no P (*P*=0.06), and 4% higher in plots receiving N vs no N (*P*=0.08) across all stands.

Discussion

Ecosystem-level responses

We found evidence for P limitation of forest growth across a suite of northern hardwood forest stands in central NH, USA. In contrast, limitation by N was not detected, and the response to N+P addition together was not consistently greater than that to N or P alone. Phosphorus limitation of productivity on these relatively young, glacially derived forest soils was unexpected given the presence of apatite minerals as a P source in these soils (Blum et al. 2002). Apatite P in the B horizon at these sites is typically at least an order of magnitude greater than aboveground biomass P (Vadeboncoeur et al. 2014). In our forests, P limitation may result from historical anthropogenic N enrichment (Likens and Lambert 1998, Driscoll et al. 2003), which increases the supply of N relative to P. Although there is evidence that adding N can accelerate

P cycling by stimulating extracellular phosphatase enzyme activity (Olander and Vitousek 2000, Treseder and Vitousek 2001, Gress et al. 2007), inputs to P pools from mineral weathering or from very slowly recycling biotic pools may not be sufficient to maintain balance with high N inputs (Richter et al. 2006, Schaller et al. 2010, Vadeboncoeur et al. 2014). Such a nutritional imbalance owing to N enrichment may have alleviated N limitation of productivity in these ecosystems, inducing a transactional limitation of productivity by P (Vitousek et al. 2010).

The responses to P after four years of treatment were relatively modest (Fig. 3), even though the relatively low rates of fertilization in our factorial N x P experiment were successful in elevating soil nutrient availability and litterfall nutrient cycling (Table 2). The lack of strong overall response to P was partly a consequence of stand age. Mature forest RGR responded relatively weakly to P, consistent with high relative density (0.88); fully-stocked stands would be expected to be limited more by light than by nutrients. It is possible that fertilization enhanced canopy expansion more than diameter growth, but the lack of litterfall response (Table 2) suggests that any canopy response was small. The most marked increase in aboveground growth in response to P addition occurred in the mid-age forests, where the lower relative density (0.71) suggests greater potential for growth and, therefore, nutrient limitation. P limitation in the mid-age stands at BEF is consistent with higher root growth in response to localized P additions in the same stands (Naples and Fisk 2010).

In young stands, the average RGR suggested N rather than P limitation of growth, but growth was more variable and significant effects were not detected. The successional transition in the young forests included high mortality of both pin cherry and beech, which probably contributed to the high variation in RGR among species. Furthermore, the contrasting nutrient responses by white birch (positive response to N and slightly positive response to P, Fig. 4B) and beech (negative response to both N and P, Fig. 5A) in the young stands complicated the overall response. The relatively low number of stems >10 cm DBH in these young stands also constrained our ability to detect significant treatment effects.

Our results also show that nutrient limitation may be variable in forests of this region. Among the mid-age and mature forests, broad-scale spatial variation in the response to fertilization contributed to the relatively weak overall effects of P (Table 4). This variation must be interpreted with caution because of the lack of within-stand replication; however, these results are consistent with variation in nutrient limitation among stands as assessed by nutrient-amended root ingrowth cores (Naples and Fisk 2010). While P limitation appears most common across all but the youngest stands in our study, the variation across stands suggests that these forests are close to the threshold between P and N limitation, especially in mature forests.

If nutrient limitation in these forests is indeed relatively close to a state of either N or P limitation, it is not clear why we did not find more obvious evidence of N and P co-limitation of aboveground productivity. The lack of a significant N x P interaction does not support synergistic co-limitation, and the lack of a greater response to N+P addition together than to P alone suggests that there was not additive co-limitation. In the absence of a significant growth response to N alone, the slightly higher RGRs (P=0.08) that were observed when N+P were added together compared to N alone suggests either a primary effect of P, or weak evidence for sequential P followed by N limitation. It is possible that alleviation of limitation by one nutrient induces plant allocation responses, such as changes in fine root growth and mycorrhizal colonization, that will take time to develop before inducing secondary limitation by the other nutrient. If so, this sequential co-limitation response should be evident in future divergence of productivity between fertilization with single nutrients and N+P together.

Species-level responses

Species-level effects are potentially important for interpreting nutrient limitation of productivity. Within an ecosystem, community composition can be driven by limiting nutrients (John et al. 2007), and conversely, community composition can influence the availability or use of limiting nutrients via the traits of individual species (Chapin and Shaver 1985, Chapin et al. 1986, 1987). For example, in some tropical forests, variation in nutrient use and uptake among tree species can cause the primary limiting nutrient to differ among species, thereby contributing to community-level co-limitation of productivity by N and P (Gehring et al. 1999, Menge et al. 2008, Baribault et al. 2012, Waring et al. 2015). Northern hardwood forests are moderately diverse (Lovett et al. 2004), and component species differ in tissue nutrient ratios and nutrient acquisition and conservation strategies (Schwarz et al. 2003, Bigelow and Canham 2007, See et al. 2015), and are segregated along axes of soil nutrient availability (Finzi et al. 1998). We did not find that differences in life history traits associated with successional status corresponded with differences in limiting nutrients, as both white birch (early successional, rapid growth) and beech (late successional, slow growth), responded to P. However, mycorrhizal type may have mediated nutrient responses, as both of the species that responded to P are ECM. RGR was high for all ECM species, but varied more among AM species (Table 3). Growth rates of ECM species were 13% higher in plots receiving P compared to no P (P=0.06), and 4% higher in plots receiving N vs no N (P=0.08) across all stands. ECM fungi tend to specialize in N acquisition (Smith and Read 2008), with substantial C cost to the host plant (Hobbie 2006), whereas AM fungi, which lack extracellular enzyme production for decomposition, are associated with conditions of higher soil NO_3^- availability (Phillips et al. 2013), and may be more essential for P acquisition. Hence, if ECM species more effectively acquire N when P availability is enhanced, then AM species may be more prone to secondary N limitation under these conditions. Alleviating N limitation is generally known to shift functional groups (Lilleskov et al. 2012), and can reduce ECM colonization, although responses vary among ecosystems (Treseder 2004). Any fertilizer-induced decline in mycorrhizal C costs are likely to allow re-allocation to aboveground growth more for ECM than for AM tree species, but less is known about colonization or functional group responses to P availability in forest systems. Our results indicate the need to evaluate ECM species responses under P limiting conditions, and also to examine species-level nutritional responses to better evaluate these mechanisms.

One exception to the greater RGR response to P was the response of white birch to N in the youngest stands (Fig. 4B). Enhanced growth by this species in response to N contributed to the slightly higher average RGR response to N for all species combined in the young forests

(Fig. 3B). Increased growth rates in response to N addition were consistent with pre-treatment observations of high N resorption by white birch foliage in young stands (See et al. 2015). Thus, white birch appeared to be limited primarily by N at age 20-25 yr and by P at age 30-40 yr, suggesting the possibility of a shift in nutrient limitation early in succession, which provides tentative support for predictions of the MEL model of N limitation in early stages of recovery post-harvest (Rastetter et al. 2013). The competition for canopy dominance in young forests promotes aboveground allocation in early successional, shade-intolerant species such as white birch and pin cherry (Fahey et al. 1998). Our results also suggest a shift between belowground allocation for acquisition of N vs P, with a relatively high demand for N to support aboveground growth during early succession, followed by an increase in P limitation as early successional species decline and forests transition to middle age with higher relative density and higher relative importance of slower-growing species such as beech and maple.

The response by beech differed between young and mid-age forests, in this case from a negative (but non-significant) to a significantly positive effect of P on growth (Fig. 5A). Beech is a relatively slow-growing, shade-tolerant, “resource-conservative” species and could therefore be expected to be less responsive to changes in nutrient availability (Eskilinen et al. 2015). The negative response of beech to P fertilization in young forests could arise from the faster-growing species out-competing beech for light under conditions of higher nutrient availability. In the mid-age stands, as early successional species declined (Fig. 1), the trend toward increased beech growth response to both N and P additions (Fig. 5) raises the possibility that this species is co-limited in the mid-age forests. While it may be surprising that a species with conservative traits such as beech showed the strongest response to P addition in mature forests, this pattern is consistent with pre-treatment observations of greater P conservation by beech via foliar resorption compared to other species in mature stands (See et al. 2015).

Conclusions

After four years of fertilizing thirteen northern hardwood stands of varying ages, we found evidence for P limitation in most mid-age and mature stands but no clear indication of widespread N limitation or N x P co-limitation. It is possible that P limitation of productivity has developed as a consequence of the legacy of anthropogenic N deposition in this region, consistent with the idea of anthropogenically induced transactional P limitation (Vitousek et al. 2010). At the same time, the broad extent of our study reveals important spatial variation in P vs N limitation, which cautions against generalizing about regional nutrient limitation. Observing longer-term responses to our treatments will be valuable for assessing whether sequential co-limitation develops as P limitation is alleviated by treatment and biotic demand for N increases. Species-specific responses were generally consistent with whole-ecosystem responses, but highlighted interesting shifts in limitation by N vs P as young forests mature.

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Tables and Figures

Table 1. Characteristics of 13 northern hardwood forest stands in central New Hampshire, USA.

Stand	Forest age	Year clearcut	Elevation	Aspect	Slope (%)	Pre-treatment (2011) basal area (m^2/ha)
BEF-C1	Young	1990	570	SE	5-20	25.1
BEF-C2	Young	1988	340	NE	15-30	23.4
BEF-C3	Young	~1982-1985	590	NNE	8-20	30.4
BEF-C4	Mid-age	1979	410	NE	20-25	32.4
BEF-C5	Mid-age	1976	550	NW	20-30	27.2
BEF-C6	Mid-age	1975	460	NNW	13-20	29.9
BEF-C7	Mature	1890	440	ENE	5-10	32.1
BEF-C8	Mature	1883	330	NE	5-35	34.6
BEF-C9	Mature	1890	440	NE	10-35	32.7
HB-mid	Mid-age	1970	500	S	10-25	28.9
HB-mature	Mature	1911	500	S	25-35	33.9
JB-mid	Mid-age	~1975	730	WNW	25-35	27.9
JB-mature	Mature	1915	730	WNW	30-40	35.6

Table 2. Resin-available N and P in 2015. litterfall N and P concentrations in 2012, and litterfall mass in 2012 and 2014 (standard errors of the mean are in parentheses; *n*=13 for resin-available nutrients and *n*=11 for litterfall).

	Resin- N (µg strip^{-1} 14d^{-1})	Resin- P (µg strip^{-1} 14d^{-1})	Litterfall N (mg/g)	Litterfall P (mg/g)	Litterfall mass (g/m^2)	
					2012	2014
Control	84.4 (28.4)	7.2 (1.7)	14.5 (0.6)	0.46 (0.03)	300 (8)	281 (8)
N	257.6 (70.6)	9.0 (2.7)	15.5 (0.6)	0.48 (0.29)	296 (9)	271 (15)
P	24.8 (34.1)	14.7 (2.6)	13.9 (0.5)	0.60 (0.03)	287 (10)	272 (13)
N+P	123.7 (34.1)	17.3 (3.5)	14.7 (0.6)	0.55 (0.03)	294 (13)	269 (14)

Table 3. Ecosystem- (all species) and individual species-level results (F-values and significance) of mixed effects model showing the main effects of nutrient addition on RGR (%/year) of ≥ 10 cm DBH trees across stands.

Predictor	Degrees of freedom	F-value	*P* value
Ecosystem-level RGR			
Forest age	2, 8	39.83	**≤0.001**
N	1, 36	0.23	0.73
P	1, 36	9.07	**0.02**
N x P	1, 36	0.09	0.67
White birch RGR			
Forest age	1, 4	31.22	**≤0.01**
N	1, 21	0.003	0.91
P	1, 21	8.45	**0.02**
N x P	1, 21	0.80	0.28
Beech RGR			
Forest age	2, 5	23.16	**≤0.01**
N	1, 24	0.11	0.57
P	1, 24	0.23	0.14
N x P	1, 24	0.09	0.32
Sugar maple RGR			
Forest age	2, 5	7.10	**0.03**
N	1, 27	0.67	0.42
P	1, 27	0.90	0.35
N x P	1, 27	0.49	0.49
Yellow birch RGR			
Forest age	2, 6	11.56	**<0.01**
N	1, 27	0.12	0.73
P	1, 27	0.19	0.67
N x P	1, 27	0.35	0.56

Note: Significant *P* values are indicated in bold

Table 4. Percent differences in RGR between plots receiving and plots not receiving the nutrient (i.e., N vs no N) in each stand, and means in all stands and in the BEF. Standard errors of the mean are in parentheses.

	Percent difference in RGR	
	Plots receiving N vs no N	Plots receiving P vs no P
Young		
BEF-C1	12.03	-3.69
BEF-C2	8.22	0.60
BEF-C3	-3.81	-1.54
Mid-age		
BEF-C4	7.74	15.49
BEF-C5	-10.00	31.40
BEF-C6	3.82	14.69
HB-mid	24.47	16.54
JB-mid	3.24	-3.94
Mature		
BEF-C7	-21.52	19.45
BEF-C8	3.32	8.16
BEF-C9	20.63	21.88
HB-mature	4.97	14.91
JB-mature	39.21	10.20
	All sites	
All stands	7.1 (4.3)	11.1 (3.0)
Young (*n*=3)	5.5 (4.8)	-1.5 (1.2)
Mid-age (*n*=5)	5.8 (5.5)	14.8 (5.6)
Mature (*n*=5)	9.3 (10.1)	14.9 (2.6)
	BEF site only	
All stands	2.3 (4.2)	11.8 (3.9)
Young (*n*=3)	5.5 (4.8)	-1.5 (1.2)
Mid-age (*n*=3)	0.5 (5.4)	20.5 (5.4)
Mature (*n*=3)	0.8 (12.2)	16.5 (4.2)

Table 5. The type of mycorrhizal association and the mean relative growth rate of the major tree species in young, mid-age and mature northern hardwood forest stands after four years of nutrient fertilization.

Species	Representation (number of plots out of 52 possible)	Relative growth rate (%/year)		
Arbuscular-mycorrhizal species		Young	Mid-age	Mature
Sugar maple (*Acer saccharum* Marsh.)	41	3.9	3.6	1.1
Red maple (*Acer rubrum* L.)	29	6.7	4.3	1.9
Pin cherry (*Prunus pensylvanica* L.f.)	29	3.6	1.6	-
White Ash (*Fraxinus americana* L.)	13	11.2	5.7	2.0
Striped maple (*Acer pennsylvanicum* L.)	10	4.1	1.4	-
Ectomycorrhizal species				
American beech (*Fagus grandifolia* Ehrh.)	44	7.2	5.4	2.6
Yellow birch (*Betula alleghniensis* Britton.)	42	6.6	4.6	2.1
White birch (*Betula papyrifera* Marsh.)	32	8.2	3.4	-
Eastern hemlock (*Tsuga canadensis* (L.) Carriere)	13	4.8	1.9	2.7

Note: Species present in less than 10 out of 52 plots are not presented here.

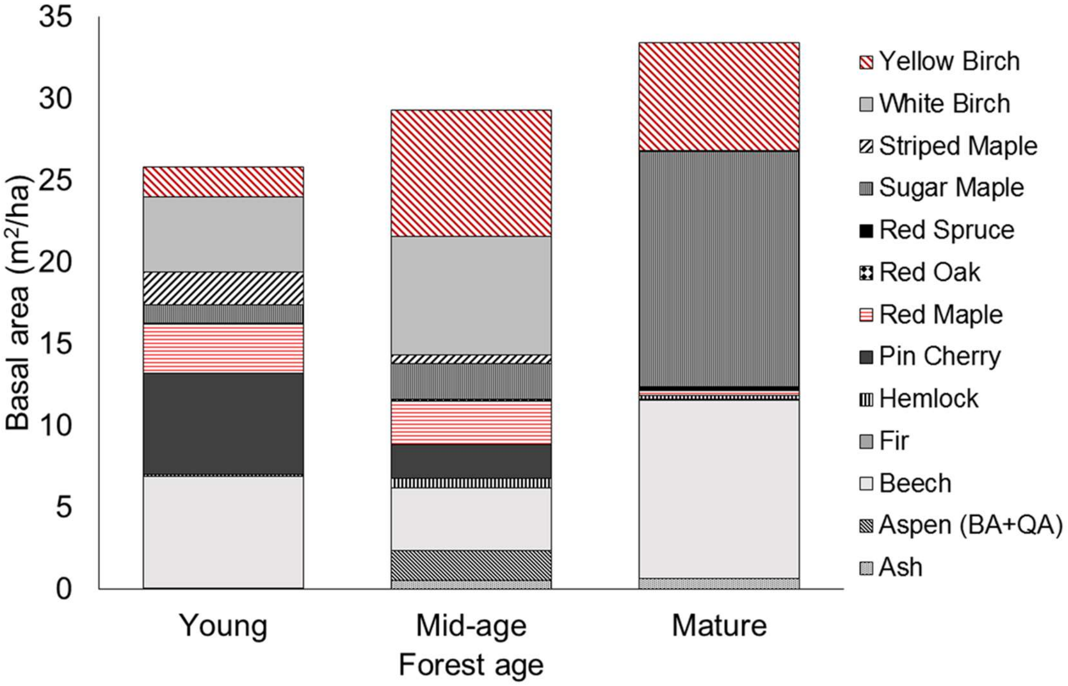

Fig 1. Basal area of live trees by species in 2011 in young, mid-age and mature forest stands. Note: Bigtooth aspen and quaking aspen have been combined.

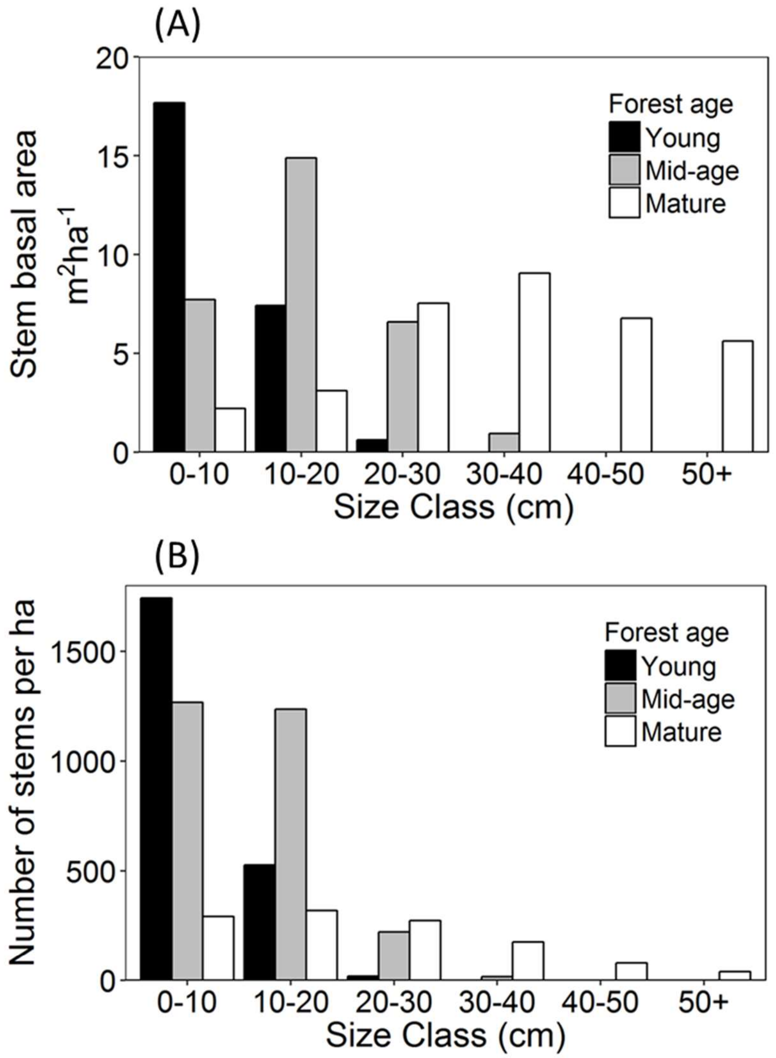

Fig 2. Basal area (panel A) and number of stems/ha (panel B) of live trees ≥10 cm DBH by size class in young, mid-age and mature forest stands.

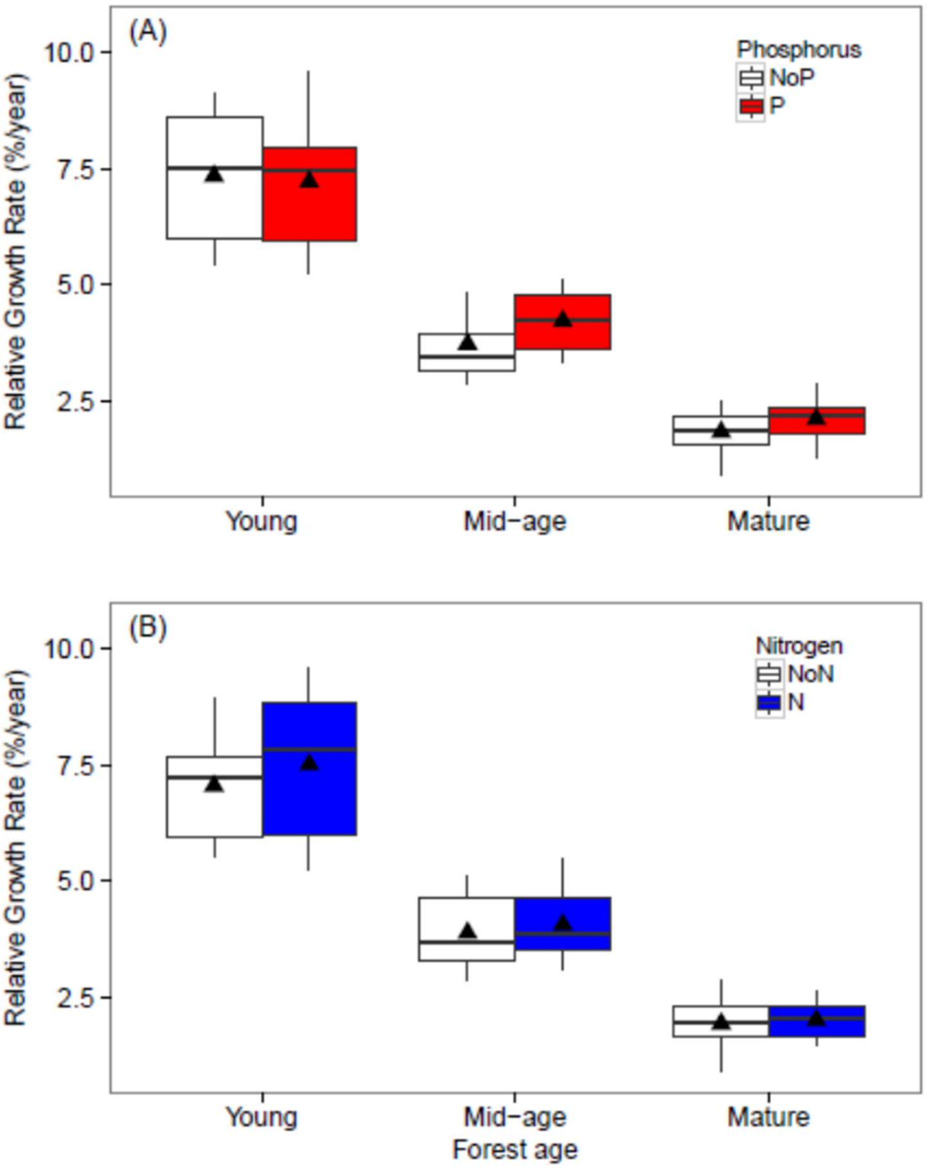

Fig 3. Relative growth rate (per tree) of live ≥10 cm DBH trees in plots receiving P vs no P (panel A) and N vs no N (panel B) in young, mid-age and mature forest stands. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

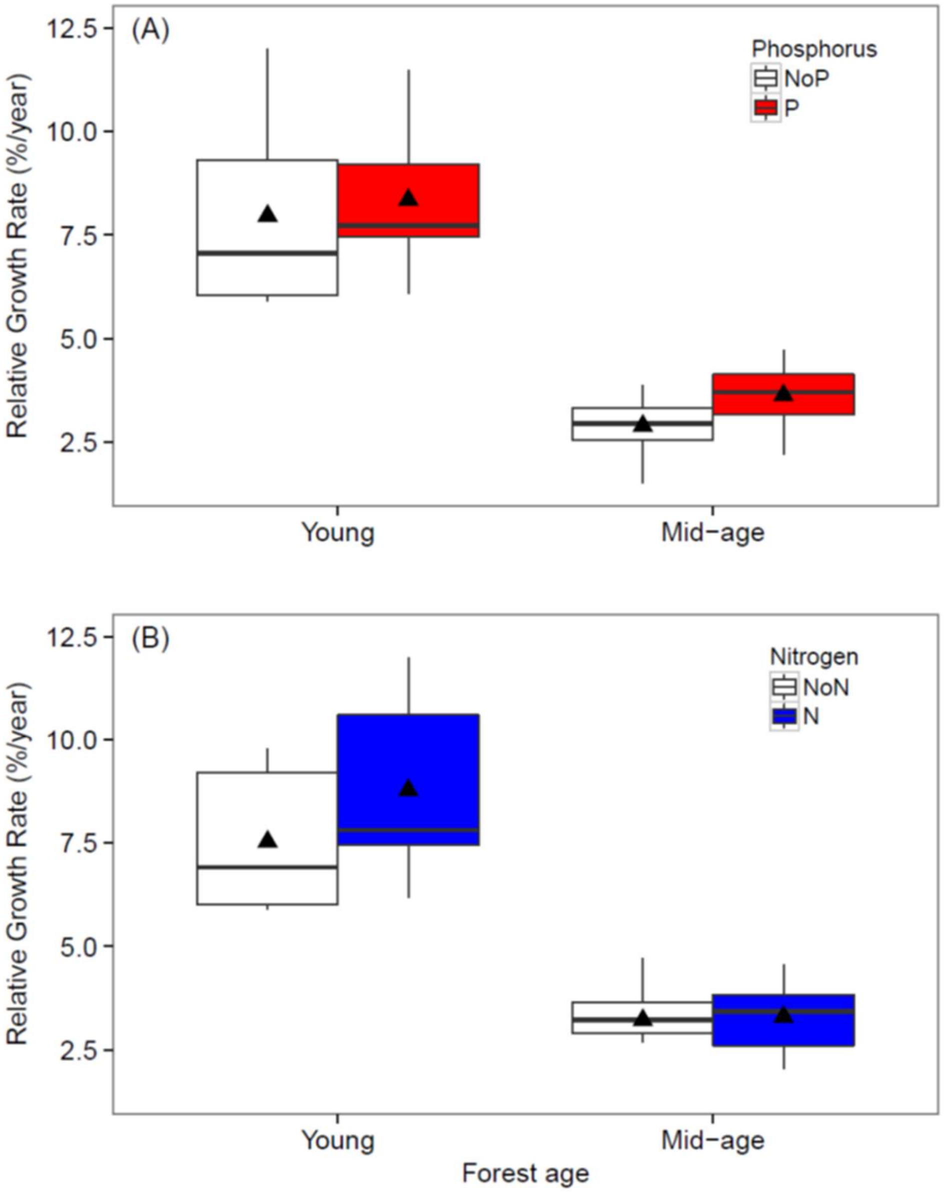

Fig 4. Relative growth rate (per tree) of live ≥10 cm DBH white birch trees in plots receiving P vs no P (panel A) and N vs no N (panel B) in young and mid-age forest stands. Boxes represent the interquartile range (25-75%) and whiskers represent the 5 and 95%, with the horizontal line as the median and the triangle as the mean.

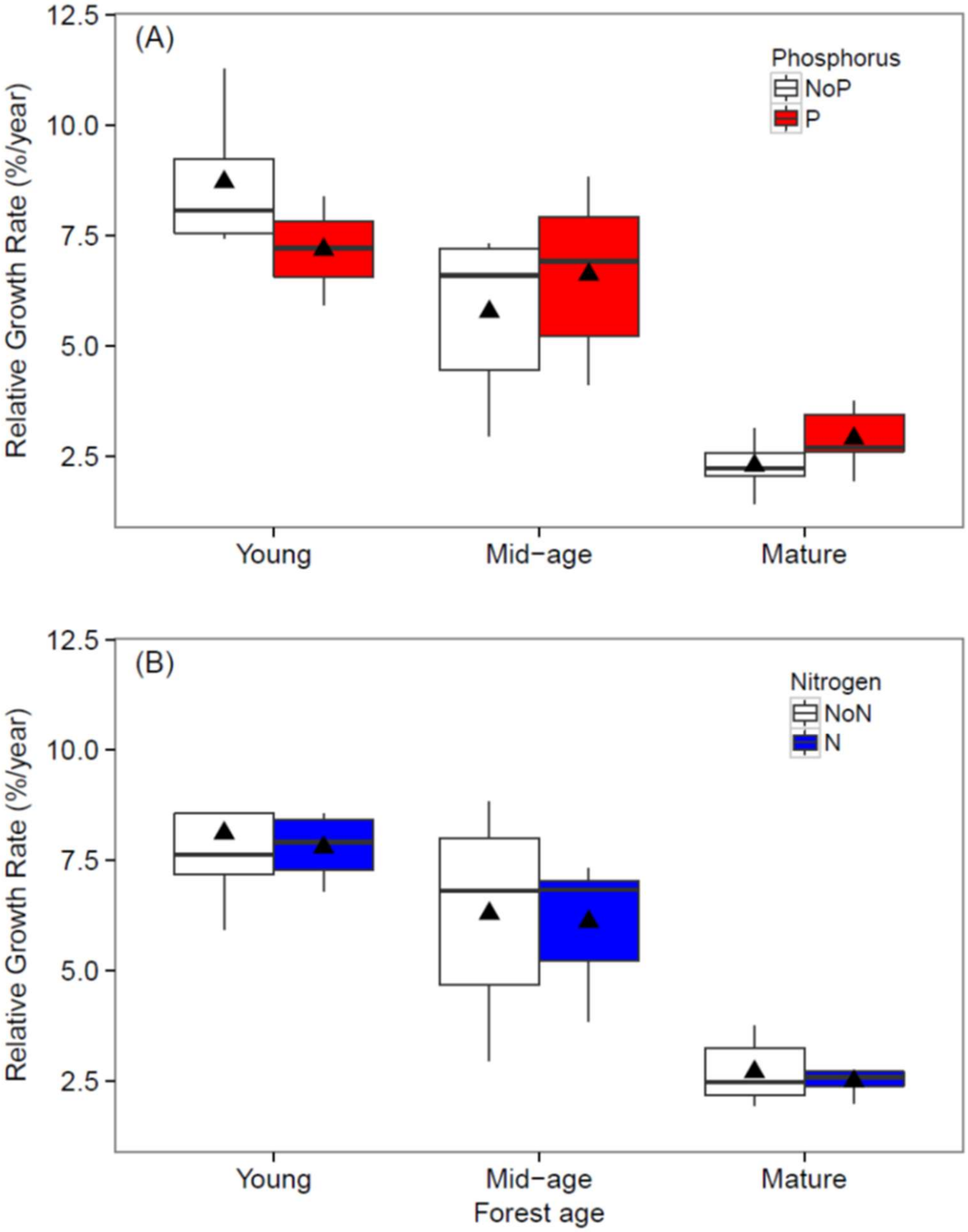

Fig 5. Relative growth rate (per tree) of live ≥10 cm DBH beech trees in plots receiving P vs no P (panel A) and N vs no N (panel B) in young, mid-age and mature forest stands. Boxes represent the interquartile range (25-75%) and whiskers represent the 5 and 95%, with the horizontal line as the median and the triangle as the mean.

Chapter 2

Phosphorus reduces nitrogen availability in northern hardwood forests

Abstract

Uptake and recycling by plants and soil microorganisms exert control on the stoichiometry of available nutrients, potentially influencing ecosystem responses to nitrogen enrichment and other perturbations that alter resource availability. We tested whether an excess of one nutrient influenced the availability of another, to learn the net outcome of various feedbacks on nutrient mineralization and biotic uptake processes, in a factorial nitrogen (N) x phosphorus (P) fertilization study in northern hardwood forest ecosystems. We examined relative availabilities of soil N and P (resin-available inorganic N and P and potentially-mineralizable N and P) in nine northern hardwood forest stands over five years of fertilizing with 30 kg/ha N, 10 kg/ha P, or N and P together. Fertilizing with N increased N availability but had no effect on P availability. Fertilizing with P increased P availability and decreased the availability of N, especially when P was added in combination with N. This effect of P on N availability could arise from increased tree growth and uptake of N but is most likely a response to elevated soil microbial uptake, and suggests that plant and soil processes can feedback and alter soil nutrient availability and recycling. That P interacts with N to reduce N availability, by whatever mechanism, could help explain observations of N and P co-limitation in ecosystems and calls attention to the need to carefully elucidate mechanisms underlying co-limitation of forest productivity.

Introduction

Over time, we expect recycling of different nutrient elements to become synchronized to provide available nutrients in ratios consistent with biotic demand (Field 1995; Rastetter et al. 2013). Synchronization of nutrient recycling occurs over time in ecosystems when processes mediating mineralization of nutrients interact with the fates of those nutrients. These fates include losses from the ecosystem in gaseous or solution form, losses from recycling pools in soil-fixed forms, and biotic uptake followed by sequestration or recycling in organic forms. These fates could all be constrained by longer-term changes (inputs and losses; Fig 1.) for example, accumulation of soil nitrogen (N) through atmospheric/biological fixation or decline in soil phosphorus (P) through weathering and soil fixation processes (Walker and Syers 1976; Vitousek and Farrington 1997) or factors like denitrification losses and post-disturbance hydrologic nutrient exports (Federer et al. 1989). Hence, the total pools of potentially-available organic N and P (pool of microbes and organic matter; Fig 1.) that mediate nutrient availability develop over time in response to the balance between ecosystem inputs and losses. However, shorter-term biotic controls of recycling processes also exist, that respond to biotic demands and can drive nutrient availability (red arrows; Fig 1.). These controls can influence the available pool of inorganic nutrients (resin-available inorganic N and P; Fig 1.), and experience different feedbacks that both accelerate (Vitousek 2004) and suppress availability (Fisk and Fahey 2001) of N and P. Over time, such short-term biological feedbacks could lead to nutrient redistribution and therefore, modify interactions between N and P availability.

Northern hardwood forests soils are characterized by heterogeneity in nutrient availabilities at different temporal and spatial scales. On the long-term, as these soils age, we expect N availability to be constrained by the balance between inputs and losses, and potentially-available P pools to be adequate to avoid P limitation (Walker and Syers 1976). At shorter time scales, anthropogenic disturbances associated with forest harvest, N deposition, CO_2 enrichment, and changing climate influence nutrient availability. Furthermore, less intense disturbances for example, inter-annual climate variability, insect defoliations, or ice storms could also disrupt the N:P balance (Lovett et al. 2002; Houlton et al. 2003; Rastetter et al. 2013). While abundant research addresses the effects of these types of disturbances on single-element recycling, it is not clear how effects on multiple elements alter the balance in availability of nutrients. Simulations with the Multiple Element Limitation model (Rastetter el al 2013) suggest a shift from N to P limitation to N-P co-limitation of productivity in northern hardwood ecosystems as forests undergo successional change. The historic N deposition could result into greater P deficiency relative to N, and force P limitation on these ecosystems as the accumulation of N relieves N limitation (Vitousek et al. 2010; Rastetter et al. 2013). Therefore, the idea of balanced nutrition should be considered at multiple time scales to evaluate short-term vs longer-term biogeochemical processes, and whether such short-term processes could mediate outcomes related to long-term changes.

Frequent short-term changes in the environment might prevent balanced recycling of N and P. The rate at which the various soil processes (such as nutrient mineralization and uptake) vs plant processes (shifts in allocation by trees) adjust relative to the frequencies of change in their driving factors, influence the relative availabilities of N and P. The driving factors could operate through natural variation in the ecosystem environment or via natural (such as ice storms) and anthropogenic disturbances at short- (nutrient fertilization), intermediate- (progression of succession following forest harvest) and longer time scales (N deposition). Mechanisms that promote short-term processes are resource allocation and changes in soil microbial stoichiometry. The concept of resource optimization suggests that organisms allocate resources to maximize the acquisition of the most limiting resource (Bloom et al. 1985, Chapin et al. 1987, 2002). Plants can adjust relative allocation above- vs belowground to respond to the co-limitation between resources like, light and nutrients (Fahey et al. 1998). Likewise, resource allocation by soil microbes is facilitated via enzyme production (via decomposition and nutrient mineralization processes; Asmar et al, 1994; Sinsabaugh et al, 1994; Allison et al. 2011) in order to maintain balance among the relative availabilities of carbon (C), N and P. Soil microbes require organic C for growth (Powlson et al. 1987; Smith & Paul 1990; Wardle 1992); however, in soils with abundant C availability, nutrients limit microbial growth and activity (Melillo et al. 1982; Dilly 2001; Demoling et al, 2007; Milcu et al. 2011; Reed et al, 2011). A recent comprehensive study of microbial biomass across multiple ecosystems suggests that N limits microbial biomass synthesis whereas P limits metabolism (Hartman and Richardson 2013). Therefore, nutrient requirements of soil microbes could act as a buffer between plants and soil, promoting rapid short-term interactions that influence the balance between N and P. However, the extent to which resource allocation can balance the acquisition of most limiting nutrients is unclear and requires further investigation.

It is also important to consider short-term processes because nutrient imbalances could lead to losses of one nutrient when availability of the other is constrained, or losses of one nutrient when the other is present in excess. Short-term processes contributed to changes in nutrient limitation over successional time in MEL model simulations (Rastetter et al. 2013). Shorter-term interactions between N and P availability can be tested using nutrient fertilization and could illustrate that adding one nutrient could alleviate its own limitation to microbes and increase the microbial demand for the other nutrient, therefore reducing the availability of this other nutrient by promoting its biotic uptake. In this study, we addressed successional change in the balance of N and P availability. Within this context, we focused on the outcome of short-term processes to learn effects of one nutrient on the availability of the other for either loss or biotic uptake. This is illustrated by Fig 1. (tan arrows), which indicates how processes mediate availability (mineralization) and the net outcome of balance between mineralization and uptake (resin-available inorganic nutrients) in response to N and P fertilization. We manipulated nutrients via factorial N x P fertilization to explore patterns of nutrient interactions, as part of efforts to understand nutrient limitation in northern hardwoods (Fisk et al. 2014; Goswami et al. 2017). We asked the following questions:

i. Does the relative availability of N and P change through succession?

ii. What is the effect of excess of one nutrient on availability of the other?

Methods

Study system and experimental design

We studied nine northern hardwood forest stands at the Bartlett Experimental Forest (BEF; elevation 250-500 m) located on soils formed in glacial drift in central New Hampshire, USA. Climate in the study region is humid continental with the mean temperatures of -9 ºC in (January) and 19 ºC in (July) (at 450 m elevation). The mean annual precipitation is approximately 140 cm evenly distributed throughout the year. Soils are Typic and Aquic Haplorthods overlying glacial drift originating primarily from granitic rock. The soils have thick surface organic horizons and low pH, varying from 4.2 – 4.9 in the top 10 cm of the mineral soil in the different forest stands (Ratliff and Fisk 2016). These nine forests stands included three each of young (21-25 yr), mid-age (30-40 yr) and mature (>100 yr) forests (Table 1). Forest age is given as the time between clear-cut harvest and the year 2011 when treatments began. Young, mid-age and mature forest ages were chosen to represent different stages of stand development in which nutrient demand might differ (Rastetter et al. 2013).

Forest composition is typical of northern hardwood stands in each age class, mature forests having an overstory dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghniensis* Britton), with occasional white ash (*Fraxinus americana* L.), white birch (*Betula papyrifera* Marsh.) and red maple (*Acer rubrum* L.). Mid-age forests were generally dominated by white birch, yellow birch, American beech, followed by pin cherry (*Prunus pensylvanica* L.f.), red maple and bigtooth aspen (*Populus grandidentata* Michx.). Pin cherry, white birch and red maple dominated the young forests. Species composition of northern hardwood forests in this region vary owing to differences in native soil fertility associated with mineralogy and texture of glacial tills and variation in hydrology and soil development (Leak 1991), as well as to past management (Vadeboncoeur et al., 2012).

Four 50 x 50 m plots were established in each stand, and randomly assigned to control, N, P, and N+P fertilizer treatments. Fertilizer was applied to the entire 50 x 50 m plot; measurements were made in the inner 30 x 30 m area. Nutrient additions began in spring 2011. N was added at a rate of 30 kg ha^{-1} $year^{-1}$ as pelletized NH_4NO_3 and P was added at a rate of 10 kg ha^{-1} $year^{-1}$ as powdered or granulated NaH_2PO_4. N and P amendments were applied twice (early June and mid-July) in the first three years and once (early June) annually thereafter. Fertilizer was pre-weighed for 2.5 x 10 m sub-plots and spread evenly by hand.

Post-treatment inorganic soil N and P availability (soil solution pool of N and P, Fig 1.) were tested using ion-exchange resin strips incubated in the organic horizon for two weeks in summer of year-one (2011), year-two (2012), year-four (2014) and year-five (2015) in six out of nine forest stands. These six forests stands included two each of young, mid-age and mature stands. The annual sampling period of July was chosen to be as close as possible to the peak period of N mineralization in northern hardwoods (Bohlen et al. 2001), and to allow enough time for fertilizers to dissolve and be incorporated into soil following application. These strips adsorb ions from soil solution as they come in contact with the surface of the resins in the strip, therefore, we assumed that the extracted pools of resin-available N and P would be similar to the available N and P in soil solution that are used by plant roots and microbes.

We used anion exchange resin strips (Ionics AR-204-SZRA; Maltz Sales) to quantify available PO_4^- and NO_3^-, and cation exchange resin strips (Ionics CR67-HMR; Maltz Sales) to quantify available NH_4^+ in soils. Resin strips (2 x 6 cm) were prepared by rinsing in weak HCl and DI H2O and then soaking in 1M NaCl (cation strips and anion strips for NO_3^-) or alternating rinses in DI H2O and 0.5M $NaHCO_3$ (anion strips for PO_4^-). All strips were rinsed with DI H_2O immediately prior to placement in the field. We deployed resin strips by inserting them under the blade of a knife used to cut the surface organic horizon at a 30 - 45° angle. Two strips per soil subplot, making a total of eight strips per plot were deployed for each nutrient. Strips were retrieved after 14 days and rinsed in deionized H_2O prior to extraction for nutrient analyses. Anion exchange resins were extracted by shaking rinsed strips in 30 mL of 1M KCl for NO_3^- and 30 mL of 0.5M HCl for PO_4^- for 1 hour each. Cation strips were extracted by shaking rinsed strips in 30 mL of 1M KCl for 1 hour. We used a phenolate-hypochlorite method to quantify NH_4^+ (method 351.2, US EPA 1983) and a cadmium reduction method to quantify NO_3^- (method 353.2, US EPA 1983) in extracts. Extract PO_4^- (henceforth resin-P) concentration was analysed by the ammonium-molybdate-ascorbic acid method (Murphy and Riley, 1962).

In addition to resin-available soil N and P, net N mineralization potentials and bicarbonate-extractable P were used as indices of potentially available N and P, and C mineralization (microbial respiration) was used as an index of microbial activity. These were analyzed on soil samples collected from all four plots per stand in all nine stands in the first week of July 2014. For each plot, we extracted 3-4 soil cores (2 cm diameter) in each of the four (5 x 5 m) soil sampling subplots, to a depth of 10 cm in the mineral horizon. We separated Oe, Oa, and mineral horizons by applying visual criteria in the field. Decaying plant matter that appeared fibrous and reddish- to dark-brown in color was considered Oe horizon and non-fibrous amorphous organic matter that was dark to black in color, with occasional mineral flecks, was identified as Oa horizon. The remaining soil sample was identified as mineral horizon. Samples from the four subplots were composited by horizons within each plot. Each composited sample was homogenized and inorganic N was extracted from an initial subsample (t_i) within a day of collection and from a final subsample (t_f) after 21 days of incubation in the laboratory at 18-20 ^{o}C. Subsamples were shaken in 2 M KCl and extracts were filtered through Whatman #1 qualitative grade paper and stored at 4°C until analysis. We used a phenolate–hypochlorite method to quantify NH_4^+ (method 351.2, US EPA 1983) and a cadmium reduction method to quantify NO_3^- (method 353.2, US EPA 1983) in extracts. Net N mineralization (henceforth N_{min}) was estimated as the difference in KCl-extractable NH_4^+ and NO_3^- between initial and final incubated soil subsamples. We also estimated C mineralization (henceforth C_{min}) by quantifying

CO_2 evolved from incubated soils throughout the 21 day incubation period. Glass scintillation vials containing 10 mL of 0.1 M NaOH were sealed in jars and replaced at 2 to 6 day intervals. The NaOH in these vials was titrated in the presence of a saturating concentration of $BaCl_2$ to quantify the amount of CO_2 that reacted with NaOH. Bicarbonate-extractable P (henceforth Bic-P) was measured by extracting a subsample of each soil sample in 0.5 M $NaHCO_3$ (pH 8.5) for 30 min, filtering through Whatman #2 qualitative grade paper, digesting filtrate with persulfate oxidation (method 365.1, US EPA, 1983) and analyzing total dissolved P using the ammonium-molybdate-ascorbic acid method (Murphy and Riley, 1962).

Data analysis

We tested whether the balance of N and P availability changed over forest succession independent of treatment using a linear mixed effects model in the nlme package in R (Pinheiro et al. 2016) on resin-available N:P in control plots only for all nine stands, with forest age (young, mid-age or mature) as a fixed effect and forest stand as a random effect. Treatment effects on resin-available soil parameters (NO_3^-, NH_4^+, total inorganic N ($NO_3^- + NH_4^+$), resin-P and N:P) were tested using a linear mixed effects model in the nlme package in R (Pinheiro et al. 2016), with treatment (N or P addition) and year as fixed effects and forest stand as a random effect. Forest stand was the unit of replication (*n*= six). Resin-available soil parameters were log-transformed prior to running the temporal analysis to meet the assumptions of normality of the residuals. We initially fit full models that included N, P, year, forest age as fixed effects (main effects + interactions) but the full models had higher Akaike Information Criterion (AIC with a correction for finite sample sizes, AICc; Bozdogan, 1987); therefore, we excluded the factor forest age and the subsequent interaction terms in the final analysis to select the appropriate models (having the lowest AICc value) for resin-available soil parameters. Similar to resin-available parameters, treatment effects on net N_{min}, C_{min}, N_{min}:C_{min} and Bic-P were tested using a linear mixed-effects model with treatment (N or P addition) as fixed effects and plots nested within forest stand as a random effect. Forest stand was the unit of replication (*n*= nine). We also tested for N x P interaction in our models for N_{min}, C_{min}, N_{min}:C_{min} and Bic-P. Similar to resin-available soil parameters, we initially fit full models that included N, P, forest age as fixed effects (main effects + interactions) but the full models had higher AICc values; therefore, we excluded the factor forest age and the subsequent interaction terms in the final analysis to select the appropriate models (having the lowest AICc value) for all the soil variables.

N or P effects were inferred if resin-available parameters and additional soil variables in plots receiving a nutrient differed from those in plots not receiving that nutrient. This factorial approach compares response variables in plots with N addition (i.e., N and N+P plots) to those with no N addition (i.e., control and P plots) and plots with P addition to those with no P addition, and also tests the interaction between N and P additions. We used post-hoc Tukey comparisons of least-squares means to test the differences between addition of N+P and either N or P alone. For resin-available soil parameters, a statistically significant year x N or P interaction would indicate interaction of N or P availability over time. We considered P values < 0.05 to be significant but also report trends for which P values were between 0.05-0.10.

Results

Forest age classes did not affect the relative availability of resin-available N and P in the control plots (Table 2), and N:P was highly variable within each forest age.

Resin-available N and P responded to treatments, in a pattern that changed over time from 1 to 5 years of N and P additions (Table 3). Resin-available total inorganic N was greater in plots receiving N vs no N (P=0.01; Fig 3A), and resin-P was greater in plots receiving P vs no P (P<0.0001; Fig 3B) over the course of five years. Although we did not detect a significant N x P interaction on total inorganic N, we detected a N x year (P=0.04), and a P x year effect (P=0.002, Table 3); adding P alone and together with N suppressed total inorganic N availability by the fifth year (Fig 3A.). Resin-available total inorganic N availability was sensitive to P availability in control and P-only plots as well. In the fifth year (2015) a threshold P availability was evident, above which N availability was clearly suppressed (Fig 4.). We detected N x year (*P*=0.01) and P x year (*P*=0.03) interaction on resin-available NO_3^- (Table 3), and by the fifth year NO_3^- availability was suppressed (Fig 5.). Interestingly, the effects of P addition on NO_3^- availability were more pronounced in successional forests (young and mid-age) compared to mature forests. Resin-available NH_4^+ was greater in plots receiving N vs no N (*P*=0.002, Fig 6.), and we detected a significant P x year interaction (*P*=0.002), where NH_4^+ availability declined in plots receiving P vs no P over time (Fig 6.). There was no interaction of N x time on resin P (Table 3). Resin-available N:P was greater in plots receiving N vs no N (*P*=0.01), and was lower in plots receiving P vs no P (*P*=0.01, Fig 7.). We did not detect a N x P interaction, but we detected a P x year interaction for resin N:P (Table 3) and after five years, resin N:P was much lower in P added plots.

Mineralization of N and C, and Bic-P pools responded to N and P additions after three years of N and P additions (Table 4). Net N_{min} (the process that supplies N to the solution pool from the potentially-available organic matter pool) was greater in plots receiving N vs no N in Oe (*P*=0.02, Fig 8A) and Oa horizon (*P*=0.01, Fig 8B). We detected a significant N x P interaction on net N_{min} in the mineral horizon (*P*=0.02, Fig 8C): the effect of P in the mineral horizon was primarily driven by the N+P plots where net N_{min} was significantly lower than N only plots (*P*<0.001). KCl-extractable inorganic N was greater in organic horizons in plots receiving N vs no N (*P*=0.001 for Oe and *P*=0.01 for Oa horizon; data not shown), and was marginally suppressed by P additions (*P*=0.10) in the mineral horizon, which is consistent with the patterns we detected in resin-available inorganic N.

Microbial respiration (C mineralization) was not affected by N or P additions in the organic soils (Fig 9 A-B), but was suppressed by N addition (*P*=0.02, Fig 9C) in the mineral horizon. The mineralization of N per unit microbial respiration (N_{min}:C_{min}) was higher in plots receiving N vs no N in Oe (*P*=0.003) and Oa horizon (*P*=0.01), and was marginally higher in N plots in the mineral horizon (*P*=0.08, Table 4). In organic horizons, N_{min}:C_{min} in N+P plots were significantly higher than P only plots (*P*=0.01 for both horizons). In the mineral horizon, we detected a significant N x P interaction which was primarily driven by the lower N_{min}:C_{min} in N+P plots compared to the N only plots (*P*<0.001).

Bicarbonate-extractable P, which is the index of potentially mineralizable P, was greater in plots receiving P vs no P in Oe (*P*=0.02, Fig 10A) and Oa (*P*=0.04, Fig 10B) horizon. The effect of P addition on organic soil Bic-P was primarily driven by the N+P plots which were significantly higher than N only plots (*P*<0.001 for Oe and *P*<0.01 for Oa). Bic-P in mineral horizon was not affected by N or P additions (Fig 10C).

Discussion

Our study allowed us to test for changes in N and P availability over succession (in ambient soils) and also to test for effects of excess of one nutrient on the availability of the other (in nutrient treated soils). Independent of treatment, we did not detect a change in resin-available N:P through succession. A slight peak in resin-available N:P in the mid-age stands is consistent with MEL model predictions (Rastetter et al. 2013), which suggests a change in the balance of relative availability of N and P as young forests transition to mid-successional stages. With increasing forest age, N is mineralized in excess of biotic demand, thereby increasing the build-up of inorganically-available N and also enhancing potential for N losses.

Elevated P availability suppressed N availability (resin-available inorganic N) after five years of fertilization across northern hardwood stands, suggesting the importance of rapid shorter-term plant-soil feedback processes for maintaining balanced nutrition. In contrast, N addition did not influence P availability. Fertilizing with a single nutrient elevated the soil availability of that nutrient (resin-available N and P and also potentially-available net N_{min} and Bic-P) indicating that nutrient addition exceeded biotic demand for that nutrient.

Plant and soil-microbial feedbacks are potential mechanisms by which N and P availability interact either by maximizing the processes that increase acquisition or availability of the nutrient in higher demand, or by minimizing the processes that contribute towards the availability of the nutrient present in excess. Suppression of soil N availability when P is abundant could have several explanations. Firstly, alleviating P limitation of microbes could potentially suppress N availability in the resin-available inorganic pool in soil. If microbes are P limited, and once the microbial demand for P is alleviated, we would expect microbial uptake of both N and P to increase. Lower net N mineralization could be caused by alleviating P limitation of microbes, and enhancing immobilization (Fig 1.). This mechanism seems likely and is supported by lower mineralizable N per unit mineralized organic C (lower N_{min}:C_{min}), which indicate increase in immobilization. Although potentially mineralizable N:C was lower in mineral horizon, we did not detect lower N:C in response to P addition in organic horizons. Lower net N mineralization could also be caused by lower microbial activity; however, we did not detect lower microbial respiration (C mineralization) with P addition. Also, we did not detect a decline in net N mineralization in organic horizons with P addition. However, we did detect a decline in net N mineralization in the mineral horizon which was likely due to higher microbial immobilization.

There are experimental results suggesting microbial P limitation in temperate hardwood forest soils. For example, Fisk et al (2015) reported that adding P enhanced C mineralization and microbial respiration in surface organic soils in similar forest stands, which highlights the importance of P for microbial processes in these soils. Although the short-term nutrient effects were only observed in combination with a labile C source, there is enough experimental evidence to suggest that microbial processes are primarily C limited but the relative availability of C, N and P is critical to drive microbial growth and functioning in forest soils (Barantal et al. 2012; Fannin et al. 2015, 2016; Nottingham et al. 2012, 2015, Popleau et al. 2016; Jing et al. 2017). Moreover, soil enzyme activity from the same stands indicate a high microbial demand for P (Shan et al. in prep). Additionally, field observations of increased microbial biomass P in P fertilized plots (Fisk et al. in prep) and enhanced microbial growth in response to P addition in laboratory incubations (Fisk et al. in prep) indicate P limitation of microbial growth in these forest soils.

Another possible explanation of N suppression could be higher aboveground tree growth in response to P addition. While northern hardwood forests are still considered to be primarily N limited, recent observations conducted in the same forest stands report that aboveground forest productivity, especially in mid-age and mature forests, is P limited (Goswami et al. 2017), therefore suggesting higher N uptake by trees. However, tree responses to P were moderate, and in four out of nine stands post-treatment plant N uptake (foliar N) was lower with P additions (Gonzales 2017). Lower foliar N with P addition, combined with lack of nutrient effect on litterfall mass (Goswami et al. 2017), suggests that limitations to soil microbial growth and activity (belowground processes) regulate the relative balance of available nutrients, and constrain P effects on aboveground growth.

If biotic uptake of N is limited by P, the potential for losses (via denitrification) is increased. In contrast, if denitrification is limited by P, it promotes N retention in the system. However, as P availability is low relative to that of N in the northern hardwoods owing to long-term anthropogenic N deposition, short-term biotic feedbacks can allow for losses of the nutrient that is available in excess. For example, increased denitrification losses (consistent with the suppression of resin-available NO_3^- with P additions) could be another possible mechanism that could lead to a reduced available N pool in the soil.

Fertilization is likely to cause abrupt short-term changes especially in the soil, however, a decline in resin-available P in P plots across all stands between the fourth and fifth year warrant additional explanations other than time since fertilizer application. It is possible that there were random differences in the amount of soil water which could affect the movement of available P to resin strips. Alternatively, potential desorption of P from resins over time could lead to an underestimation of available P (Fisk et al. 2014).

The temporal shift observed here suggests that different mechanisms operate in response to short-term disturbance vs long-term change or spatial variability. Responses detected over multiple years are relevant to understanding effects of spatial variability or long-term change, while short-term response facilitates understanding of immediate responses to disturbances like insect irruptions, ice storms, drought stress, soil frost, soil acidification, nitrogen enrichment and forest harvest. Microbial resource allocation via mineralization and uptake processes, facilitated by enzyme production, is a rapid process that could constrain plant-availability of nutrients and therefore, influence nutrient interactions that could lead to secondary nutrient limitation over time. Microbial suppression of plant available N in this study indicates that adding P alleviates P limitation, which increases growth and biotic N uptake, until eventually N availability is low enough that plant growth becomes N limited (as suggested by low foliar N in response to P in a subset of stands; Gonzales 2017). If alleviation of limitation by P induces a secondary limitation by N, this sequential co-limitation response should be evident in future divergence of plant and soil responses between fertilization with single nutrients (N or P) and N+P together. It is possible that these ecosystems are on the onset of N and P co-limitation and over time nutrient interactions will contribute to biotic responses in N+P to exceed those in N or P.

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Tables and Figures

Table 1. Characteristics of nine northern hardwood forest stands in the Bartlett Experimental Forest, central New Hampshire, USA and the resin-available N:P measured in control soils in summer 2011 and 2015.

Stand	Forest age	Year clearcut	Elevation (m)	Resin-available N:P in Control in 2015
C1	Young	1990	570	7.0 (0.2)
C2	Young	1988	340	0.2 (0.06)
C3	Young	~1982-1985	590	43.7 (3.8)
C4	Mid-age	1979	410	0.4 (0.1)
C5	Mid-age	1976	550	51.2 (4.5)
C6	Mid-age	1975	460	59.9 (15.9)
C7	Mature	1890	440	0.5 (0.1)
C8	Mature	1883	330	10.8 (3.4)
C9	Mature	1890	440	50.1 (6.4)

n = 8 strips per plot in 2015; standard errors of the mean are in parentheses.

Table 2. Results (F-values and significance) of mixed effects model showing the effect of forest age (young, mid-age or mature) on resin-available soil parameters measured in summer 2015 in control plots only across nine stands.

Response	Predictor	Degrees of freedom	F value	*P* value
Resin-available inorganic N	Forest age	2, 6	0.25	0.79
Resin-available inorganic NO_3^-	Forest age	2, 6	0.23	0.80
Resin-available inorganic NH_4^+	Forest age	2, 6	0.34	0.72
Resin-available P	Forest age	2, 6	0.16	0.85
Resin-available N:P	Forest age	2, 6	0.06	0.94

Table 3. Results (F-values and significance) of mixed effects model showing the main effects of nutrient addition (N or P addition) and year (main effects + interaction) on resin-available soil parameters measured in summer 2011, 2012, 2014 and 2015 across six stands.

Source	DF	F-value (*P* value) Resin-available soil parameters				
		Total N	NO_3^-	NH_4^+	Resin P	N:P
N	1, 78	6.72 (**0.01**)	1.64 (0.20)	9.96 (**0.002**)	0.68 (0.41)	7.12 (**0.01**)
P	1, 78	0.46 (0.49)	0.61 (0.44)	0.03 (0.86)	28.41 (**<0.001**)	7.47 (**0.01**)
Year	3, 78	3.08 (**0.03**)	7.11 (**0.0003**)	1.91 (0.13)	0.93 (0.43)	1.06 (0.19)
N x P	1, 78	1.69 (0.19)	0.01 (0.90)	2.25 (0.13)	0.26 (0.61)	0.11 (0.74)
N x Year	3, 78	2.89 (**0.04**)	4.47 (**0.01**)	1.80 (0.15)	0.64 (0.59)	0.43 (0.73)
P x Year	3, 78	5.03 (**0.002**)	3.13 (**0.03**)	5.63 (**0.002**)	11.05 (**<0.001**)	3.37 (**0.02**)

Note: Significant *P* values are indicated in bold.

Table 4. Results (F-values and significance) of mixed effects model showing the effects of nutrient addition (N or P addition; main effects + interaction) on soil variables (N_{min} = Net N mineralization, C_{min} = C mineralization and Bic-P= Bicarbonate-extractable P) measured in organic (Oe and Oa) and mineral horizons in summer of 2014 across nine stands.

Source	Degrees of Freedom	Response	F-value (*P* value)		
			Oe	Oa	Mineral
N	1, 24	N_{min}	6.01 (**0.02**)	7.40 (**0.01**)	1.71 (0.20)
		C_{min}	1.15 (0.29)	0.32 (0.58)	5.70 (**0.02**)
		N_{min}: C_{min}	10.57 (**0.003**)	7.28 (**0.01**)	3.20 (0.08)
		Bic-P	1.05 (0.31)	0.002 (0.96)	<0.001 (0.97)
P	1, 24	N_{min}	0.23 (0.64)	0.02 (0.88)	0.20 (0.66)
		C_{min}	0.03 (0.86)	0.02 (0.89)	0.25 (0.62)
		N_{min}: C_{min}	0.67 (0.42)	0.32 (0.57)	0.45 (0.50)
		Bic-P	6.55 (**0.02**)	4.47 (**0.04**)	0.76 (0.39)
N x P	1, 24	N_{min}	0.02 (0.89)	1.15 (0.29)	5.53 (**0.02**)
		C_{min}	0.27 (0.61)	0.78 (0.38)	0.17 (0.68)
		N_{min}: C_{min}	0.04 (0.83)	0.02 (0.87)	7.55 (**0.01**)
		Bic-P	1.19 (0.29)	0.49 (0.49)	0.001 (0.98)

Note: Significant *P* values are indicated in bold.

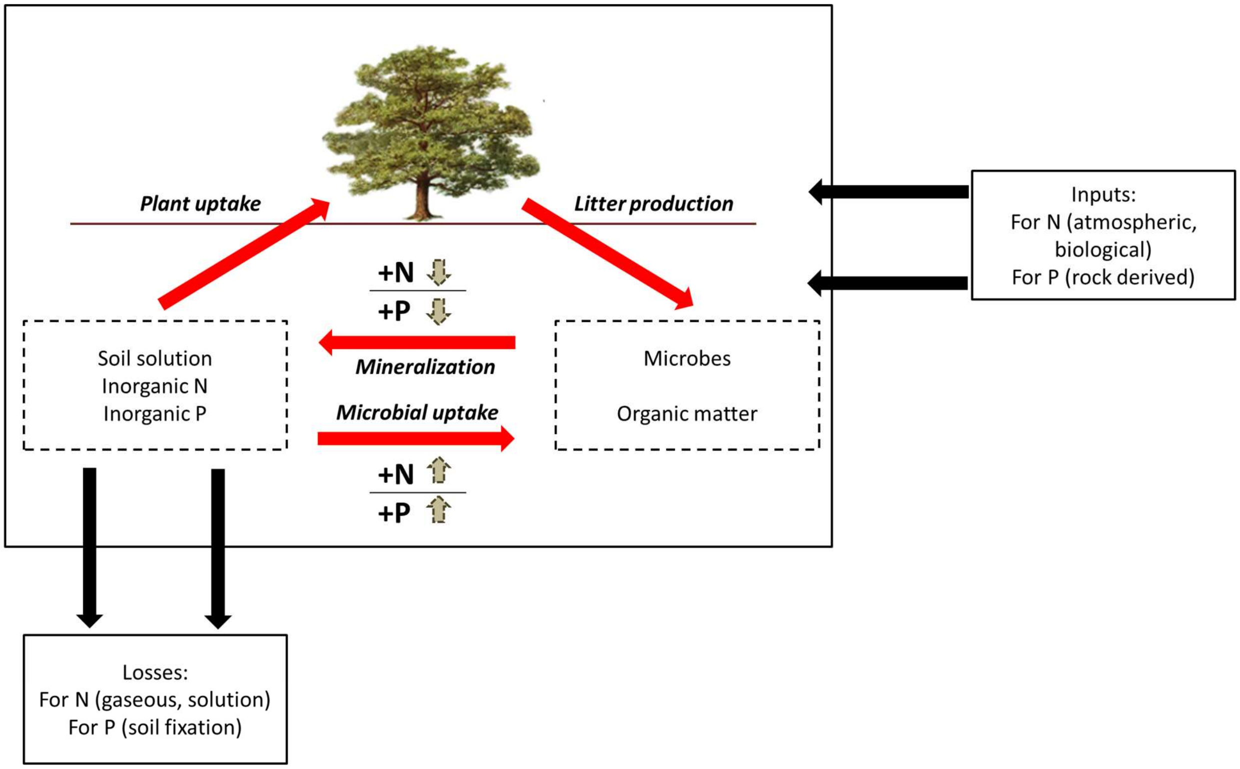

Fig 1. Conceptual generalized model system showing N and P cycling in a temperate forest ecosystem. Dashed boxes represents soil solution pool of inorganic nutrients (which is the balance between mineralization supply, biotic uptake and losses), and pool of microbes and organic matter (potentially-available pool of N and P). Red arrows indicate within-system nutrient recycling fluxes and black arrows indicate fluxes into (abiotic/biotic inputs) and out (losses) of the model system. Tan arrows indicate how net nutrient mineralization and microbial uptake processes could change in response to N and P fertilization (experimental treatments).

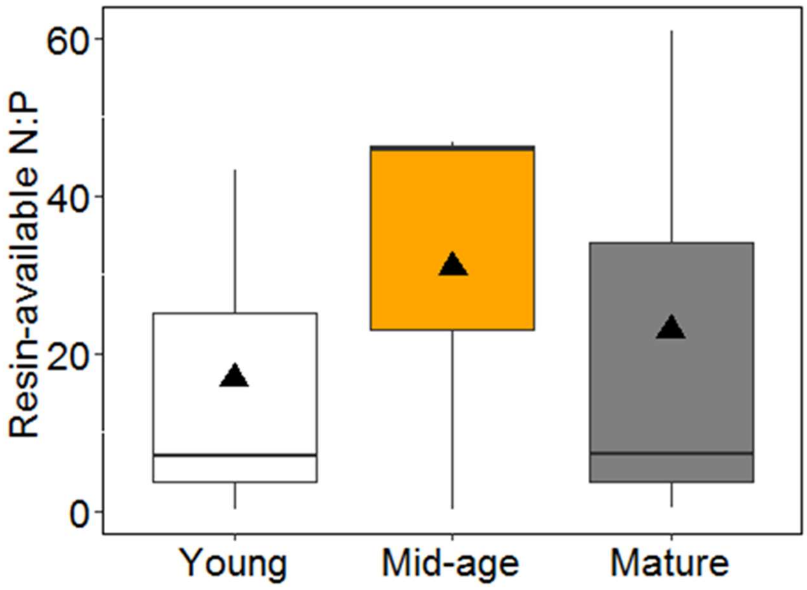

Figure 2. Resin-available inorganic N:P in control plots at nine stands (three each of young, mid-age and mature) in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

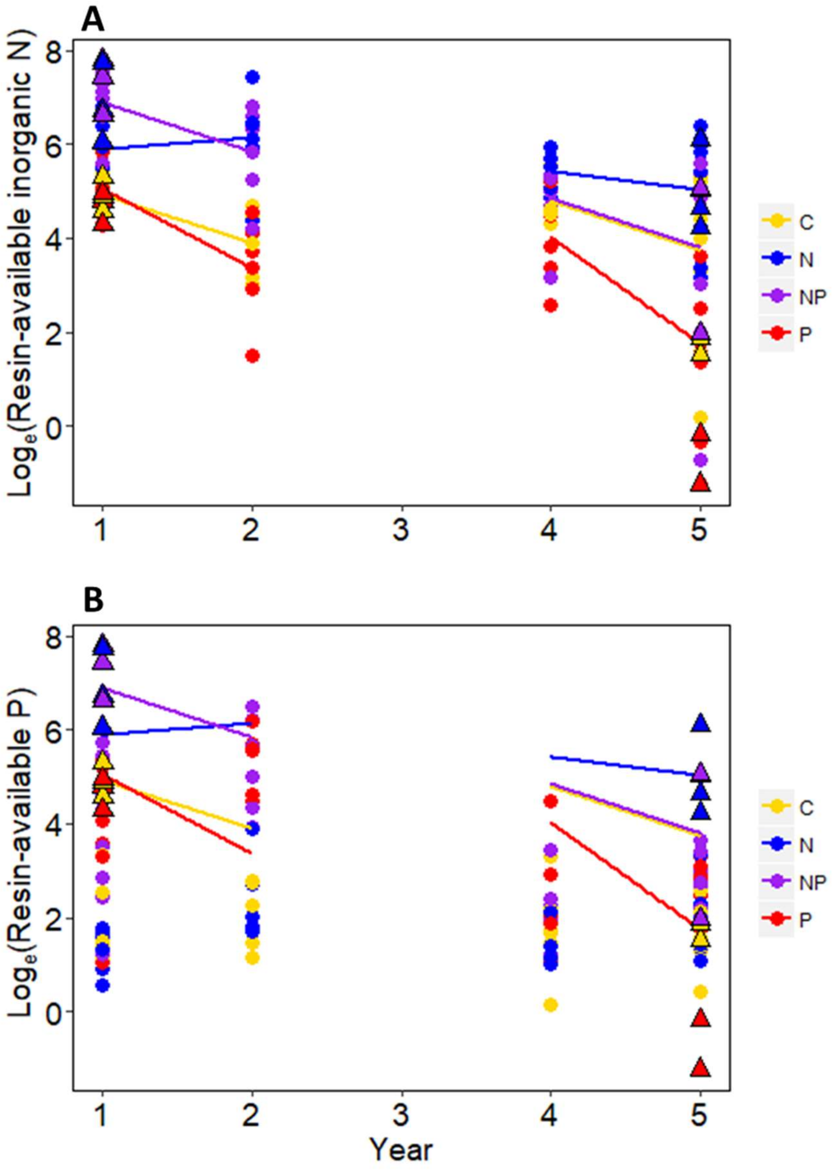

Figure 3. Resin-available inorganic N (panel A) and resin-available P (panel B) in C (control), N (nitrogen), NP (nitrogen + phosphorus) and P (phosphorus) at young, mid-age and mature stands (three of each) in northern hardwood forests over the course of five years. *Note*: three stands: C3 (young), C4 (mid-age) and C7 (mature), denoted by triangles were not measured in years 2 and 4.

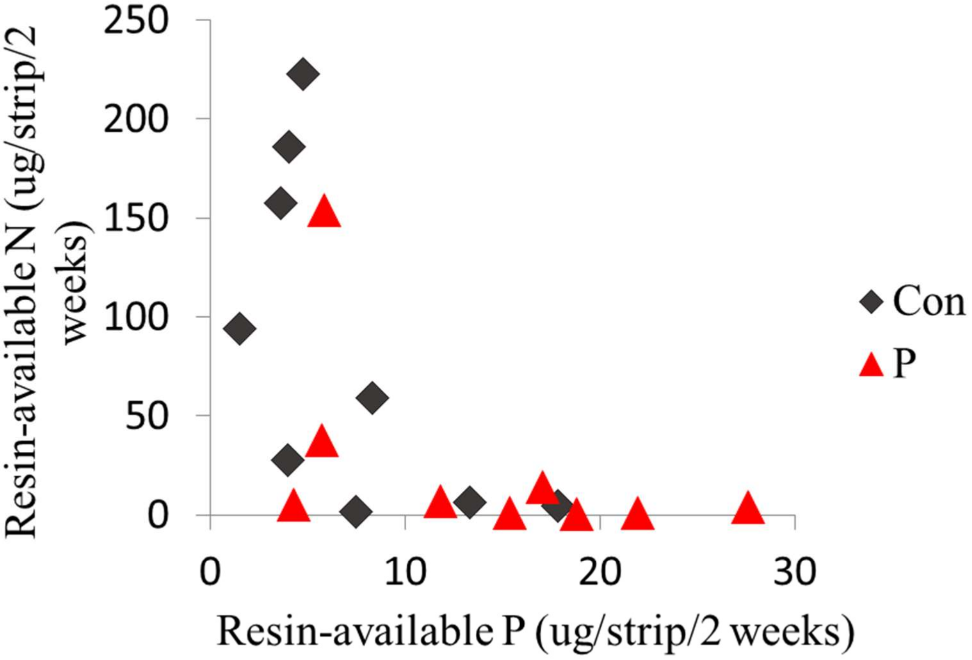

Figure 4. Resin-available inorganic N (NO_3^- + NH_4^+) vs resin-available P in control and P-only plots in nine forest stands (three each of young, mid-age, and mature forest) in fifth-year (2015) of N and P additions.

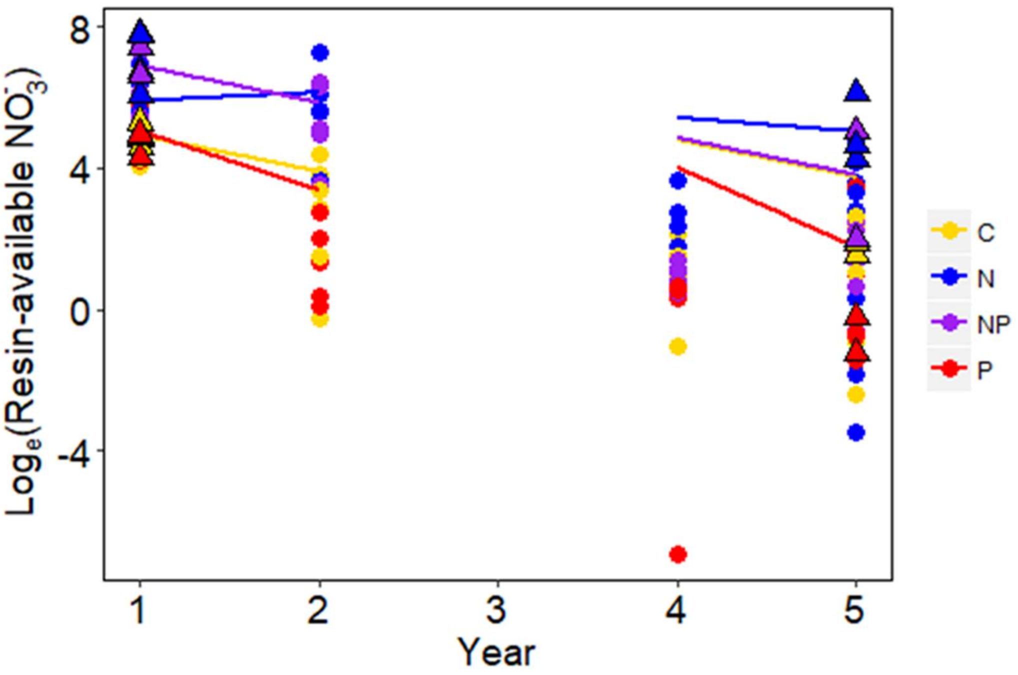

Figure 5. Resin-available NO$_3^-$ in C (control), N (nitrogen), NP (nitrogen + phosphorus) and P (phosphorus) at young, mid-age and mature stands (three of each) in northern hardwood forests over the course of five years. *Note*: three stands: C3 (young), C4 (mid-age) and C7 (mature), denoted by triangles were not measured in years 2 and 4.

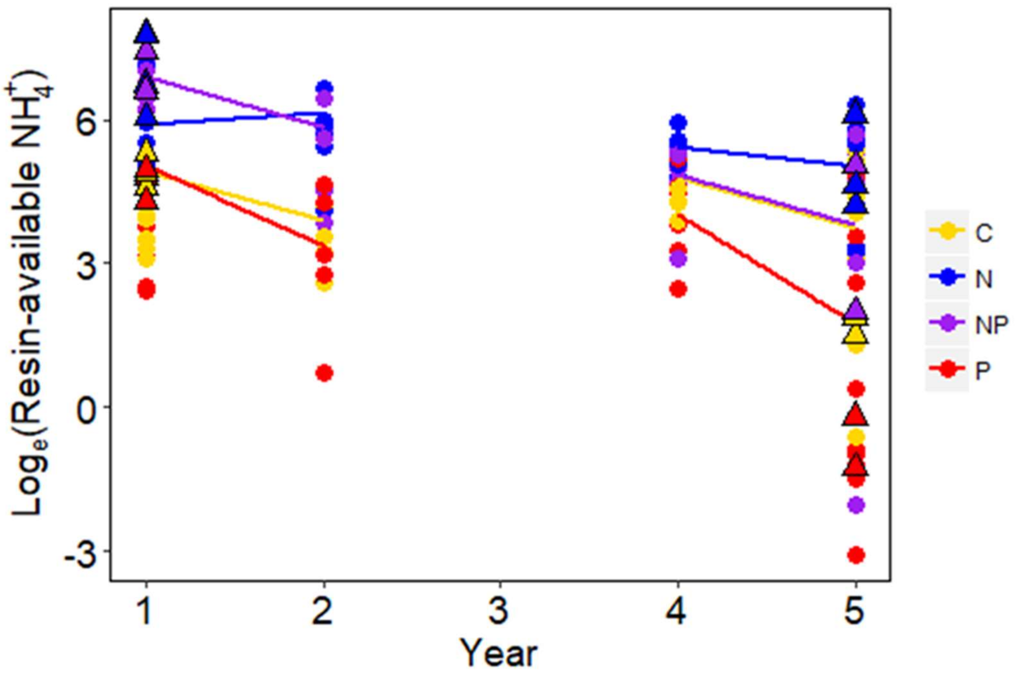

Figure 6. Resin-available NH$_4^+$ in C (control), N (nitrogen), NP (nitrogen + phosphorus) and P (phosphorus) at young, mid-age and mature stands (three of each) in northern hardwood forests over the course of five years. *Note*: three stands: C3 (young), C4 (mid-age) and C7 (mature), denoted by triangles were not measured in years 2 and 4.

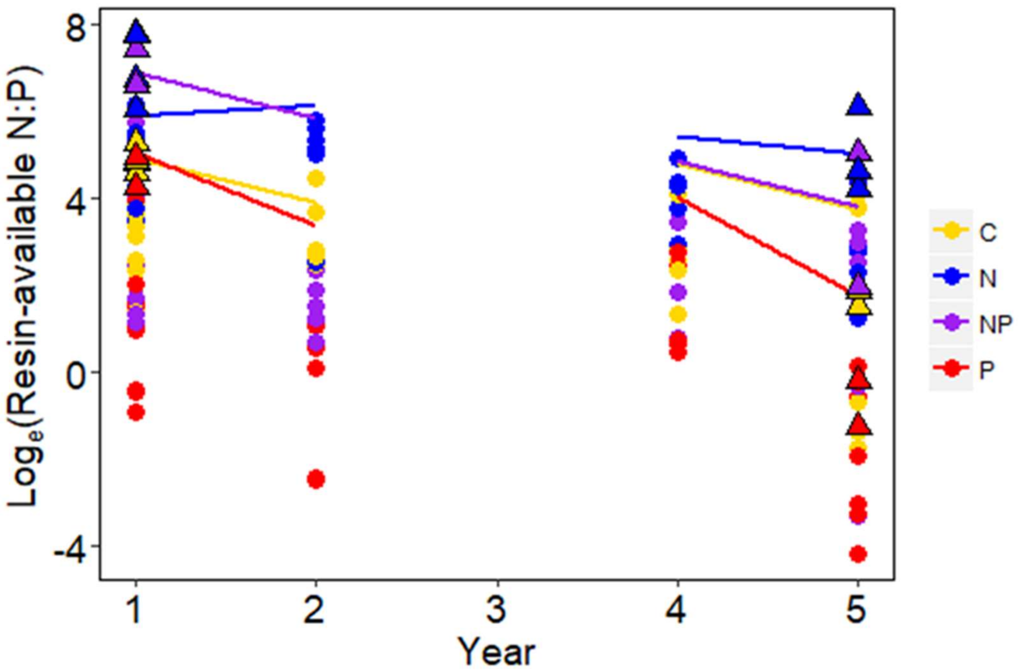

Figure 7. Resin-available N:P in C (control), N (nitrogen), NP (nitrogen + phosphorus) and P (phosphorus) at young, mid-age and mature stands (three of each) in northern hardwood forests over the course of five years. *Note*: three stands: C3 (young), C4 (mid-age) and C7 (mature), denoted by triangles were not measured in years 2 and 4.

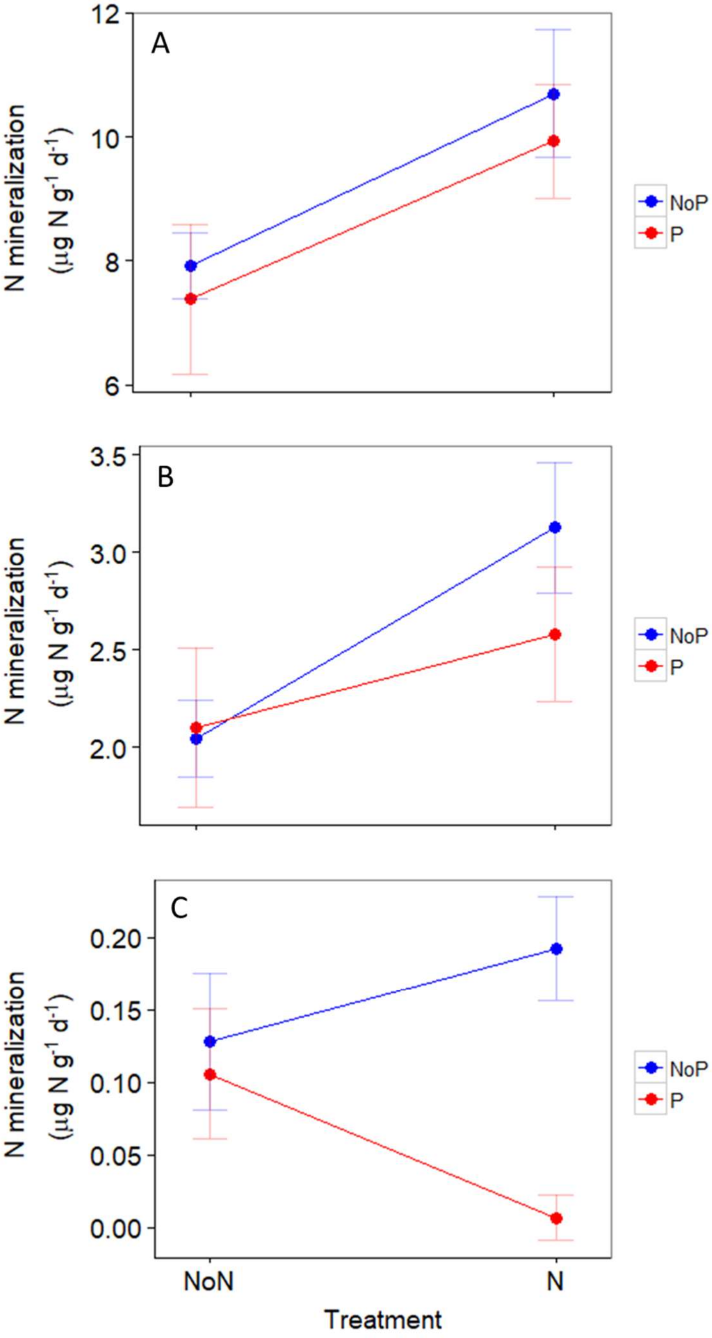

Figure 8. Net N mineralization in plots receiving N vs no N and P vs no P in Oe (panel A), Oa (panel B) and mineral (panel C) horizons at nine (three each of young, mid-age and mature) stands in northern hardwood forests after three years of N and P additions.

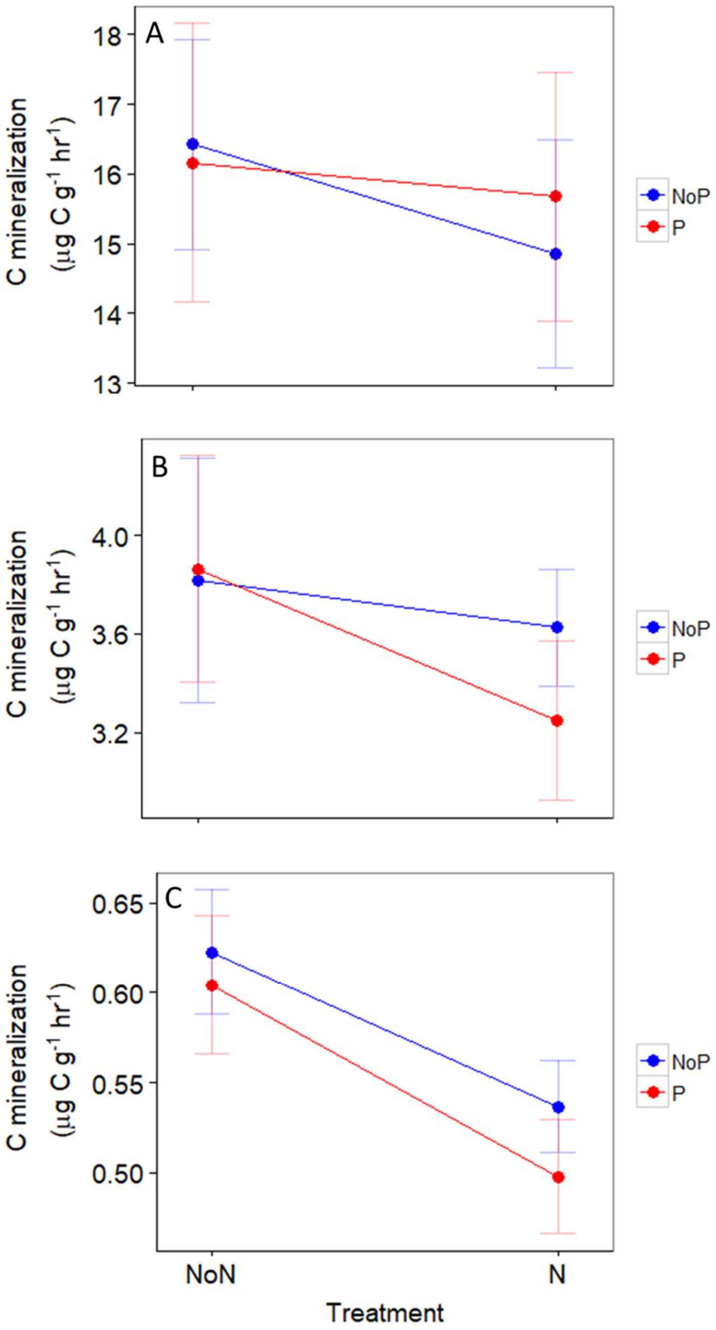

Figure 9. C mineralization in plots receiving N vs no N and P vs no P in Oe (panel A), Oa (panel B) and mineral (panel C) horizons at nine (three each of young, mid-age and mature) stands in northern hardwood forests after three years of N and P additions.

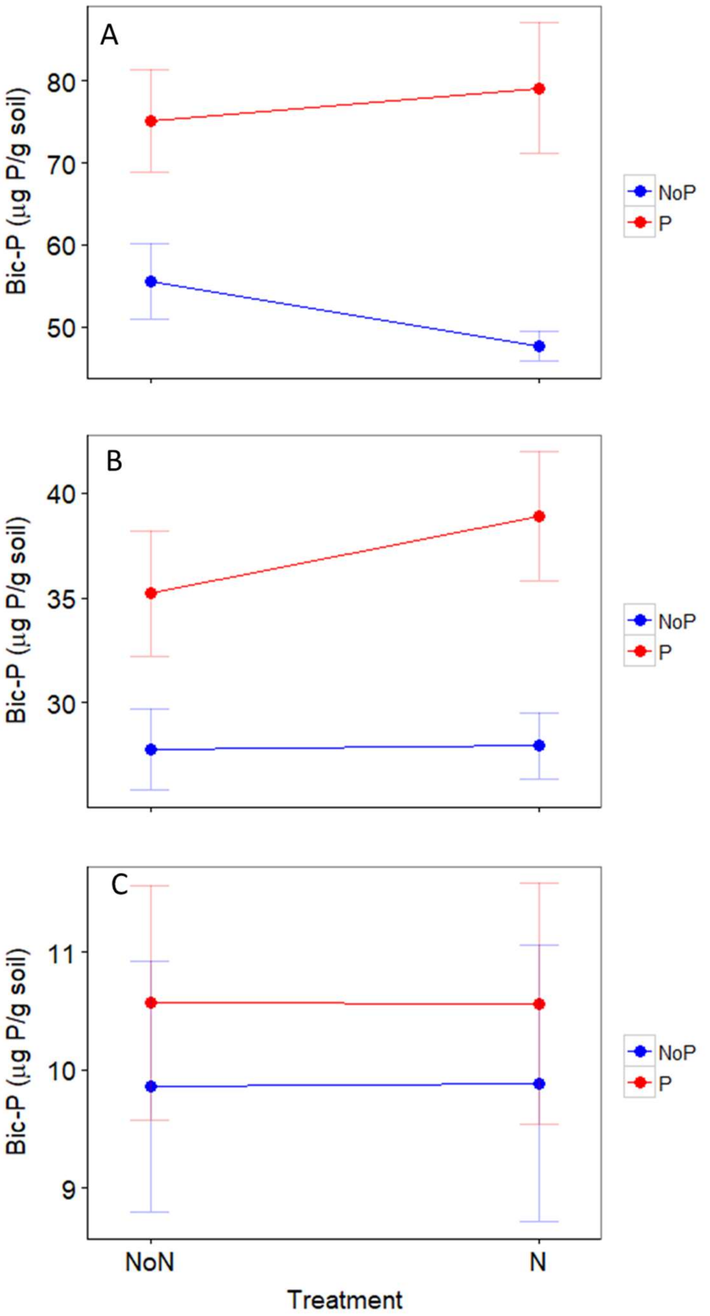

Figure 10. Bicarbonate-available P in plots receiving N vs no N and P vs no P in Oe (panel A), Oa (panel B) and mineral (panel C) horizons at nine (three each of young, mid-age and mature) stands in northern hardwood forests after three years of N and P additions.

Chapter 3

Seedling survival and allocation responses to nutrient additions in northern hardwood temperate forests

Abstract

The heterogenous and dynamic hardwood forest understory contains the bulk of the future regeneration potential and thus has important influences on tree species recruitment. Light is a crucial resource for regeneration success in the understory. However, high variability in survival during the early phases of recruitment suggests that other factors like water and nutrients may be important to survival, especially for shade tolerant species. In this study, we asked the following questions- (i) is seedling survival nutrient limited? ii) is seedling biomass limited by the availability of N or P, And, (iii) Is seedling biomass related to allocation patterns or to root and foliar morphology? We tested the above questions in mature (>100 years age) northern hardwood forest stands where plots in each stand had been fertilized with either 30 kg N ha^{-1} yr^{-1}, 10 kg P $ha^{-1}yr^{-1}$, the same amounts of N+P, or neither (control) since 2011. We followed a single cohort of naturally regenerating American beech (*Fagus grandifolia* Ehrh.) and sugar maple (*Acer saccharum* Marsh.) seedlings in these forest plots from 2012 to 2014and monitored survival and allocation responses to light, soil moisture, and nutrients for three years. N addition decreased seedling survival in both species and sugar maple survival substantially declined with nutrient additions. Allocation by survivors over time was relatively unresponsive to elevated nutrients, despite increased foliar nutrient content. Furthermore, shallow rooting by seedlings likely caused a soil moisture sensitivity. Also, greater seedling leaf damage suggested higher herbivory in response to elevated nutrients. It is uncertain in the earlier stages of seedling establishment whether higher aboveground allocation increases seedling survival or if improved nutrition encourages herbivory.

Introduction

Understanding controls on early stages of tree recruitment and regeneration is essential as it can lead to future shifts in species composition. It is well established that regeneration and recruitment of forest tree species exhibit large-scale spatial and temporal variability (Clark et al. 1999), however mechanistic studies following early phases of tree recruitment in combination with anthropogenically accelerated environmental changes are limited. Early phases of recruitment are associated with high variability (Clark et al. 1999; Cleavitt et al. 2011, 2014) and a steep decline in the first few years of seedling survival (Beckage et al. 2005; Cleavitt et al. 2011). Therefore, it is important to understand what factors influence the final survivors, to assess potential limitations to seedling recruitment.

Light is considered as the most limiting resource for plant growth, however, environmental changes altering nutrient availability could influence recruitment and establishment. For tree species in the forest understory, light has been established as a crucial resource for regeneration success (Canham et al. 1996; Pacala et al. 1996; Walters and Reich 1996; Coomes and Grubb 2000; Antos 2009). In addition to slightly higher light microsites, factor in forest understories that could increase seedling survival and growth is greater belowground resource availability. Under relatively low light, resources like nutrients and water have been found to influence forest tree seedling survivorship (Juice et al. 2006; Cleavitt et al. 2011; Santiago et al. 2012; Fisichelli et al. 2013) and growth rates (Peace and Grubb 1982; Walters and Reich 1997, 1999, 2000; Coomes and Grubb 1998; Kobe 2007; Walters et al. 2014). Spatial dominance of overstory trees could constrain nutrients and water availability for smaller seedlings (Antos 2009), possibly intensifying nutrient and water limitation early during seedling establishment.

Temperate forest ecosystems in the north-eastern US have been impacted by a history of anthropogenic nitrogen (N) deposition (or N enrichment; Fenn et al., 1998; Aber et al., 2003) and calcium depletion (from acid deposition; Likens et al., 1996, 1998). Soil properties and nutrient cycling processes have been altered as a consequence (Fenn et al. 1998, Aber et al. 2003), and could have potential effects on tree demography and rates of tree recruitment (St. Clair et al. 2008), given the influence of nutrition on seedling growth and regeneration. N is considered to be the most important limiting nutrient in temperate ecosystems (Vitousek and Howarth 1991), and evidence exists that temperate forest tree seedlings and saplings vary in their growth responses to N (Walters and Reich 1997, 1999, 2000; Kobe 2007; Walters et al. 2014). However, growth responses to ecosystem scale N fertilization were not tested. Moreover, studies have shown that high N availability under low light can improve photosynthetic potentials in some species (Euliss et al. 2007; Bloor et al. 2008). The importance of P to regenerative performance of seedlings has received less attention, despite recognition that it can influence aboveground productivity (Vadeboncoeur 2010; Goswami et al. 2017). General attention to N and P co-limitation of productivity has grown in response to meta-analyses suggesting that productivity in most ecosystems is N and P co-limited (Elser et al. 2007, Harpole et al. 2011). More specifically, in this ecosystem, model simulations predict that P interacts with N to limit forest growth over successional time (Rastetter et al. 2013).

Sugar maple (*Acer saccharum* Marsh.), a dominant in the northern hardwoods is of particular importance given the above trends in changes in relative availabilities of soil nutrients. Base cation depletions and soil calcium (Ca) deficiencies have been shown to affect sugar maple regeneration (Kobe et al. 2002; Juice et al. 2006). Experimental addition of Ca to the soils have greatly enhanced seedling survival (Juice et al. 2006; Cleavitt et al. 2011) and have highlighted sugar maple sensitivity to changes in soil ph. The other co-dominant tree species in mature northern hardwoods is American beech (*Fagus grandifolia* Ehrh.) and a lower light threshold (Canham 1990; Nolet et al. 2008) for survival in beech could give this species a long-term advantage in regenerative success over sugar maple. Studies on American beech seedlings and saplings in northern hardwoods suggest that the early stages of this species are primarily light limited (Finzi and Canham 2000; Cleavitt et al. 2008) and not nutrient limited, but there is experimental evidence that beech seedlings (grown in greenhouse) respond to nutrient additions at intermediate and higher light levels (Latham 1992). Furthermore, nutrient effects on growth, but not survival was reported in European beech (*Fagus sylvatica*) seedlings that were grown in high light conditions (Monotta and Pinzauti 1996). Although American beech is morphologically and genetically isolated from European beech (Denk et al. 2005), it is important to investigate whether nutrient effects are observed under low light. In contrast, sugar maple responsiveness to N availability in northern hardwoods vary depending on the availability of other nutrients like Ca, on light availability, and also on the stage of tree development (Duchesne et al. 2002; Finzi and Canham 2000; St. Clair et al. 2008). Additionally, there is evidence that sugar maple survival and regeneration potential is primarily influenced by biotic factors, especially herbivory damage by fungi and caterpillars, and secondarily by other abiotic factors (Gardescu 2003; Cleavitt et al. 2014). Therefore, it is important to examine how recruitment in these co-dominant species respond to increased nutrient availability.

Biomass and energy conservation traits like carbon use efficiency (greater growth per unit C acquired), could confer a competitive advantage and maximize seedling survival in forest understories, by minimizing the tradeoffs associated with above- vs belowground allocation. Allocation to roots vs shoots is a key means by which plants respond to the relative demand for above- vs belowground resources, in order to maximize acquisition of the most limiting resource (Chapin et al. 1986, 1987; Grime 1994; Coomes and Grubb 2000). Alleviating belowground resource limitation could shift allocation to shoots (Harris, 1992) and under low light conditions, having more leaf area increases potential for photosynthesis, thereby enhancing growth potential (Coley 1988; Kitajima 1994; Kobe 1997). Therefore, when light and water availability are not limiting seedling growth, various seedling allocation efforts could shift in response to elevated nutrients to maintain a positive C balance (Fig 1.). However, the above- vs belowground allocation responses to nutrients that are expected in mature trees might not be advantageous in seedlings whose root systems are far less extensive. Changes in morphology could accompany shifts in allocation but minimize the associated tradeoff in resource acquisition. Some of the ways that plants can improve their C use efficiency for resource acquisition are through morphological changes in specific leaf area (leaf area per unit dry leaf mass) or specific root length (root length per unit dry root mass), that improves leaf area or constructs more roots (that exploit greater soil volume), without having to use more C. Shifting morphology could increase aboveground growth per unit C investment in either roots or leaves. However, morphological shifts like increased leaf area could be associated with incremental costs/risks for example, higher probabilities of damage (Coley 1988) due to physical as well as biotic factors (herbivory), and could considerably affect growth and survival.

In this study, we took advantage of a large forest ecosystem experiment which manipulated nutrients via factorial N x P fertilization in an effort to understand nutrient limitation in northern hardwoods (Fisk et al. 2014; Goswami et al. 2017). Nutrient additions (fertilized with either 30 kg N ha^{-1} yr^{-1}, 10 kg P $ha^{-1}yr^{-1}$, the same amounts of N+P, or nothing - control) in the forest stands began in summer 2011, which coincided with exceptionally high seed production of American beech and sugar maple in some mature forest stands (N. Cleavitt, personal communication), resulting in a large cohort of beech and sugar maple germinants in 2012; this provided an opportunity to study effects of nutrients on regeneration and above- vs belowground allocation by seedlings of these two species. The objectives of the current study were to quantify survival and allocation responses of this cohort of beech and sugar maple seedlings to nutrient additions and answer the following specific questions:

(i) Is seedling survival nutrient limited?
(ii) Is seedling biomass limited by the availability of N or P?
(iii) Is seedling biomass related to allocation patterns or to root and foliar morphology?

Methods

Study system and experimental design

We studied three northern hardwood forest stands at two different sites located on soils formed in glacial drift in central New Hampshire, USA: two at the Bartlett Experimental Forest

(BEF) and one at Hubbard Brook Experimental Forest (HBEF). Climate in the study region is humid continental with the mean temperatures of -9 °C in (January) and 19 °C in (July) (at 450 m elevation). The mean annual precipitation is approximately 140 cm evenly distributed throughout the year. Soils are Typic and Aquic Haplorthods overlying glacial drift (deposited approximately 14,000 years ago) originating primarily from granitic rock at BEF and granodiorite and schist at HBEF. The soils have thick surface organic horizons (~ 5 cm average depth; Vadeboncoeur et al. 2012) and low pH, varying from 4.2 – 4.9 in the top 10 cm of the mineral soil depending on the stand (Ratliff and Fisk, 2016).

The forest stands were mature (>100 years) and were dominated by sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghniensis* Britton), with occasional white ash (*Fraxinus americana* L.), white birch (*Betula papyrifera* Marsh.) and red maple (*Acer rubrum* L.). Four 50 x 50 m plots were established in each stand, and randomly assigned to control, N, P, and N+P fertilizer treatments. Fertilizer was applied to the entire 50 x 50 m plot; measurements were made in the inner 30 x 30 m area. Nutrient additions began in spring 2011. N was added at a rate of 30 kg ha^{-1} $year^{-1}$ as pelletized NH_4NO_3 and P was added at a rate of 10 kg ha^{-1} $year^{-1}$ as powdered or granular NaH_2PO_4. N and P were applied twice (early June and mid-July) in the first three years and once (early June) annually thereafter. Fertilizer was pre-weighed for 2.5 x 10 m sub-plots and spread evenly by hand.

Within each 50 x 50 m plot, ten 1 x 1 m seedling subplots were randomly established in May 2012 and seedling counts were conducted for American beech and sugar maple (stems < 50 cm in height) that germinated in spring 2012, following a strong mast year for both these species. Seedlings of this cohort were categorized as first-year germinants and were marked using zip-ties for following up on periodic survival counts over the successive years. Seedling counts for one-year-old (2013) and two-year-old (2014) seedlings (i.e. originated in 2012) for beech and sugar maple were conducted in summer of 2013 and 2014 in each of the seedling subplots for the three forest stands to understand whether nutrient treatments affect seedling survival. We quantified seedling survival (%/year) for both species. To understand whether abiotic factors like moisture and light availability affected seedling survival, we quantified soil moisture and canopy closure (amount of forest overstory measured directly above the seedling subplot providing an approximate inverse measure of light was used as an index of light availability; Lemmon 1956, 1957; Jennings et al. 1999) for each seedling subplots and surrounding buffer area of each treatment plot during summer in the second and third year of the study. Soil moisture of the forest floor was measured using an ECH20 moisture probe (5 cm length) and an LI-8100 FCO2 system (Licor Biosciences, Lincoln, NE) and canopy closure was estimated using a spherical densiometer (Lemmon 1956, 1957; Jennings et al. 1999; Forestry Suppliers Inc.). We did not measure abiotic factors in the first year of the study (2012).

To study mechanisms of variable allocation effort in beech and sugar maple in response to nutrient availability, 10-20 seedlings (belonging to 2012 cohort) per species were randomly chosen and destructively harvested from the buffer area around each of the nutrient-treated plots in summer of 2012. Using harvested seedlings from the buffer area along with survival counts from the seedling subplots allowed us to relate seedling survival (quantified in each seedling subplot) with the allocation efforts measured. Seedlings were carefully extracted with their root systems intact, following sampling methods described by Juice et al. (2006). Seedlings were kept moist during transport to the laboratory in plastic bags, and processed the same day. One-year-old and two-year-old seedlings were harvested in summer of 2013 and 2014 to test nutrient and resource (soil moisture and light availability) limitation of seedling growth.

We quantified the destructively measured variables: seedling biomass, seedling root:shoot, seedling leaf area, seedling root length and seedling foliar chemistry over consecutive sampling periods. Images of all seedlings were taken using a flatbed scanner in the laboratory and these images were used to quantify root and shoot length and leaf area of each seedling. All seedlings were divided into root and shoot tissues. Root and shoot biomass were quantified after drying to a constant mass at 60ºC. Root to shoot ratios were calculated on a mass basis. Seedling leaf area (cm^2) was quantified from scanned images using ImageJ software (ImageJ 1.x, Schneider et al. 2012), and leaf area damage for each individual was estimated as the percent difference between leaf area present during sampling and projected leaf area (drawn on the scanned images based on the remaining shape of each leaf). Seedling root length (cm) was estimated from scanned images using a line intercept method (Tennet 1975). Dried samples of seedling leaves were used for chemical analysis. Samples were powdered in a ball mill and analyzed for C and N in a combustion analyzer. Foliar tissue was ashed at 470ºC, digested in 6M HNO3, and P concentrations were analyzed using ICP-OES. Specific leaf area (hereafter, SLA; cm^2/mg) and specific root length (hereafter, SRL; cm/mg) were estimated by dividing projected leaf area (complete) and root length by respective dry tissue masses. SLA and SRL were quantified only for year-one germinants.

Data analysis

All statistical analyses were performed using R software (Version 3.1). Because we had a mix of destructive and non-destructive measurements, we performed separate analysis of variance for each variable. The analysis utilized repeated measurements on individual tagged seedlings for the non-destructive variables (survival, canopy cover, soil moisture) but not for the destructive variables (seedling biomass, seedling root:shoot, seedling leaf area, SLA seedling root length, SRL and seedling tissue foliar chemistry).

Nutrient additions were the experimental treatment (main predictor variable) for seedling survival and allocation efforts. Soil moisture and light availability were used as additional predictor variables for one-year-old and two-year-old seedlings. Seedling survival (%/year) at the end of each year was analyzed through a binomial logistic regression using mixed models with nutrients (N or P addition) and additional predictor variables as fixed effects and seedling subplots nested within stands as random effects (R glmer model with binomial error and logit link function, using the lme4 package; Bates el al. 2015). Significance was determined by maximum likelihood (Laplace Approximation). We considered *P* values < 0.05 to be significant but also report trends for which *P* values were between 0.05-0.10. Our factorial approach compares response variables in plots with N addition (i.e., N and N+P plots) to those with no N addition (i.e., control and P plots) and plots with P addition to those with no P addition, and also tests the interaction between N and P additions. We used post-hoc Tukey comparisons of least-squares means to test the differences between addition of N+P and either N or P alone. Year-one survival for both species was not included in the final model because the predictor variables moisture and light were not available for year one. We used the Akaike Information Criterion (AIC with a correction for finite sample sizes, AICc; Bozdogan, 1987) and compared models to select the best predictor variable (nutrients, moisture or light availability; having the lowest AICc value). We evaluated competing models that best explained variation in seedling survival using the change in AICc values and a model was considered competing if Δ AICc was less than 2.0. Due to very high mortality of sugar maple in fertilized plots (especially in plots with P addition), the models testing the interaction between N or P and light and soil moisture failed to converge. Therefore, we were unable to test nutrient and abiotic factor interactions for sugar maple survival.

Destructively measured variables that were available for year-one germinants and one- and two-year-old seedlings (seedling biomass, seedling root:shoot and leaf area damage) were analyzed using linear mixed effects models in the nlme package (Pinheiro et al. 2017), with N and P addition and year as fixed effects and stands as random effects. N or P effects were inferred if destructively measured variables in plots receiving a nutrient exceeded those in plots not receiving that nutrient. We also tested for N x P and N or P x year interactions in our models. We used post-hoc Tukey comparisons of least-squares means to test the differences between addition of N+P and either N or P alone. We considered *P* values < 0.05 to be significant but also report trends for which *P* values were between 0.05-0.10. Some of the destructively measured variables e.g. tissue foliar chemistry, could not be quantified in year one for both species on a plot level due to the limited amount of tissue in year-one germinants. Therefore, those variables were analyzed for one- and two-year-old seedlings using similar analysis as the above variables. We initially fit full models that included N, P and year as fixed effects (main effects + interactions) but the full models had higher Akaike Information Criterion (AIC with a correction for finite sample sizes, AICc; Bozdogan, 1987); therefore, we excluded the factor year and the subsequent interaction terms in the final analysis to select the appropriate models (having the lowest AICc value) for foliar tissue chemistry variables. SLA and SRL variables were only available for year-one germinants, so treatment effects on these two variables were analyzed only for year-one using similar analysis as other destructively measured variables.

Results

Survival

Seedling survival responded to nutrients after two and three years of fertilization. Nutrients were the best predictor that explained survival patterns in beech seedlings (lowest AICc value; Table 1), and nutrients and light combined together best explained sugar maple survival (lowest AICc value; Table 1). There were some competing models for seedling survival (Table 1); for beech, the additive and interactive effects of soil moisture combined with nutrients were the second- and third-best predictors of survival (Δ AICc < 2.0; Table 1). For sugar maple, nutrients alone and soil moisture added with nutrients were the next two competing models that explained seedling survival (Δ AICc < 2.0; Table 1).

Beech seedling survival responded to N (*P*=0.006, Fig 2.) but not to P addition (*P*=0.2; Fig 2.), with a significant N x P interaction (*P*=0.016; Table 2). After three years of nutrient additions, beech survival was lower by 48% in plots receiving N vs no N. Survival in N only plots was much lower than in controls (*P*=0.01), but was not significantly lower in plots receiving N+P compared with controls (*P*=0.18), and survival in N+P plots were not significantly different from N only plots (*P*=0.16). Sugar maple seedling survival declined with nutrient additions (Table 2, Fig 3.), and was lower by 54%, in plots receiving N vs no N (*P*=0.016) Although survival showed a substantial reduction by year three (Table 2, Fig 3.), our model was unable to detect a significant effect of P addition due to very high mortality of sugar maple seedlings in their second and third years in P plots in two out of three stands. Sugar maple survival was not lower in N+P plots than N alone, and we were unable to detect the difference between addition of N+P and P alone plots given the high mortality of this species in these plots.

Allocation efforts and tissue morphology and chemistry

Seedling biomass and seedling root:shoot in both species varied among years. Beech seedling biomass and seedling root:shoot did not respond to nutrient additions (Table 3 and 4). There were differences in these responses between years, but no year x nutrient interactions were detected for beech seedling biomass and root:shoot. As with beech, sugar maple seedling biomass and seedling root:shoot did not respond to N or P additions. However, there was year to year variation in these responses (Table 4), and we detected a significant P x year interaction for sugar maple root:shoot (P=0.02), likely owing to an increase in root:shoot in year two and then a subsequent decrease in root:shoot in year three in response to P (Table 4). The only response detected for SRL was in year-one sugar maple germinants (Table 5, Fig 4.): SRL was greater by 27% in N vs no N plots (P=0.10). SRL in year-one beech germinants did not respond to N or P addition (Table 5). We did not detect any N x P interaction for SRL in either of the species. SLA in year-one beech and sugar maple did not respond to N or P additions (Table 5), and we did not detect any N x P interaction for SLA in either of these two species.

One- and two-year-old seedling foliar tissue chemistry responded to nutrient additions (Table 6). Foliar C:N was lower in plots receiving N, by 10.3% for beech (Fig 5.) and 10.4% for sugar maple (Fig 6.). Foliar C:N did not respond to P for both species (Figs 5 and 6.). Foliar C:P did not respond to N in either species (Table 6). Foliar C:P was 20% lower in plots receiving P vs no P (P=0.02) for beech seedlings, but did not respond to P in sugar maple seedlings. Foliar N:P in beech was not affected by N addition (Table 6), but was lower by 17.5% in plots receiving P vs no P (P=0.06). We did not detect any N x P effect for foliar N:P in beech, and

N+P plots were not significantly different from N only and P only plots. Foliar N:P in sugar maple was not affected by either N or P addition (Table 6).

After three years of nutrient additions beech seedling leaf-area damage was marginally greater in plots receiving N vs no N (*P*=0.07), but was not different in plots receiving P vs no P, and there was no N x P interaction detected. The effect of N addition was greater over time (N x year interaction *P*=0.04; Table 7), leaf-area damage in two-year old beech seedlings was markedly greater by 184% in plots receiving N vs no N (Fig 7.). Unlike beech, sugar maple leaf-area damage did not significantly respond to either N or P addition (Table 7), and we did not detect any N x P interaction or nutrient by year interaction. Seedling survival was not affected by leaf area damage in either of the species, however, there was a weak negative trend (*P*=0.15) detected between seedling survival and leaf area damage in one- and two-year-old sugar maple seedlings.

Discussion

Our stand-level fertilization experiment established that survival was suppressed by elevated nutrient availability in a single cohort of naturally regenerating beech and sugar maple seedlings in mature northern hardwood forests. Seedling survival of both species was strongly suppressed by N availability. Seedling survival was sensitive to soil moisture, survival increased with higher soil moisture in the surface soils which was likely due to small root systems of seedlings. Light availability had a minimal effect on seedling survival and surprisingly, we detected a negative effect of light on seedling survival. It is likely that both these species being highly shade tolerant (Walters and Reich 1999) can develop better under moderate light levels than they do under open areas where the surface soils could dry out below the depth of these shallow rooted seedlings (Tubbs 1978).

Nutrient effects on seedling survival were observed in both co-dominant species in northern hardwood forests. Beech survival declined with N addition and was slightly higher (although not significantly) with P addition, thus contributing to the N x P interaction that we observed. Sugar maple survival was strongly suppressed in both N and P fertilized plots. Sugar maple is a sensitive species and there is evidence that sugar maple survivorship has been affected by reduction in soil pH associated with soil Ca depletion (Horsley et al. 2000; St.Clair and Lynch 2005; Juice et al. 2006; St.Clair et al. 2008). In this study, in two out of three stands, we observed a slight but not significant reduction in surface soil pH with N and P additions, and we detected a significantly higher seedling survival in sugar maple with increasing surface soil pH (P=0.004; data not shown). This observation supports the idea that changes in soil pH could be an important factor that determines how sensitive species like sugar maple respond to soil nutrient variability. Decline in seedling survival with elevated nutrients is inconsistent with the findings of some previous studies in similar forest ecosystems (Juice et al. 2006; Cleavitt et al. 2011; Fisichelli et al. 2013).

Despite the strong evidence for N suppression of seedling survival, these effects are most likely indirect acting through factors such as species ability to tolerate stress and/or determinants like pathogens and physical damage. Decline in survival with higher N availability could be attributed to mechanisms that are more varied and species-specific (Canham and Murphy 2017). Beech is a low-resource specialist and a stress-tolerator (Grime 1977), and has usually been unresponsive to N additions (Lea et al. 1979; Tripler et al. 2002; Bigelow and Canham 2007). Beech survives well in low nutrient soils (Pacala et al. 1996), but it is sensitive to drought stress (Caspersen et al. 1999). High N availability could contribute to seedling mortality indirectly by increasing the susceptibility of drought stress in this species in our study sites, through declining root:shoot. This is consistent with experimental observations of increased drought sensitivity with N fertilization in European beech (Dziedek et al. 2016). Conversely, seedling survival in sugar maple in northern hardwood forests may be more susceptible to factors such as pathogens, herbivores and physical damage (Gardescu 2003; Hane 2003; Cleavitt et al. 2014; Urli et al. 2016) compared to negative impacts resulting from nutrient imbalances (St.Clair et al. 2008; Cleavitt et al. 2014).

In general, it is considered that foliar N concentrations and photosynthetic capacities are strongly correlated in broadleaf deciduous trees (Ellsworth and Reich 1992). In this study, adding N improved foliar nutrition (reduced foliar C:N) in both species, while adding P improved foliar nutrition (reduced foliar C:P) in beech, therefore suggesting seedlings are experiencing improved nutrition with N and P fertilization, and are possibly showing higher photosynthesis. St.Clair and Lynch (2005) observed higher photosynthetic rates and foliar N in sugar maple seedlings in greenhouse soils that were base cation enriched, suggesting Ca to be of primary importance to nutrition in sugar maples; however, we understand that nutrient fertilization effects in a forest understory would vary from greenhouse conditions.

Higher foliar content of nutrients indicate that both beech and sugar maple seedlings increased nutrient uptake in response to elevated nutrient availability. Seedling biomass was not promoted by availability of N or P and nutrient fertilization did not reduce allocation to roots. Allocation by survivors over time was relatively unresponsive to elevated nutrients, despite increased foliar nutrition. Many of our allocation predictions (Fig 1) were not supported. However, plants can alter allocation to resource acquisition without changing allocation on a mass basis, through changes in morphology such as SRL or SLA. Improved nutrition did not substantially affect allocation, but it improved the use of C for belowground resource acquisition in sugar maple. The marginally higher SRL in year-one sugar maple in N plots suggests a morphological change in roots to improve C use efficiency, without a change in belowground biomass. This would be an efficient way to use C to acquire water, by increasing the soil volume exploited without increasing C costs to construct more roots. Constructing a higher proportion of very fine roots would be associated with a higher N cost compared with thicker roots, because of the greater functional activity of finer roots (Pregitzer et al. 1998), which is likely why we detect changes in root morphology in plots receiving N vs no N. In contrast, no nutrient effects were observed in SLA in year-one sugar maple and beech suggesting that a lack of effect of nutrients on biomass was not related to changes in foliar morphology.

Typically forest trees increase SLA in response to higher nutrition, however potential incremental costs/risks of increasing SLA could be associated with increase in herbivory damage. Seedling mortality was very high in the first few years across treatment, and those patterns are in agreement with previous studies of seedling survival in similar northern hardwood stands (Gardescu 2003; Hane 2003; Cleavitt et al. 2011, 2014). In our study, evidence for improved foliar nutrition and an increase in leaf area damage following N addition in beech suggests increased potential costs associated with herbivory. Additionally, a weak negative relationship of sugar maple survival with higher levels of leaf area damage, suggest effects of mechanical injury and/or herbivory on seedling mortality. Cleavitt et al (2014) observed a strong influence of damage agents (pathogenic fungi and caterpillar) on sugar maple regeneration in similar stands. The extent to which soil nutrient availability interacts with soil pathogens and insect defoliators is not clear but could have considerable implications on long-term impacts of N and P availability on seedling survival and recruitment in northern hardwoods.

Our results indicate that higher nutrient availability can have negative effects on seedling survival, which emphasizes the potential sensitivity of regeneration to anthropogenic nutrient enrichment. Long-term fertilization studies in northern hardwood forests show that N enrichment can occasionally induce mortality (Magill et al. 2004; Wallace et al. 2007). The lack of nutrient limitation on growth and the relative lack of plasticity (with the exception of foliar chemistry), suggests that seedling growth in the early stages is not very sensitive to nutrient variability in the immediate environment. These results demonstrate that changes in foliar chemistry along with potential indirect effects of improved nutrition such as herbivory damage, which could be related to a negative effect of N, deserve more attention.

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Tables and Figures

Table 1. AICc and change in AICc values (Δ AICc) results of mixed effects models showing the effects of different predictor variables – nutrients (main effects + interaction), light availability (main effects), soil moisture (main effects), and additive (nutrients + light or soil moisture) and interactive (nutrients x light or soil moisture) effects of the three predictor variables on one- and two-year-old beech and sugar maple seedling survival (% year^{-1}) across three stands.

Predictor	AICc	Δ AICc	AICc weight
American beech survival (% year^{-1})			
Null	183.8	12.9	0.00
Nutrients (N x P)	**170.9**	**0.00**	**0.30**
Light	184.5	13.6	0.00
Soil moisture	181.4	10.6	0.00
Nutrients + Light	172.8	1.9	0.12
Nutrients x Light	177.2	6.3	0.01
Nutrients + Soil moisture	171.0	0.1	0.29
Nutrients x Soil moisture	171.1	0.2	0.28
Sugar maple survival (% year^{-1})			
Null	119.6	17.5	0.00
Nutrients (N x P)	102.2	0.12	0.33
Light	121.1	19.0	0.00
Soil moisture	118.2	16.1	0.00
Nutrients + Light	**102.1**	**0.00**	**0.35**
Nutrients x Light	-		
Nutrients + Soil moisture	102.3	0.22	0.32
Nutrients x Soil moisture	-		

Note: For sugar maple survival, models having nutrients and/or light, soil moisture interactions failed to converge due to parameter issues dealing with high levels of mortality.

Table 2. Results of nutrients added and year (main effects + interaction) on one- and two-year-old beech and sugar maple seedling survival (% year^{-1}) across three stands. Results based on generalized binomial mixed effects model. Significant effects are indicated in bold.

Response	Species	Treatment	Estimate	Std. Error	z value	Pr(>|z|)
Survival	American beech					
		N	**-2.971**	**1.097**	**-2.706**	**0.006**
		P	-0.764	0.647	-1.182	0.237
		Year	-0.478	0.601	-0.796	0.426
		N x P	**2.875**	**1.205**	**2.387**	**0.016**
		N x Year	-0.622	1.002	-0.621	0.534
		P x Year	0.332	0.905	0.368	0.713
	Sugar maple					
		N	**-2.603**	**1.089**	**-2.389**	**0.016**
		P	-42.71	988.9	0.000	1.000
		Year	**-1.504**	**72.14**	**-2.085**	**0.037**
		N x P	42.831	988.9	0.006	0.993
		N x Year	1.504	1.611	0.934	0.350
		P x Year	-29.03	196.3	0.000	1.000

Note: For sugar maple survival, model failed to detect P effect and P x Year effect due to parameter issues dealing with high levels of mortality in two out of three stands.

Table 3. Results (F-values and significance) of linear mixed effects model showing the effects of nutrients added and year (main effects + interaction) on seedling biomass and seedling root:shoot in year-one germinants, and one- and two-year-old beech and sugar maple across three stands.

Source	DF	F value (*P* value)			
		Biomass		Root:shoot	
		Beech	Sugar Maple	Beech	Sugar Maple
N	1, 24	0.07 (0.79)	0.67 (0.42)	<0.0001 (1.00)	0.13 (0.72)
P	1, 24	1.07 (0.31)	0.11 (0.74)	0.001 (0.99)	0.17 (0.68)
Year	2, 24	4.71 (**0.01**)	5.49 **(0.01)**	8.80 (**0.002**)	6.03 (**0.01**)
N x P	1, 24	0.94 (0.34)	0.09 (0.76)	0.27 (0.61)	0.98 (0.33)
N x Year	2, 24	0.41 (0.67)	0.01 (0.98)	0.72 (0.50)	0.27 (0.76)
P x Year	2, 24	0.57 (0.57)	0.57 (0.57)	0.61 (0.55)	4.61 **(0.02**)

Note: Significant *P* values are indicated in bold.

Table 4. Mean seedling biomass and root:shoot in treatment plots in year-one, and one- and two-year-old beech (BE) and sugar maple (SM) across three stands. Standard errors of the mean are in parentheses.

Treatment	Year 1				Year 2				Year 3			
	Biomass		Root:shoot		Biomass		Root:shoot		Biomass		Root:shoot	
	BE	SM	BE	SM	BE	SM	BE	SM	BE	SM	BE	SM
Control	0.25 (0.03)	0.13 (0.03)	0.35 (0.02)	0.34 (0.02)	0.29 (0.01)	0.11 (0.003)	0.46 (0.05)	0.40 (0.02)	0.39 (0.02)	0.21 (0.01)	0.59 (0.06)	0.57 (0.02)
N	0.24 (0.03)	0.11 (0.01)	0.35 (0.03)	0.36 (0.03)	0.24 (0.01)	0.09 (0.01)	0.49 (0.08)	0.40 (0.05)	0.40 (0.06)	0.19 (0.03)	0.52 (0.07)	0.53 (0.02)
N+P	0.25 (0.006)	0.11 (0.005)	0.31 (0.005)	0.29 (0.03)	0.24 (0.02)	0.13 (0.03)	0.51 (0.05)	0.45 (0.11)	0.38 (0.04)	0.20 (0.03)	0.53 (0.03)	0.50 (0.08)
P	0.20 (0.02)	0.12 (0.01)	0.35 (0.01)	0.37 (0.03)	0.24 (0.03)	0.13 (0.01)	0.47 (0.03)	0.61 (0.04)	0.40 (0.05)	0.18 (0.02)	0.52 (0.02)	0.49 (0.05)

Table 5. Results (F-values and significance) of linear mixed effects model showing the effects of nutrients added (main effects + interaction) on seedling specific root length (SRL; cm/mg) and specific leaf area (SLA; cm^2/mg) in year-one beech and sugar maple across three stands.

Source	DF	F value (*P* value)			
		SRL		SLA	
		Beech	Sugar Maple	Beech	Sugar Maple
N	1, 6	1.26 (0.31)	3.72 (0.10)	0.67 (0.45)	0.26 (0.62)
P	1, 6	3.07 (0.13)	0.13 (0.74)	1.81 (0.23)	0.11 (0.75)
N x P	1, 6	1.51 (0.26)	0.80 (0.40)	0.89 (0.38)	0.27 (0.62)

Table 6. Results (F-values and significance) of linear mixed effects model showing the effects of nutrients added (main effects + interaction) on seedling foliar C:N, C:P and N:P on one- and two-year-old beech and sugar maple across three stands.

Source	DF	F value (*P* value)					
		Foliar C:N		Foliar C:P		Foliar N:P	
		Beech	Sugar Maple	Beech	Sugar Maple	Beech	Sugar Maple
N	1, 18	5.69 (**0.02**)	5.32 (**0.03**)	1.86 (0.19)	0.30 (0.59)	0.17 (0.68)	0.11 (0.75)
P	1, 18	0.09 (0.76)	0.78 (0.39)	5.72 (**0.02**)	0.38 (0.54)	3.75 (0.06)	0.23 (0.64)
N x P	1, 18	0.08 (0.78)	0.16 (0.70)	0.61 (0.44)	0.36 (0.55)	0.39 (0.54)	0.29 (0.59)

Note: Significant *P* values are indicated in bold.

Table 7. Results (F-values and significance) of linear mixed effects model showing the effects of nutrients added and year (main effects + interaction) on seedling leaf area damage (%) in year-one, and one- and two-year-old beech and sugar maple across three stands.

Source	DF	F value (*P* value)	
		Leaf area damage (%)	
		Beech	Sugar Maple
N	1, 24	1.97 (0.07)	0.09 (0.76)
P	1, 24	1.32 (0.19)	0.01 (0.89)
Year	2, 24	1.51 (0.13)	1.48 (0.21)
N x P	1, 24	0.90 (0.29)	0.11 (0.76)
N x Year	2, 24	3.97 (**0.04**)	0.32 (0.72)
P x Year	2, 24	0.17 (0.84)	0.54 (0.58)

Note: Significant *P* values are indicated in bold.

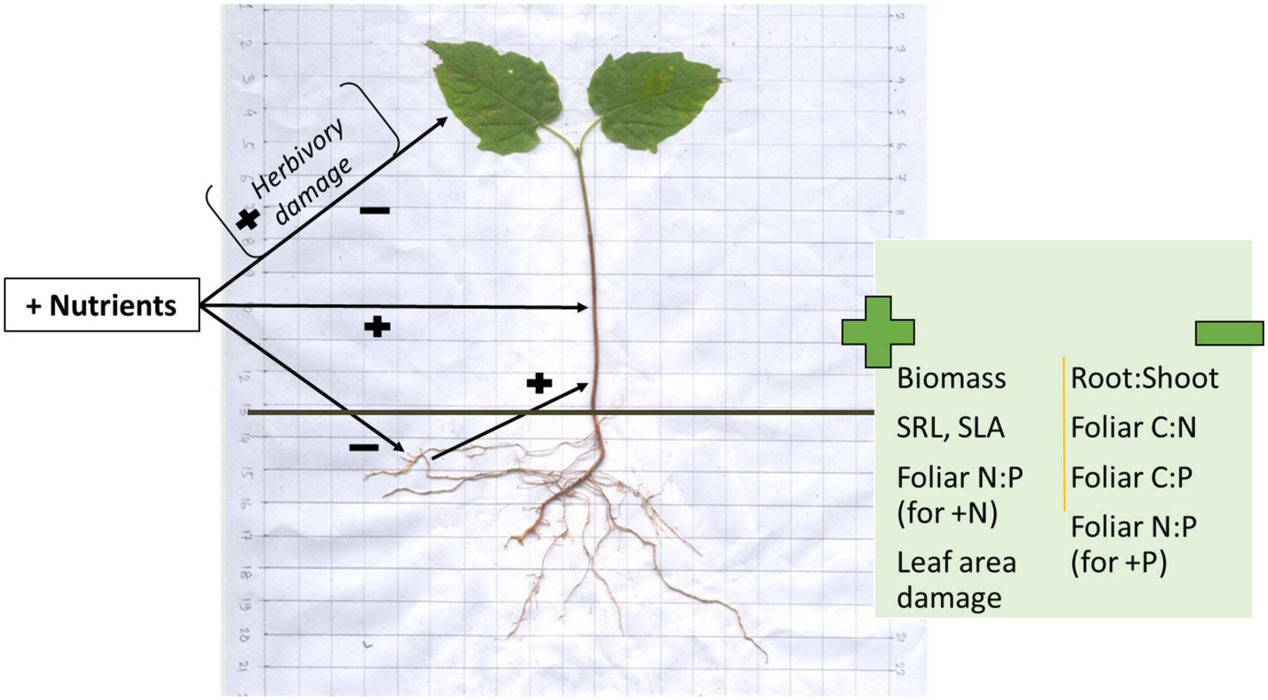

Figure 1. Simplified conceptual framework followed in this research, demonstrating links between various allocation responses at an individual plant level. Predictions are based on the assumption that seedling growth is nutrient limited.

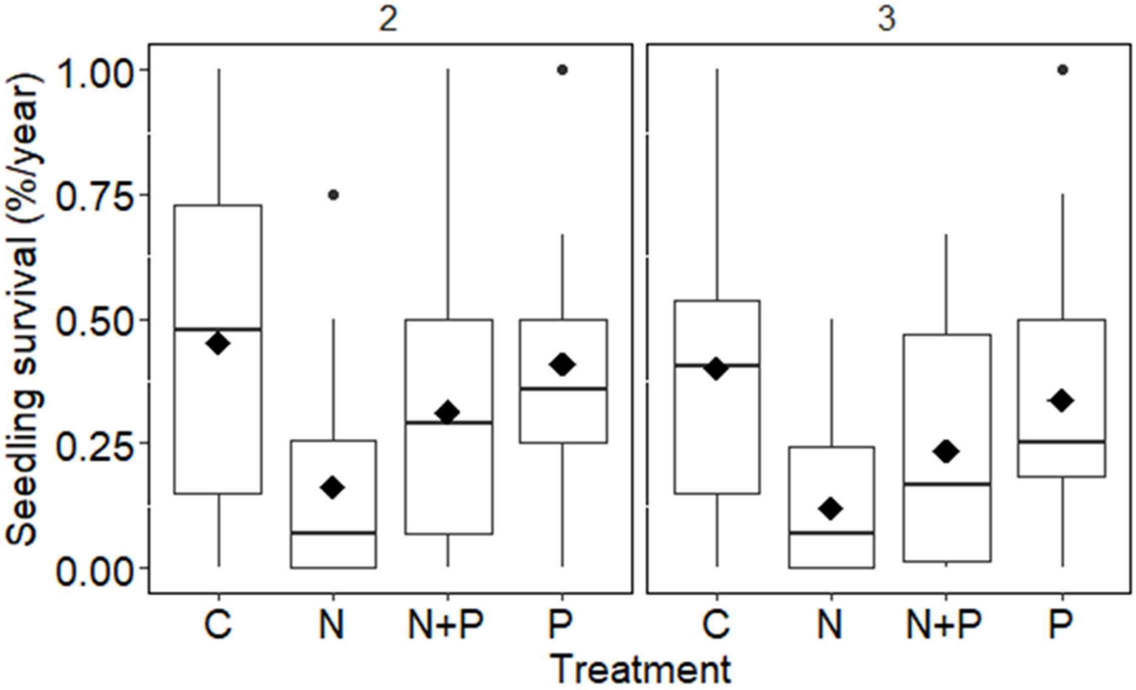

Figure 2. Survival of beech seedlings (%/year) in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) in year 2 (2012 to 2013) and 3 (2013 to 2014) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

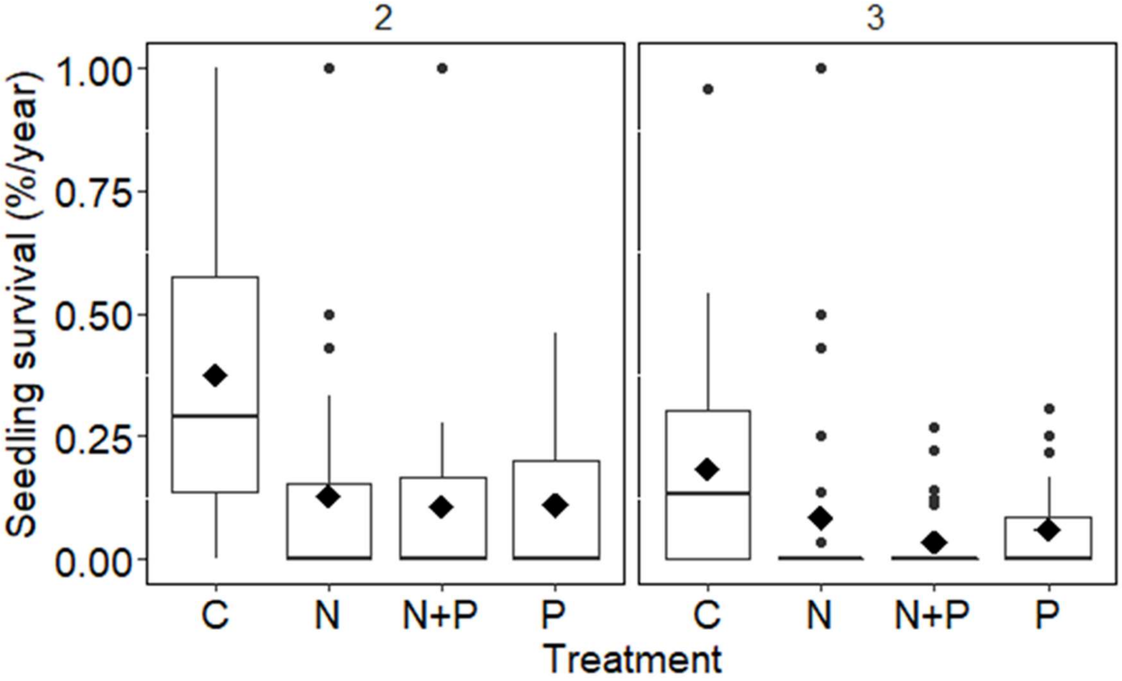

Figure 3. Survival of sugar maple seedlings (%/year) in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) in year 2 (2012 to 2013) and 3 (2013 to 2014) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

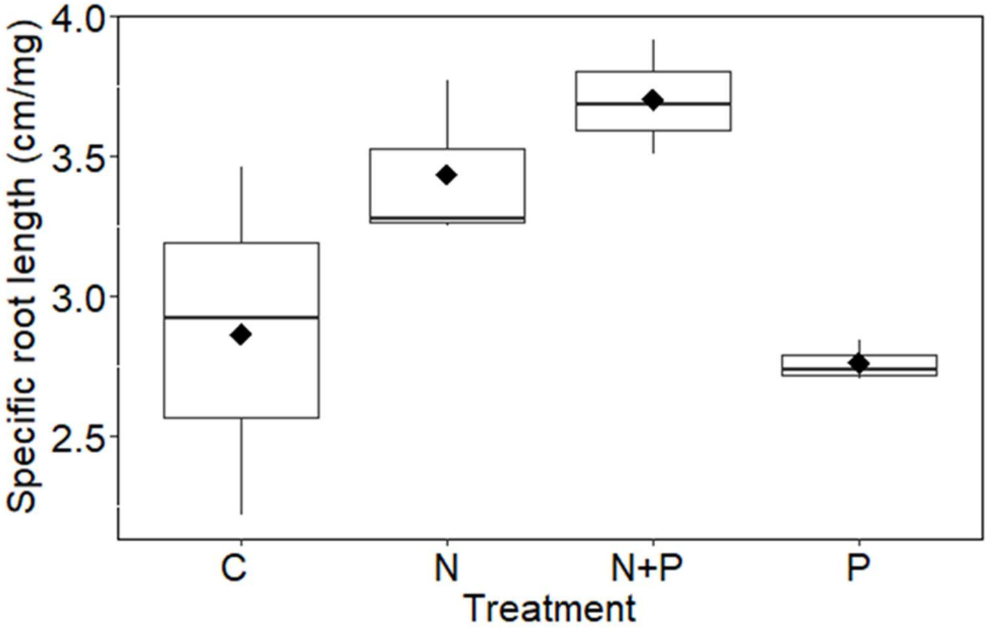

Figure 4. Specific root length in year-one sugar maple germinants in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

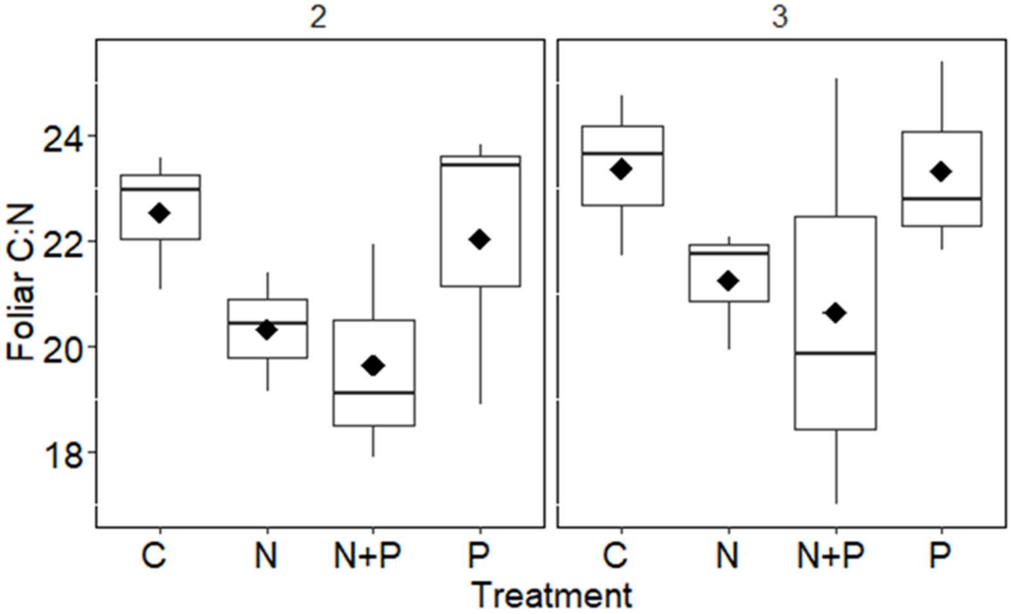

Figure 5. Foliar C:N in one- (panel 2) and two-year-old (panel 3) beech seedlings in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

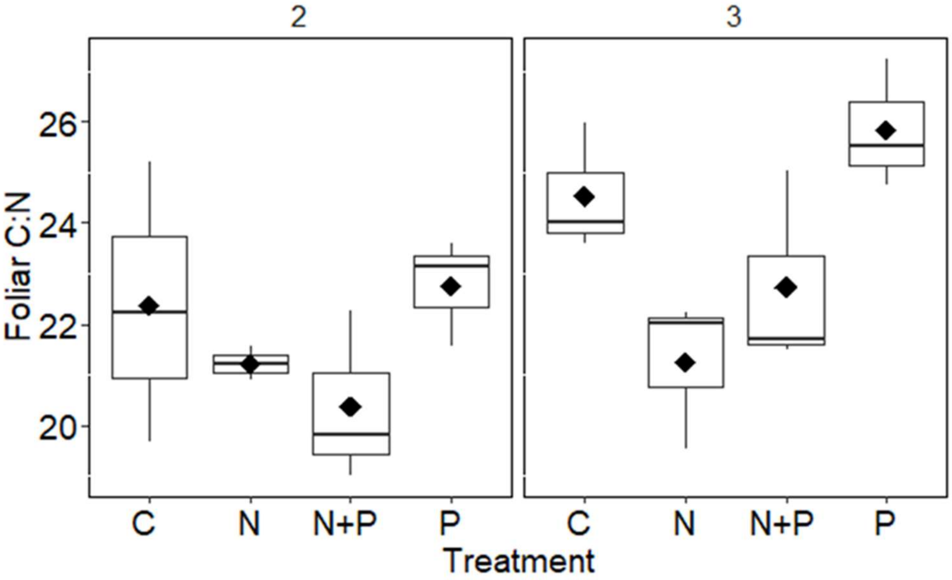

Figure 6. Foliar C:N in one- (panel 2) and two-year-old (panel 3) sugar maple seedlings in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

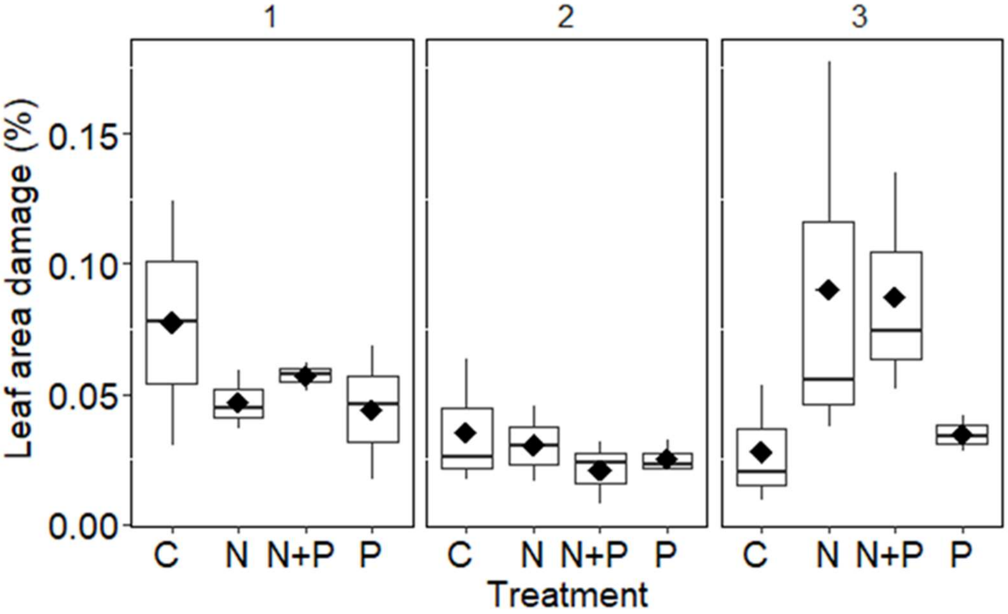

Figure 7. Leaf area damage (%) in year-one germinants (panel 1), and one- (panel 2) and two-year-old (panel 3) beech seedlings in C (control), N (nitrogen), N+P (nitrogen + phosphorus) and P (phosphorus) at three replicated mature stands in northern hardwood forests. Boxes represent the interquartile range and whiskers represent the 5th and 95th percentile, with the horizontal line showing the median and the triangle the mean value.

General Conclusions

My dissertation research identified profound independent and interactive effects of N and P that have implications for our understanding of the mechanisms that mediate N-P co-limitation (Fig 1., general introduction) of productivity in northern hardwood temperate forest ecosystems. Traditional single nutrient limitation theory considers northern hardwood forest productivity to be N limited, while resource optimization theory suggests I would detect N and P co-limitation however, I found evidence for P limitation of aboveground productivity on these relatively young, glacially derived forest soils (Chapter 1). I did not detect N and P co-limitation of aboveground productivity in these ecosystems. Defining and detecting co-limitation is challenging because it depends on the time scale of observations (Davidson and Howarth 2007), and the mechanisms contributing to a synergistic or additive response to N+P added together than to either nutrient alone, could differ depending on what specific ecosystem processe or sub-processes are studied. Organisms (plants and soil microbes) require both N and P in stoichiometrically balanced proportions and they have physiological mechanisms that enable them to maintain this balance between the required nutrients. Irrespective of whether elevated nutrient availability stimulates processes such as soil-microbial feedbacks or increases aboveground growth of tree species, ecosystem level N and P co-limitation should be evident when biological activity is higher in response to N and P added together than to either added alone.

Experimental tests of N vs P limitation in temperate forest ecosystems are few and short-term with nutrient manipulation that are typically at much higher rates than what is experienced by natural variation and short-term anthropogenic perturbations. My dissertation research used a long-term factorial N x P study with moderate rates of nutrient application that better mimicked perturbations caused by natural and anthropogenic changes. The study design also spanned a gradient of forest ages to better understand whether time since forest harvest interacts with long-term change such as N deposition to induce a transient limitation by another nutrient in these ecosystems. I found a greater response of aboveground production in response to P addition compared to N addition in the mid-age and mature forests. Consistent with the Multiple Element Limitation (MEL) model, my results indicate N limitation in younger forests. Species-specific responses were generally consistent with whole-ecosystem responses, but highlighted interesting shifts in limitation by N vs P as young forests mature. My research suggests that a nutritional imbalance owing to long-term N enrichment in northern hardwoods may have alleviated N limitation of productivity in these ecosystems, and have induced a transactional limitation of productivity by P (Chapter 1). Increased aboveground production with P addition in these successional forests is consistent with substantial reduction in foliar N:P in similar forests following five years of nutrient fertilization (Fahey et al. 1998), suggesting P limitation in these forests. However, it is important to consider whether this observation of P limitation after four growing seasons with elevated nutrient availability would continue to persist (which is likely as a consequence of historic N deposition), or whether the ecosystem will start resynchronizing the cycles of N and P as forest succession progresses, and eventually shift to a stage where clear observations of N-P co-limitation would be evident.

To address whether these ecosystems can maintain balance of nutrient availability in response to shorter-term disruptions within the context of long-term change, I tested short-term responses by one nutrient to an excess of another (Chapter 2). I found microbial suppression of plant-available N in response to P addition, which suggests alleviation of P limitation with P addition and a subsequent increase in biotic uptake of N. However, it is likely that excess of P relative to N over time could increase N losses via biotic uptake of N or via denitrification losses of N. Such N losses will eventually constrain the availability of N, and mediate the on-set of sequential N-P co-limitation of productivity in these ecosystems.

Another important contribution of this dissertation was to investigate whether N and P availability regulate recruitment in the northern hardwoods and examine mechanistic links that show how co-dominant species in these forests allocate C in response to changes in nutrient availability (Chapter 3). Although, not necessarily representative of mature trees, seedlings are expected to respond to environmental changes more rapidly than mature trees. Additionally, it was possible to study whole-plant allocation in seedlings, which is central to understanding how plants meet competing needs for multiple resources such as light, water and nutrients. Seedling survival in American beech and sugar maple was strongly suppressed by N availability and allocation was relatively unresponsive to elevated nutrients, despite increased foliar nutrition. My findings suggest that nutrient addition leads to improved nutrition and a potential increase in nutrition is generally associated with incremental costs (e.g. herbivory damage). Within the context of the forest understory, favorable microsites with greater belowground resource availability (nutrients and water) regulate recruitment and growth in these shade tolerant species. Although there were few notable seedling responses to P addition, it is evident that N is still the most crucial nutrient for seedling recruitment and this study suggests the importance of further investigations on contribution of long-term N deposition on the failure of tree species regeneration, especially for sensitive species like sugar maple.

It is important to recognize the paradigm shift from single nutrient effects towards considering effects of multiple nutrients on dynamic ecosystems. Concurrent with resource optimization processes of biota, single nutrient limitation is expected to be minimized whether in terrestrial or aquatic ecosystems. Recent syntheses (Elser et al. 2007; Harpole et al, 2011; Kaspari and Powers 2016) and experimental observations (Arrigo 2004; Craine et al. 2008, Sperfeld et al. 2012, Bracken et al. 2015; Eskelinen and Harrison 2015; Fay et al. 2015; La Pierre et al. 2016) provide examinations of nutrient fertilization studies across different ecosystems and suggest that co-limitation is an important mechanism which could mediate the effects of nutrient additions on productivity in different ecosystems. This dissertation adds to that growing body of literature that has found important and interactive effects of nutrients on forest responses. This study recognizes that not all of the detected responses to nutrient additions in this experiment lead towards N and P co-limitation, and it is very likely that co-limitation will not be attained even in presence of mechanisms favoring balance in nutrient availability. However, it is important to consider that as nutrient availability continue to change as a result of natural and anthropogenic disturbances, examining forest responses is necessary to predict future responses to nutrient alterations and understand potential limitations to forest production.

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