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Co-limitation of Fine Root Growth by Nitrogen and Phosphorus in Early Successional Northern Hardwood Forests

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Abstract

Functional balance theory predicts that plants will allocate less carbon belowground when the availability of nutrients is elevated. We tested this prediction in two successional northern hardwood forest stands by quantifying fine root biomass and growth after 5–7 years of treatment in a nitrogen (N) x phosphorus (P) factorial addition experiment. We quantified root responses at two different levels of treatment: the whole-plot scale fertilization and small-patch scale fertilization of ingrowth cores. Fine root biomass was higher in plots receiving P, and fine root growth was highest in plots receiving both N and P. Thus, belowground productivity did not decrease in response to long-term addition of nutrients. We did not find conclusive evidence that elevated availability of one nutrient at the plot scale induced foraging for the other nutrient at the core scale, or that foraging for nutrients at the core scale responded to addition of limiting nutrients. Our observations suggest NP co-limitation of fine root growth and indicate complex interactions of N and P affecting aboveground and belowground production in early successional northern hardwood forest ecosystems.

Key words: Fine root; Belowground carbon allocation; Co-limitation; Phosphorus; Nitrogen; Northern hardwood forest.

Highlights

- Fine root growth was stimulated by combined N and P additions
- Fine root biomass was higher in plots receiving P addition
- Belowground production may be N and P co-limited

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Author Contributions TJF, MCF, RDY, and SL designed the study. SL and TJF collected the data. SL performed analyses and drafted the manuscript, and TJF, MCF, and RDY contributed substantially to writing and revisions.

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Introduction

Plant growth is constrained by a variety of environmental resources that plants require, including light, carbon dioxide, soil water and mineral nutrients. The multiple limitation hypothesis states that plants adjust their growth patterns such that they are limited by several resources simultaneously (Ågren and others 2012; Bloom and others 1985). The nature of this ''co-limitation'' of plant growth has attracted recent attention because environmental changes in atmospheric CO_2, N deposition, rainfall, and soil base cation depletion are disrupting the natural balance of these resources.

Theoretically, plants should achieve maximum productivity by allocating their assets to maintain an optimal balance and to facilitate further acquisition of various resources in the face of differing availability or supply (Rastetter and others 2013). The acquisition of C by foliage can be balanced with that of water and nutrients by roots and mycorrhizae by the adjustment of root/shoot ratio to assure sufficient light and soil resource capture in the face of competition and soil resource scarcity. Thus, root/shoot ratios are expected to decline in response to fertilization or irrigation, and aboveground growth is maximized by increasing proportional allocation of C to shoots, according to the functional balance theory (Thornley 1991).

This allocation theory has been questioned by Simon and others (2017), who suggested that increases in soil nutrient supply might enhance the C sink strength of roots and C allocation belowground such that root growth remains proportional to aboveground growth. In fact, fine root growth and biomass have been seen to increase in response to N and P additions in some tropical forests (Alvarez-Clare and others 2013; Zhu and others 2013). However, a meta-analysis across 48 experiments in tropical forests did not find that nutrient addition consistently altered fine root biomass (Wright 2019). The inconsistency of belowground C allocation responses to elevated nutrient availability might be due to the nature of nutrient limitation—it is possible that forests experiencing single-element limitation of growth will allocate less to root growth in response to alleviating that limitation, whereas those experiencing nutrient co-limitation of growth will respond in more complex ways.

Reviews of the literature have indicated that co-limitation by N and P is common in many global aquatic and terrestrial biomes (Elser and others 2007; Harpole and others 2011). Unfortunately, few direct tests of N-P co-limitation have been conducted in temperate forests, and those that have been reported (Finzi 2009) were short-term with levels of nutrient addition likely to disrupt the natural balance among nutrient acquisition mechanisms and other processes. Fertilization experiments in eastern deciduous forests commonly show N limitation of aboveground growth and some evidence for P limitation, but there were insufficient data to test for co-limitation by multiple nutrients (Vadeboncoeur 2010). The Multiple Element Limitation model suggested that young, successional northern hardwood forests should be most responsive to N addition because of large losses of N from labile pools that commonly occur after intensive forest harvest (Rastetter and others 2013). Thereafter, according to the model, co-limitation by N and P should develop over time as nutrient supply becomes re-synchronized via recycling mechanisms. On the other hand, long-term, high atmospheric N deposition in northeastern North America (Driscoll and others 2003) could induce P limitation in what would otherwise be an N limited system, a condition designated ''transactional P limitation'' (Vitousek and others 2010). Finally, changes in the availability of one limiting nutrient could induce stoichiometrically imbalanced nutrition, interfering with any growth response to single nutrient addition. Resolution of these complexities is needed before a better understanding of the nature of temperate forest responses to soil nutrient limitation is possible.

We initiated a long-term nutrient addition experiment to test for Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE). Since 2011 we have added relatively low levels of N and P in a full factorial design to a suite of 13 forest stands in the White Mountains, NH (Goswami and others 2018). The treatments have increased soil available N and P (Fisk and others 2014; Fisk 2019) and altered foliar N and P (Hong and others 2022), soil respiration (Mann 2021), and tree growth (Goswami and others 2018). In the present study, we examined the effects of nutrient addition on standing fine root ($<$ 1 mm diameter) biomass and growth in control and treated plots in two early successional stands (age 20–22 years at the start of the experiment). Our study design included N, P as well as N + P addition to investigate possible co-limitation. We also measured aboveground production in these plots to better characterize nutrient limitation and to infer relative carbon allocation aboveground vs belowground. We studied two replicate stands in hopes of demonstrating consistent responses to the treatments.

Our overarching idea was that N and P might be co-limiting to forest productivity in these early successional stands, and we sought evidence on the mechanisms underlying such co-limitation. On one hand, according to the functional balance theory, we hypothesized that increased availability of these limiting nutrients would allow increased aboveground production by reducing the belowground allocation (H_1). Alternatively, addition of limiting nutrients could directly stimulate aboveground production along with higher total carbon allocation belowground and increased root growth and biomass (H_{1a}) (Hendricks and others 1993; Nadelhoffer 2000). We addressed this hypothesis and its alternative by testing treatment effects, including an N and P interaction, on fine root growth and biomass, aboveground production, the ratio of fine root growth to aboveground production, and total forest production. We also evaluated fertilization effects on fine root turnover and soil respiration as additional indicators of carbon allocation belowground.

As further evidence about the nature of nutrient co-limitation we examined fine root growth into patches of nutrient enrichment, so-called ''fine root foraging.'' Fine root foraging has been widely used to identify growth-limiting nutrients (Raich and others 1994; Gleeson and Good 2003; Naples and Fisk 2010; Giehl and von Wirén 2014). We predicted greater root growth into soil cores enriched with growth-limiting nutrients in control (unfertilized) plots (H_2), and we expected this fine root foraging response to reflect any differences in total forest production across the treatments. Finally, to connect the belowground allocation responses to changing nutrient availability (H_1) with the mechanisms of co-limitation, we sought further evidence regarding possible nutritional imbalances induced by single nutrient additions. We evaluated the hypothesis that addition of a single limiting nutrient at the whole-plot scale could induce a nutritional imbalance and thereby stimulate fine root foraging for the other nutrient(H_3).

Methods

Site Description

The analyses in this report are from the two youngest study stands (C1 and C2) in the Bartlett Experimental Forest (BEF) within the MELNHE long-term fertilization experiment in the White Mountains of New Hampshire, the USA (Goswami and others 2018). Both stands regenerated naturally following clear-cutting (C1 in 1990, C2 in 1988) and are classified as early successional northern hardwoods (Table 1). Although the composition of the two stands differs slightly, the dominant tree species in both stands are white birch (*Betula papyrifera* Marsh.), pin cherry (*Prunus pensylvanica* L.f.), American beech (*Fagus grandifolia* Ehrh.), red maple (*Acer rubrum* L.), sugar maple (*A. saccharum* Marsh.), and yellow birch (*B. alleghaniensis* Britton) (Table 1). Stand C1 is located at 510 m elevation on a gentle, south-facing slope (5–15%), while stand C2 is at 340 m on a moderate northeast-facing slope (10–30%). Soils are well-drained Spodosols (Typic Haplorthods) formed in granitic glacial drift. The climate is humid continental; average annual mean temperature is 6.2 °C and average annual precipitation is 1270 mm (Bartlett Experimental Forest NEON, n.d.). Between 1979 and 2003, wet N deposition in this area was 4–7 kg N ha^{-1} $year^{-1}$, but it has declined to about 2–4 kg N ha^{-1} $year^{-1}$ since 2008 (NADP Program Office, 2017). Atmospheric deposition of P in this region is negligible, at$\sim$0.04 kg P ha^{-1} $year^{-1}$ (Yanai 1992).

Nutrient Addition Scheme

In each stand four 50 m × 50 m experimental plots were delineated and assigned to one of four treatments: control, N, P, and NP (that is, 2 × 2 full factorial). The 30 m × 30 m central measurement area is surrounded by a 10 m buffer. Beginning in 2011, each plot received its designated treatment at the start of growing season: N in the form of NH_4NO_3 (30 kg N ha^{-1} y^{-1}), P in the form of $NaHPO_4$ (10 kg P ha^{-1} y^{-1}), and NP plots with both at the same rates. Control plots were not fertilized but received trampling associated with fertilization activity as a control. Treatment effects on soils were measured using resin strips (Table 2).

Ingrowth Cores

We estimated rates of fine root growth in each plot by using ingrowth cores containing soils from the same plot (that is, C, N, P or NP). We studied fine root foraging by including two types of ingrowth cores with ''transplanted'' soils (Figure 1). In the control plots, soil from each of the nutrient-treated plots was used in ingrowth cores. Additionally, in the N plots, soil from P treatment plots was used in ingrowth cores, and in P plots, soil from the N treatment plots was used in ingrowth cores (that is, reciprocal transplant). In each case 10 replicate cores were installed at equal spacing along transects in the designated plot. Thus, 90 ingrowth cores were installed in each stand: 40 in control plots

Table 1. Site Descriptions for Two Forest Stands at the Bartlett Experimental Forest, New Hampshire

Stand	Year cut	Basal area (m^2 ha^{-1})	Fine root biomass 0–30 cm (g m^{-2})	Leaf litterfall (g m^{-2} $year^{-1}$)	Soil N mineralization (μg g^{-1} soil d^{-1})	Soil resin P (μg g^{-1} soil)	Dominant species
C1	1990	25.2	407 ± 37	314 ± 18	8.7 ± 2.1	9.3 ± 1.7	WB, PC, AB
C2	1988	23.4	362 ± 25	331 ± 9	7.8 ± 2.1	7.4 ± 1.4	RM, WB, AB

Dominant species are listed in order of importance by their contribution to stand basal area (WB = white birch, PC = pin cherry, AB = American beech, RM = red maple). Pre-treatment fine (< 1 mm diameter) root biomass was measured in 2010. Pre-treatment leaf litterfall mass and soil N mineralization and resin available P (average of Oe, Oa, and mineral horizon) were measured in 2009 (Fisk 2019).

Table 2. Post-treatment Soil Resin N and P (Average of 2015, 2016, 2017, and 2019)

Stand	Treatment	Resin-available N (μg $strip^{-1}$ d^{-1})	Resin-available P (μg $strip^{-1}$ d^{-1})
C1	Ctrl	63 ± 31	13 ± 5
	N	1127 ± 401	12 ± 4
	P	18 ± 5	88 ± 53
	NP	426 ± 170	186 ± 118
C2	Ctrl	20 ± 12	6 ± 2
	N	308 ± 219	6 ± 3
	P	16 ± 7	132 ± 94
	NP	150 ± 128	65 ± 41

Resin strips were incubated in the Oa horizon for approximately 3 weeks beginning 2 to 5 weeks after fertilizing. Values for N are the sum of available N from NO_3 and NH_4 resin strips (Fisk 2019).

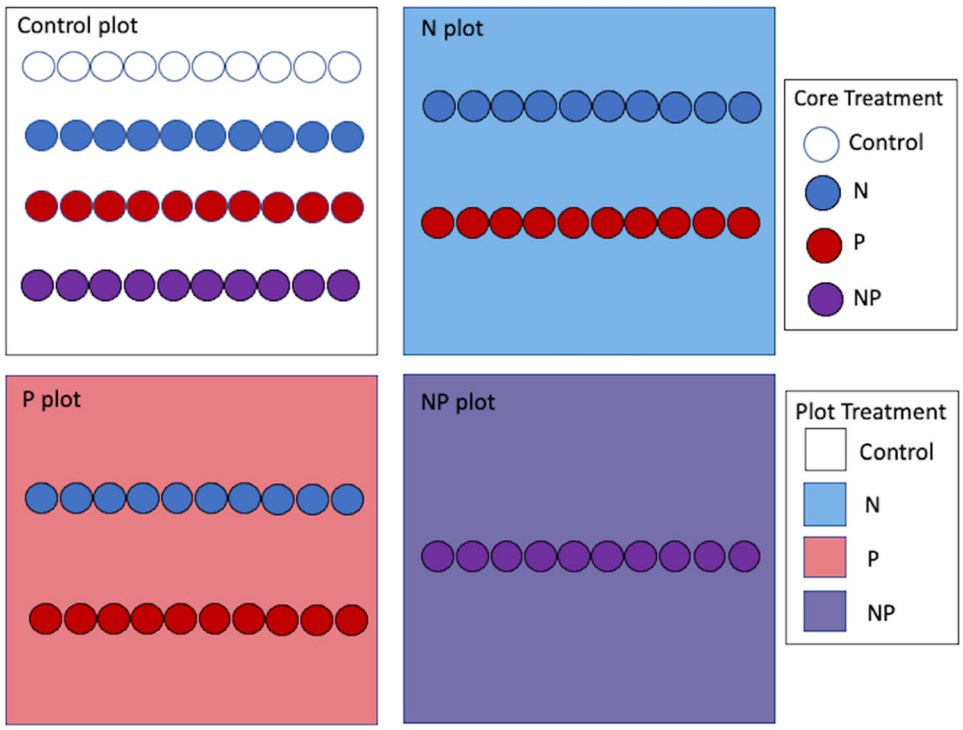

Figure 1. A conceptual diagram showing the experimental design of the ingrowth cores experiment.

(with soil originating from C, N, P, and NP plots), 20 in N plots (with N- or P-treated soils), 20 in P plots (with P- or N-treated soils), and 10 in NP plots (with NP soil only) (Figure 1).

We installed the ingrowth cores in the two stands in early October 2017 in the treated buffer zones, to minimize disturbance in the measurement areas. Ingrowth cores were established in autumn to maximize the time for soil to settle before the growing season begins. Soil cores were removed to 20 cm depth with a 5-cm-diameter corer, and the soils were discarded. Three steel rods (0.5 cm diameter) were positioned vertically on the wall of the core holes to assist with later retrieval, and the holes were filled with soil taken from the appropriate plot (See above Figure 1) that had been sieved to remove roots and coarse fragments. Each ingrowth core received the designated nutrient treatment (See above Figure 1) at the same rate and time of plot-level nutrient additions in early June 2018.

At the time of harvest in October 2018, a 4-cm-diameter corer was used to extract root-colonized soil from inside the rods. Harvested ingrowth cores were returned to the laboratory cold and then frozen at −20 °C until processing. Live fine roots of 0–1 mm diameter were collected from each core; dead roots were distinguished by their dark color and low tensile strength. Fine roots were cleaned of adhering soil over a fine sieve, dried to constant mass at 70 °C and weighed.

Fine Root Biomass

Fine root biomass was measured in each plot in late August 2010 (pre-treatment) and in August 2015 by soil coring and manual dry sorting of live roots from soil. Twelve soil cores were collected in each plot in 2010, and ten soil cores were collected in each plot in 2015 from locations near our permanent soil respiration collars, adjusted to avoid large roots and rocks. After removing the litter layer (Oi horizon), a 5-cm-diameter split-PVC pipe corer was hammered into the soil with a rubber mallet. The nominal depth of sampling was 30 cm but because of obstructions the actual depth of sampling averaged 27 cm. Each core was divided in the field into two depth increments, 0–10 cm (including the Oe and Oa horizon and usually some mineral material) and 10–30 cm (dominantly E and B mineral horizons with varying amounts of organic matter). Samples were transported to the lab for storage at −20 °C until laboratory processing.

Live fine roots of 0–1 mm diameter were hand sorted from each sample; dead roots were distinguished by their dark color and low tensile strength. For roots in the 0–10 cm depth increment, fine root biomass was estimated from the diminishing root mass recovered during sequential, timed picking intervals (Metcalfe and others 2007). For this approach, we manually extracted roots from soil cores for four intervals of 10 min each, and the cumulative biomass extracted over time was predicted by fitting a logarithmic curve. Total fine root biomass was estimated at the point at which the predicted incremental root mass extracted in the next 10 min time interval was $< 2\%$ of the cumulative total. On average, this required 6 more picking intervals, representing a time savings of one hour per core. For 10–30 cm samples, which had less root mass, all roots were hand sorted from each core. Sorted roots were washed free of adhering soil on a fine sieve, dried to constant mass at 70 °C and weighed. Total fine root biomass of each soil core was calculated by summing the biomass from the two soil depths.

Fine Root Turnover

Annual fine root turnover for each treatment plot was estimated as the ratio of annual fine root growth to fine root biomass (Table 3).

Soil Respiration

Flux of CO_2 from the soil (soil respiration) was measured in each plot using a LI-8100 system (Li-cor Biosciences, Lincoln, NE). Seven PVC collars (10 cm diameter) were systematically installed in each plot, avoiding any large tree roots and boulders. Collars disturbed by animal activity were reinstalled at nearby locations prior to measurement early in the field season each year. Care was taken to apply fertilizer to the cores at a rate consistent with the plot-level addition. Soil respiration was measured between 9 AM and 4 PM in each plot during the warm season, June–August. In 2016, four measurements of each collar were made, and in 2017, two measurements were made. Soil temperature was measured at 10 cm depth near the respiration collars, while soil respiration rates were taken.

Forest Productivity

In each plot, we measured the diameters and recorded the species of all trees > 10 cm in diameter in the 30 m $\times$ 30 m measurement area and of trees 2–10 cm in diameter on 5 smaller 5 m $\times$ 5 m subplots in 2015 (Goswami and others 2018) and 2019. We estimated aboveground production in

Table 3. Fine Root Turnover Index, The Ratio of Annual Fine Root Growth to Aboveground Production, and Warm Season Mean Soil Respiration with Standard Errors (n = 6 Measurement Dates, Each Represented by the Median of 7 Collars in the Plot, Year 2016 and 2017) (Fahey and others 2021) in Control and Treated Plots of Two Forest Stands at Bartlett Experimental Forest, New Hampshire

Stand	Treatment	Fine root turnover index (per year)	Root growth: Aboveground production	Soil respiration(μmol CO_2 /m^2/second)
C1	Ctrl	0.27	0.08	6.1 ± 0.7
	N	0.26	0.09	5.8 ± 0.7
	P	0.28	0.09	7.0 ± 0.7
	NP	0.29	0.11	7.3 ± 0.6
C2	Ctrl	0.43	0.17	7.3 ± 0.8
	N	0.31	0.12	5.8 ± 0.6
	P	0.31	0.15	8.8 ± 1.1
	NP	0.50	0.25	6.3 ± 0.6

each plot as the sum of average annual wood production and leaf litterfall production based on measurements from 2015 to 2019. Wood production was estimated as the change in live biomass plus mortality (Kloeppel and others 2007). Biomass of live trees in 2015 and live and dead trees in 2019 was estimated using locally derived allometric equations (Fatemi and others 2011) based on tree diameter and species. Mortality was equal to the woody biomass that died between 2015 and 2019. Leaf litterfall was collected using five systematically placed litter baskets (each 0.23 m^2) in each plot in spring, late summer and late fall of 2015–2018. Litter was returned to the laboratory, dried to constant mass and weighed.

Total forest production was estimated as the sum of annual aboveground production and annual fine root growth.

Statistical Analysis

We conducted analysis of variance in R (*R: The R Project for Statistical Computing*, n.d.). To analyze root growth in each plot (H_1 and H_{1a}), we used N-amended cores in N-addition plots, P cores in P plots, control cores in control plots, and NP cores in NP plots. Fine root ingrowth per unit ground area per year was the response variable, and values were log transformed to achieve normality of residuals. Explanatory variables were the main effects of N and P and their interaction and stand was included as a blocking factor. A linear mixed-effects model (Bates and others 2015) was applied with random effect of plots nested within stands. A post hoc Tukey comparison was conducted to compare the estimates for the four treatments.

To test for N, P or NP fine root foraging for limiting nutrients (H_2), we compared fine root ingrowth per unit area per year in enriched cores and control cores in the control plots; values were log transformed to achieve normality of residuals. Linear regression was used to test the main effects of N and P and their interaction, with stand as a blocking factor.

To measure fine root foraging for one nutrient in plots fertilized at the whole-plot scale with the other nutrient, we used both N and P cores in N and P plots. Fine root ingrowth per unit area per year was the response variable; values were log transformed to achieve normality of residuals. A linear mixed-effects model was applied with the random effect of plots nested within stands. The explanatory variables were the main effects of plot-level treatment (N or P) and core-level treatment (N or P), and the interaction of plot-level and core-level treatment, and stand was included as a blocking factor. To test H_3, we used contrasts of N cores in P plots to P cores in P plots, and P cores in N plots to N cores in N plots.

For standing fine root biomass (H_1 and H_{1a}), fine root biomass (0–30 cm depth) per unit ground area was the response variable; values were log transformed to achieve normality of residuals. Linear mixed-effects models were used to test the main effects of N and P and their interaction, with stand as a blocking factor and plots nested within stands as random effects. A post hoc Tukey comparison was conducted to compare the estimates for the four treatments. We also tested the importance of the pretreatment plot-average standing root biomass as a covariate using Akaike's Information Criterion (AIC).

We tested the effect of fertilization on aboveground production and its components (woody production and leaf litterfall), fine root turnover,

the ratio of root growth to aboveground production, and total forest production in separate linear models (H_1 and H_{1a}). We used the main effects of N addition and P addition and their interaction, with stand as a blocking factor. When a treatment effect was detected, a post hoc Tukey comparison was conducted to compare the estimates for the four treatment plots.

We tested for effects of fertilization on soil respiration using the main effects of N and P addition and their interaction. We used a linear mixed-effects model with stand and year as blocking factors and soil temperature as a covariate, with the six measurement occasions and plots nested within stands as random effects.

For all our tests, ANOVA and coefficient tables and the results of comparison are provided in the supplemental material. We report p values rather than specify alpha to avoid dichotomization and promote transparency (Amrhein and others 2019).

Results

Root Ingrowth Cores

To test for a fine root growth response in each treatment plot, we compared ingrowth cores that received the same nutrient treatment as the plot-level treatment (Figure 2A). Fine root ingrowth in P-amended plots (P and NP) was 45% higher than in those not receiving P ($p = 0.03$ for the main effect of P) (Table S1 & S2). The P effect appears to be driven not by a response to P alone but by the large response to N + P (65% greater than the response to P; Figure 2A), consistent with the evidence of an N x P interaction ($p = 0.09$). We did not detect main effects of N on fine root ingrowth ($p = 0.20$). On average, stand C2 had 44% higher fine root ingrowth than C1 ($p = 0.08$).

We did not find evidence of fine root foraging for N or P in the control plots: There were no effects of core-scale N or P addition or an N x P interaction on fine root ingrowth detected (Figure 2B, Table S4 &S5). Again, the cores in C2 exhibited much higher fine root ingrowth than in C1 (57%, $p < 0.001$).

To explore the possibility that adding one nutrient at the whole-plot scale would induce foraging for the other nutrient, we compared fine root ingrowth into cores containing soil from the same plot (for instance, N cores in N plots) with ingrowth into cores containing soil from the plot treated with the other nutrient (for instance, P cores in N plots). In each of the P-addition plots, average root colonization of N cores exceeded that of P cores (Figure 2C); however, within-plot variation was high and we did not detect fine root foraging for N (Table S8). Plot-scale treatments ($p = 0.04$) explained more variation in root ingrowth than did core-scale treatments ($p = 0.95$) or the interaction between plot-scale and core-scale treatment ($p = 0.18$). Again, ingrowth was higher in C2 than in C1 (23%, $p = 0.09$) (Figure 2C, Table S6 & S7).

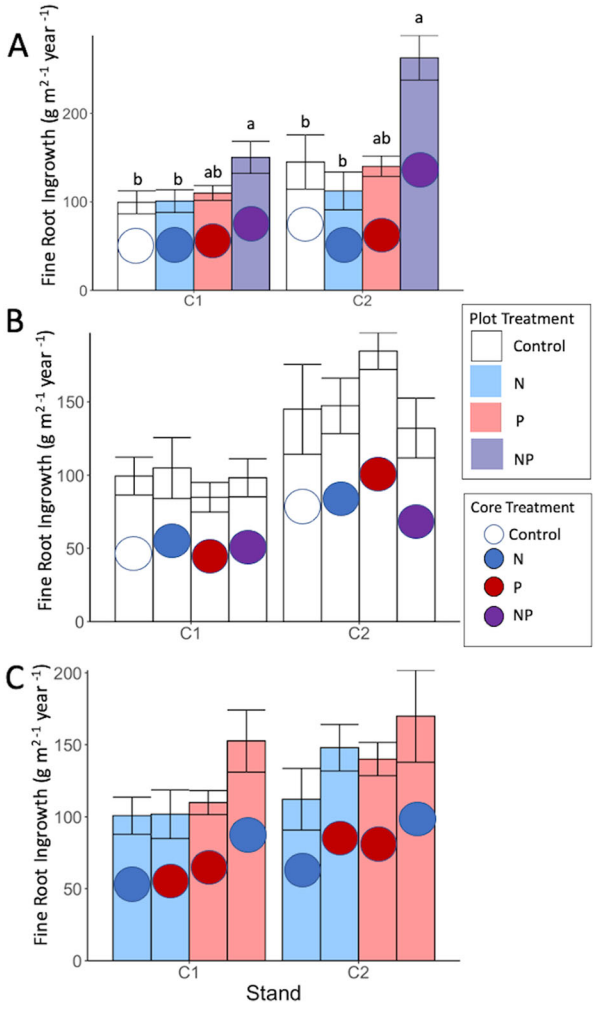

Figure 2. Fine root growth and foraging, indicated by ingrowth cores (Fahey and others 2023). Values are plot means, and the bars are one standard error of the mean ($n = 10$). Means sharing lowercase letters did not differ with $p < 0.10$ based on Tukey's test. **A** Fine root ingrowth in cores containing soil from the same treatments as the plots. **B** Fine root ingrowth in cores filled with nutrient-amended soil in control plots, indicating microsite foraging for nutrients. **C** Fine root ingrowth in N and P plots, including reciprocal transplant of soils.

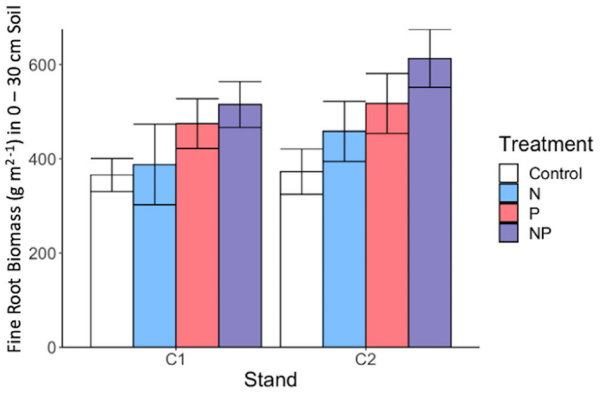

Figure 3. Standing fine root (< 1 mm) biomass of soil depth 0—30 cm measured as g/m^2 under long-term nutrient addition treatments (Fahey and others 2023). Values are treatment means, and the bars are one standard error of the mean (n = 10).

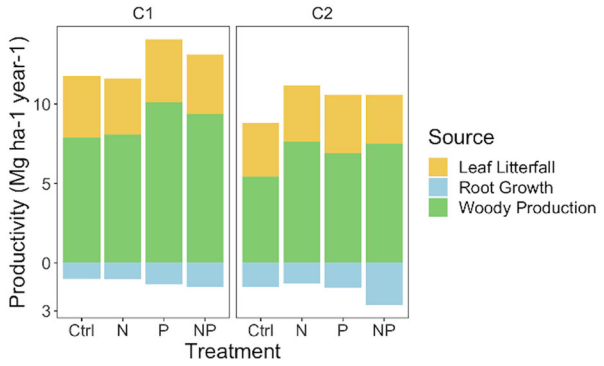

Figure 4. Primary production components in control and treated plots of two forest stands at Bartlett Experimental Forest, New Hampshire: annual woody production (average 2015–2019) (Fisk and others 2022a), annual leaf litterfall (average 2015–2018) (Fisk and others 2022b), and root production based on ingrowth cores (2018) (Fahey and others 2023).

Fine Root Biomass

As further evidence regarding the effects of nutrient additions on belowground C allocation, we measured fine root biomass (< 1 mm; 0–30 cm depth) in each stand and plot before treatment in 2010 and after five years of nutrient addition in 2015. Surprisingly, pre-treatment fine root biomass in 2010 was not a strong predictor of fine root biomass in 2015 (p = 0.43 for the covariate) and including the pre-treatment data did not improve the AIC (139.07 with the covariate and 138.25 without). Fine root biomass was 32% greater in plots receiving P than no P (p < 0.0001 for main effects of P), whereas no effects of N, N x P interaction, or stand were detected (Figure 3, Table S9 & S10).

Fine Root Turnover

Fine root turnover (Table 3) was not responsive to N (p = 0.71), P (p = 0.57), or their interaction (p = 0.15) (Table S12 & S13). Fine root turnover in stand C2 was 1.6 times higher than in C1 (p = 0.08), due to higher growth rates in C2.

Forest Production and Soil Respiration

Woody production (Figure 4) did not respond consistently to the treatments (p = 0.42 for the main effect of N; p = 0.13 for the main effect of P; and p = 0.35 for the N by P interaction) (Table S14 & S15). Notably, tree mortality was high, accounting for 20 to 40% of estimated production. Woody production was 16% higher in C1 than C2 (p = 0.04) (Figure 4, Table S14 & S15).

Litter production was not consistently affected by treatments (p = 0.16 for the main effect of N; p = 0.86 for the main effect of P; p = 0.37 for the interaction between N and P) (Table S16 & S17). On average stand C1 produced 10% more leaf litter than C2 (p = 0.08) (Figure 4, Table S16 & S17).

Aboveground biomass production, estimated as the sum of woody production and leaf litterfall, also did not respond consistently to nutrient additions (p = 0.68 for the main effect of N; p = 0.16 for the main effect of P; p = 0.32 for the interaction of N and P) (Table S18 & S19). Stand C1 had 23% higher aboveground production than C2 (p = 0.04) (Figure 4, Table S18 & S19).

The ratio between root growth and aboveground production also did not respond consistently to nutrient additions (p = 0.48 for the main effect of N; p = 0.21 for the main effect of P; p = 0.15 for the interaction term), but the ratio in stand C2 was 1.8 times higher than that in C1 (p = 0.04) (Table 3, S20 & S21). We note, however, that the ratio of root growth to aboveground production was highest in the NP plots of both stands (Table 3), indicating that the root response to N + P was out of proportion to an aboveground response.

Total forest production, estimated as the sum of aboveground production and fine root growth, was 15% higher under P addition (p = 0.08) but did not respond consistently to N (p = 0.45), and no interaction between N and P was detected (p = 0.57; Table S22 & S23). On average, C1 had 15% higher total production than C2 (p = 0.07; Table S22 & S23).

The effects of adding N or P on total soil respiration in these two stands during the warm season

(June–August) were not consistent ($p \geq 0.27$; Table 3, S25 & S26). There was no consistent difference in warm season soil respiration between the two stands ($p = 0.11$; Table 3, S25 & S26).

Stand Differences

The two stands were similar in age but differed somewhat in species composition (Table 1), and as noted above there were differences in biomass and production between the two stands. First, aboveground production ($p = 0.04$; Table S18 & S19) and total forest production ($p = 0.07$; Table S22 & S23) were higher in stand C1 than C2, primarily reflecting higher wood production (Figure 4). Second, although fine root biomass was similar between stand C1 and C2, fine root growth was much higher in stand C2 (Figure 2). Thus, the ratio of root growth to aboveground production was much higher in stand C2 ($p = 0.04$; Table S20 & S21), and the fine root turnover index (Table 3) was also higher in C2 than C1 ($p = 0.08$; Table S12 & S13); this difference was especially marked in the control and NP plots (Table 3).

Discussion

In accordance with the theory that plants adjust their above and belowground allocation to maximize growth and resource uptake (Bloom and others 1985; Thornley 1991; Hermans and others 2006), we predicted that allocation to root growth and biomass would decline in response to elevated availability of N and P. Instead, after 7 years of treatment, we observed stimulation of fine root growth in response to simultaneous addition of N and P (Figure 2A). In contrast to the traditional view that C supply regulates nutrient acquisition through belowground C allocation, we did not observe a consistent negative association between aboveground production and root growth in either of our two stands (Figure 4). This was surprising, as we hypothesized that root growth would be low where nutrient limitation was relieved by addition of a limiting nutrient.

Instead, our alternative hypothesis, that root growth increases in response to adding limiting nutrients, was supported. Nutrient effects on aboveground production were not consistent between stands (Figure 4), and root growth was not consistently related to aboveground production (Figure 4). The increase in C allocation belowground in response to NP addition was consistent between stands and was disproportionate to the aboveground growth response, as indicated by the greater root/shoot ratio in NP plots than other plots in both stands (Table 3).

One possible explanation for the greatest root growth in NP plots is that both nutrients are required in stoichiometric balance for root construction. Root growth in response to addition of one limiting nutrient could be required to obtain enough of the other limiting nutrient to maintain stoichiometric balance (Shan and others 2022). Notably, we did observe higher root ingrowth into cores enriched with the nutrient not added at the plot level (Figure 2C), but our statistical test did not yield strong evidence for this assertion, either because of the small sample size or because the degree of nutrient enrichment in these cores was too low to stimulate a strong foraging response. We have observed analogous nutrient interactions aboveground: In 2014 both foliar resorption proficiency and efficiency of N increased with P addition for the dominant tree species in these young stands (Gonzales and Yanai 2019), suggesting increased demand for N with alleviation of P limitation. Resorption of P was more proficient and efficient with N addition, consistent with exacerbated P limitation.

While we observed the greatest root growth (Figure 2A) in NP plots in early successional stands in the MELNHE study, a similar study in mature MELNHE stands found the greatest root growth in response to N addition (Shan and others 2022). Nutrient requirements for fine root production and other processes could differ with stand age owing to differences in species composition: White birch, red maple, and pin cherry were important in the young stands we studied but not the mature stands. In the mature stands, increased root growth in response to N addition without a detectable increase in root biomass stocks reflected higher fine root turnover. Increased root turnover is in keeping with the positive correlation between fine root respiration and root N concentration across biomes (Burton and others 2002). However, longer root lifespans and lower root turnover have also been reported under higher N availability (Burton and others 2000). We found the highest turnover in response to combined N and P addition in the early successional MELNHE stands (Table 3). Further study of the possible effects of NP nutrition on fine root metabolism is clearly warranted.

Consistent with our alternate hypothesis (H_{1a}) that addition of limiting nutrients would stimulate root growth and biomass, we found positive effects of P addition on fine root biomass across the two young forest stands (Figure 3), indicating primary P limitation of standing fine root stocks. Higher fine

root biomass or fine root growth in response to forest P addition has been observed in *Abies* forest in southern Spain (Blanes and others 2012), Hawaiian montane forests (Ostertag 2001), and lowland tropical forests in southern China (Yuan and Chen 2012), as well as in various other terrestrial ecosystems (Zhu and others 2013). In the mature stands of MELNHE study, fine root biomass did not respond consistently to nutrient additions (Shan and others 2022).

Our test of fine root foraging as an indicator of nutrient limitation of forest production was disappointing. Although forest production responded to P addition (Figure4), root foraging was not stimulated by core-scale nutrient treatments. Exactly why such fine-scale root foraging for nutrients was not observed is not clear, but it is possible that the degree of nutrient enrichment in these cores was too low to stimulate statistically detectable foraging.

In summary, our observations of fine root dynamics in two early successional northern hardwood forests indicate N and P co-limitation of root growth and a relatively greater importance of P than N availability in controlling belowground C allocation and fine root biomass. In the MELNHE experiment, foliar N:P ratios ranged from 20 to 31 in unmanipulated controls, suggesting P limitation (Gonzales and Yanai 2019; Hong and others 2022), whereas root growth in mature forest increased with N addition (Shan and others 2022). Different nutrients have been shown to limit aboveground vs belowground production in grasslands (Cleland and others 2019) and lowland tropical forest (Wright and others 2011). In addition, fine root biomass and morphology and mycorrhizal symbionts can respond differently to different nutrients (Waring and others 2019; Wurzburger and Wright 2015). Limitation by different nutrients in different ecosystem components may represent another mechanism for maintaining nutrient balance in addition to adjustments of carbon allocation between roots and shoots.

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DATA AVAILABILITY

Data from this manuscript are available at: fine root ingrowth and biomass: https://doi.org/10.6073/pasta/c51516c8eca9071862db5bcec0f4542woody production: https://doi.org/10.6073/pasta/377b1fd44bd88a58e9654ab207e50706leaf litterfall: https://doi.org/10.6073/pasta/8b2975a3a02cbcfb1b0a12ac954576d4 resin available nutrients: https://doi.org/10.6073/pasta/e842c6704690be8e041da48a778583e3soil respiration: https://doi.org/10.6073/pasta/eb37cd72ccaa3e9197c461f0c1c734eb.

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