ECOSYSTEMS

Fine Root Growth Increases in Response to Nitrogen Addition in Phosphorus-limited Northern Hardwood Forests

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ABSTRACT

Resource allocation theory posits that increased soil nutrient availability results in decreased plant investment in nutrient acquisition. We evaluated this theory by quantifying fine root biomass and growth in a long term, nitrogen (N) × phosphorus (P) fertilization study in three mature northern hardwood forest stands where aboveground growth increased primarily in response to P addition. We did not detect a decline in fine root biomass or growth in response to either N or P. Instead, fine root growth increased in response to N, by 40% for length ($P = 0.04$ for the main effect of N in ANOVA), and by 36% for mass, relative to controls. Fine root mass growth was lower in response to N + P addition than predicted from the main effects of N and P ($P = 0.01$ for the interaction of N × P). The response of root growth to N availability did not result in detectable responses in fine root biomass ($P = 0.61$), which is consistent with increased root turnover with N addition. We propose that the differential growth response to fertilization between above- and belowground components is a mechanism by which trees enhance P acquisition in response to increasing N availability, illustrating how both elements may co-limit northern hardwood forest production.

Key words: allocation; co-limitation; fine root; nitrogen; northern hardwood forest; phosphorus.

HIGHLIGHTS

- Neither biomass nor growth of fine roots declined in response to nutrient addition
- Fine root growth increased in response to added N unless P was also added
- Co-limitation may be mediated by interacting above- and belowground limitations

INTRODUCTION

Plants are expected to respond to variation in soil nutrient availability by adjusting biomass allocation to maximize resource uptake and growth, according to the economic theory of resource acquisition (Bloom and others 1985; Tilman 1988; Thornley 1991). This theory predicts that nutrient limitation should stimulate plants to increase carbon (C) allocation belowground (Tilman 1988; Poorter and Nagel 2000), whereas increased nutrient availability should allow C allocation to shift aboveground, promoting effective competition for light (Giardina and others 2003). However, the response by roots to altered nutrient availability is not always consistent with this expected C allocation response: Root biomass in forests has shown negative (Fahey and others 1998), neutral (Lee and Jose 2003; Burton and others 2012), positive (Li and others 2015), and mixed (Phillips and Fahey 2007; Peng and others 2017) responses to nutrient additions in different experiments.

One possible explanation is that interactive effects among multiple limiting nutrients contribute to variable belowground responses to nutrient enrichment. Expectations of allocation response to resource limitation have traditionally been framed in terms of single-nutrient limitation (Hermans and others 2006; Eyles and others 2009; De Parseval and others 2016); however, two commonly limiting macronutrients, nitrogen (N) and phosphorus (P), are known to interactively influence plant growth (Harpole and others 2011). Synergistic effects of N and P addition on net primary productivity suggest a dynamic balance in plant demand for the two nutrients, such that the addition of one could induce greater limitation by the other (Harpole and others 2011; Rastetter and others 2013). If alleviating limitation by one nutrient increases limitation by another, allocation to root growth would not be expected to decline, unless the other nutrient also was added.

Root growth could also be constrained by low nutrient availability and thus increase in response to nutrient addition. The absorptive roots, in the first several root orders (Guo and others 2008a), have high proportions of metabolically active cortex, have high N requirements to support nutrient uptake and metabolism (Pregitzer and others 2002; Lux and others 2004; Guo and others 2008b; Yahara and others 2019), and form mycorrhizal associations with fungi that also require nutrients (Johnson 2010; Nasholm and others 2013; Hasselquist and others 2016). As a consequence, allocation of C to support root growth can be constrained by limited supplies of nutrients needed for construction and metabolism (Hagedorn and others 2016; Simon and others 2017). Growth of new roots could be limited by the same nutrient that limits aboveground growth (whole-plant single-element limitation) or by a different nutrient (whole-plant multi-element limitation). Root growth has responded to the addition of different nutrients than aboveground growth in lowland tropical ecosystems (Wright and others 2011; Wurzburger and others 2015; Waring and others 2019) and wetlands (Darby and Turner 2008). This whole-plant multi-element limitation would be consistent with the varied stoichiometry of plant tissues (He and others 2015) which reflects the different functions of foliage and absorptive roots (Gargallo-Garriga and others 2014; Schreeg and others 2014).

There have been few tests of multiple element limitation in temperate forests. We established a study of Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE) in 13 stands of three age classes distributed across three sites in central New Hampshire, USA, in which N and P have been added in full factorial combination since 2011. These treatments were effective: by 2015, both soil (Fisk and others 2014; Goswami and others 2018) and foliar N and P concentrations (Gonzales and Yanai 2019; Hong 2019) reflected addition of the respective nutrient. We found that tree diameter growth responded primarily (but not uniformly) to P addition (Goswami and others 2018; reporting tree growth as of 2015). This may be surprising considering that hardwood forests on glacially derived soils have been assumed to be N limited (Walker and Syers 1976; Vitousek 2004); however, historical anthropogenic N enrichment may have shifted the ecosystem toward P limitation by increasing the supply of N relative to P (Vitousek and others 2010). Phosphorus limitation was also indicated by responses of foliar nutrient concentration and resorption in three of the young MELNHE stands in 2014 (Gonzales and Yanai 2019) and by foliar N and P concentrations across ten of the stands in 2015–16 (Hong 2019). By 2015, P addition had alleviated P limitation enough that resin-available P remained elevated (Goswami and others 2018), soil P- and N-acquiring enzyme activity shifted from P- toward N-acquisition (Shan 2020), and foliar N:P ratios declined to 14.7, compared to 21.5 in the controls (Hong 2019).

To evaluate and refine the traditional concept that C allocation to root growth decreases in response to alleviation of nutrient limitation of aboveground growth, we measured fine root bio-

mass and growth in three mature northern hardwood stands in the MELNHE experiment, 5 years after the onset of fertilization. We tested the alternative hypotheses that (1) aboveground nutrient limitation controls allocation to root growth or (2) belowground nutrient limitation controls allocation to root growth. The aboveground limitation hypothesis predicts that root growth will decline in response to addition of one or more limiting nutrients. It is also possible that root growth will increase in response to one nutrient if adding that nutrient increases the demand for co-limiting nutrients, in which case that increase will be negated by adding the other co-limiting nutrients. Alternatively, the belowground limitation hypothesis predicts that root growth will increase in response to adding one or more limiting nutrients; adding the second nutrient will not negate the effects of the first.

Materials and Methods

Site Description and Nutrient Treatments

Our study sites were three forest stands (designated C7, C8, and C9) in the Bartlett Experimental Forest, White Mountain National Forest, NH, which form part of a larger study of Multiple Element Limitation in Northern Hardwood Ecosystems (MELNHE). These stands are located at elevations ranging from 330 to 590 m, with slopes of 5 – 30%. The mean annual temperature is 6 °C, and the mean annual precipitation is 1400 mm. These stands are typical mature (> 100 years), second-growth northern hardwood forests in the northeastern United States that originated following forest harvest in the late nineteenth and early twentieth century. The overstory is dominated by sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britton), along with some white ash (*Fraxinus americana* L.) and white birch (*Betula papyrifera* Marsh) (Goswami and others 2018). Soils are predominantly Spodosols (Typic and Aquic Haplorthods) derived from glacial drift with well developed surface organic horizons 4–8 cm thick (Yanai and others 2006; Vadeboncoeur and others 2012). Soil characteristics are described in more detail by Vadeboncoeur and others (2012), See and others (2015), and Ratliff and Fisk (2016).

In each stand, we delineated four 50 × 50 m treatment plots and randomly assigned treatments to each plot. The plots were fertilized annually with N (30 kg N ha^{-1} y^{-1} as NH$_4$NO$_3$), P (10 kg P ha^{-1}y^{-1} as NaH$_2$PO$_4$), N + P (at the same rates), or nothing (control) beginning in June 2011. A 10-m wide treated buffer surrounded an inner 30 × 30 m measurement area in each plot.

Phosphorus concentrations of leaf litter, collected in 5 baskets per plot in autumn of 2016, were elevated by P addition and depressed by N addition (Table 1). Soil P availability, captured by 3-week incubation of resin strips in the organic horizon in 2014, 2015, and 2016, was elevated by P addition and depressed by N addition, and resin-available N and extractable inorganic N were elevated by N addition (Table 1). Potential N mineralization in 2-week laboratory incubations of organic and mineral horizons in 2014 was not influenced by either N or P addition and effects of nutrient addition on extractable inorganic N and N mineralization did not differ by depth (Table 1). The methods used to quantify resin-available nutrients were described by Fisk and others (2014), and the methods for N mineralization were described by Ratliff and Fisk (2016).

Fine Root Biomass

Fine root biomass was measured in each plot in late August 2015 by soil coring and manual dry sorting of live roots from soil. Two soil cores were collected near the four corners and the center of the measurement area of each plot (n = 10 cores per plot) using 5-cm diameter split-PVC pipe corers hammered into the soil with a rubber mallet. Locations were probed to avoid obstruction in the surface soil. The nominal depth of sampling was 30 cm but because of obstructions below the surface soil, the actual depth of sampling averaged 27 cm. Each core was divided in the field into two depth increments, 0–10 cm (including Oe and Oa horizons) and 10–30 cm. Samples were transported to the laboratory 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 color and low tensile strength. The sorting procedure differed between 0–10 cm and 10–30 cm depths because of the time required for many of the organic matter-rich surface cores (often exceeding 3 h). For 0–10 cm samples that were expected to take more than 1 h to sort, a timed picking approach was employed (Metcalfe and others 2007). For this temporal prediction method, fine root biomass was estimated from the diminishing root mass recovered during sequential, timed picking intervals. We used four 10-min intervals and estimated total mass by extrapolation

Table 1. Soil nutrient and litter properties in the three mature northern hardwood forest stands

Treatment	Resin-available P ($\mu g\ d^{-1}$)	Resin-available N ($\mu g\ d^{-1}$)	Litter P (%)	Litter N (%)	Extractable inorganic N ($mg\ m^{-2}$)		N mineralization ($mg\ m^{-2}\ d^{-1}$)		Ingrowth core soil inorganic N ($\mu g\ N\ g^{-1}$)		
					FF	Min	FF	Min	FF	0–10 cm	10–20 cm
Control	1.4 (0.7)	4.1 (1.4)	0.035 (0.008)	1.031 (0.047)	149 (42)	315 (128)	44 (8)	32 (18)	32.2 (10.7)	2.4 (1.1)	4.7 (2.5)
N	0.4 (0.1)	27.7 (8.1)	0.027 (0.004)	1.037 (0.049)	281 (104)	498 (88)	49 (4)	28 (4)	35.0 (14.3)	6.9 (3.4)	3.1 (0.5)
P	7.2 (3.7)	3.55 (2.9)	0.074 (0.04)	1.014 (0.04)	143 (59)	260 (95)	43 (13)	27 (9)	23.8 (16.8)	7.8 (4.1)	6.7 (4.9)
N + P	1.4 (0.4)	19.4 (4.5)	0.047 (0.005)	1.074 (0.045)	377 (120)	454 (65)	56 (11)	24 (9)	33.1 (7.7)	9.1 (3.4)	6.0 (2.2)
ANOVA results: df, F, significance (* < 0.05, ** < 0.01*** < 0.001**** < 0.0001)											
N	1,6, 8.07*	1,6, 44.83***	1,6, 12.29*	1,6, 3.65	1,14, 38.2****		1,14, 0.16		1,24, 1.63		
P	1,6, 7.71*	1,6, 1.91	1,6, 30.03**	1,6, 0.32	1,14, 0.00		1,14, 0.01		1,24, 0.75		
N × P	1,6, 0.12	1,6, 0.10	1,6, 3.35	1,6, 2.38	1,14, 0.87		1,14, 0.10		1,24, 0.20		
Depth					1,14, 23.08***		1,14, 8.20*		2,24, 14.60****		
Depth × N					1,14, 0.01		1,14, 0.77		2,24, 0.24		
Depth × P					1,14, 2.45		1,14, 0.34		2,24, 0.93		

Litter P and N were measured in October 2016. Resin-available N and P were measured with resin strips installed in the O horizon (forest floor) in midsummer 2014, 2015, and 2016. Extractable inorganic N and N mineralization rates were measured in midsummer 2014 at two depths: forest floor (FF) and 0–10 cm mineral soil (Min). Inorganic N of ingrowth core soils at the time of collection was measured at three depths: forest floor (FF), 0–10 cm, and 10–20 cm. Values are means of the three replicate stands and standard errors of the means are in parentheses; df = degrees of freedom.

to 98% of the asymptote. For 10–30 cm samples, all roots were hand sorted from each core.

Sorted roots were washed free of adhering soil on a 0.4 mm sieve, divided by size class, dried to constant mass at 70 °C, and weighed. A subsample of roots was analyzed for ash content by dry ashing at 450 °C for 4 h. All values are expressed on an ash-free dry mass basis by correcting for the average measured ash content of subsamples for 0–10 cm depth (7.0% ash) and 10–30 cm depth (15.6%); ash fraction did not differ systematically between stands or treatments. Fine root biomass was expressed on a unit area basis (to the depth of sampling) corrected for the measured area of rocks, boulders and root crowns where fine root biomass was assumed to be zero (Bae 2013, Bae and others 2015, Fahey and others 2017). The fine root biomass distribution between 0–10 cm versus 10–30 cm was compared by calculating a shallow-to-deep mass ratio (root biomass of 0–10 cm divided by that of 10–30 cm).

Fine root growth and vertical distribution

We used an ingrowth core approach to estimate fine root growth and its vertical distribution. Colonization of soil cores by fine roots, measured as root ingrowth length or biomass per core, was used as an index of fine root growth (Fahey and Hughes 1994).

In June 2013, we prepared ingrowth cores in eight systematically located positions in each treatment plot. At each location, we collected soil with a corer 5 cm in diameter and 25 cm in length. Soil was separated into three layers: forest floor (FF, Oe + Oa), 0–10 cm (mineral) and 10- 20 cm (mineral). Soil was prepared for filling each layer of the core holes in a plot by combining the 8 cores of the same layer with extra soil from that layer in the same plot, homogenizing, and gently passing through 5 mm mesh hardware cloth to remove roots and rocks. We carefully refilled each core hole with 10 cm of each of the two mineral layers and 5 cm of homogenized forest floor. We inserted a 5-cm diameter ring of hardware cloth in the top to mark the core location. Installation was completed in early July 2013.

All ingrowth cores were incubated in situ for 2 years to allow complete root colonization. During this time, the cores were fertilized individually with equal amounts of N or P per m^2 as the surrounding plot. In August 2015, all cores were extracted by re-coring the marked locations with a smaller diameter (4.5 cm) corer to a depth of 20 cm in the mineral soil. The cores were separated into three layers (forest floor, 0 – 10 cm, and 10 – 20 cm), transported to the laboratory and stored at 4 °C for up to 2 weeks prior to further processing. Fine roots (< 1 mm diameter) were sorted from soils by hand, washed with tap water, and scanned. Fine root length was quantified with the Analyze Skeleton plugin (Niemisto and others 2005) in ImageJ (Schneider and others 2012). Dry root mass was measured after oven drying at 60 °C.

Data Analysis

For fine root biomass, the response variables were the sum of root biomass per unit area in the 0–10 cm and 10–30 cm depths and the ratio of root biomass in two depths (the shallow-to-deep mass ratio). Biomass and shallow-to-deep mass ratio from the replicate cores within each plot were averaged before analysis. Analysis of variance was conducted with N (present or absent), P (present or absent), their interaction, and forest stand as fixed effects. The same analysis was conducted for resin-available N and P, using plot-level values averaged over the 3 years of measurement.

Ingrowth response variables were fine root ingrowth length and biomass per unit area and specific root length. Analysis of variance was conducted with N, P, soil depth, and all two- and three-way interactions among fertilization treatments and depth. Depth was a categorical variable with three levels: forest floor, and 0–10 cm and 10–20 cm mineral soil. Extractable inorganic N and N mineralization rates were analyzed using the same model, except that the depths were forest floor and 0–10 cm mineral soil. Tukey's post hoc test was performed for the pairwise comparison among the four fertilization treatments (control, N, P, and N + P) when a significant N × P interaction was found.

To describe patterns of ingrowth with depth, the response variables were densities of fine root ingrowth length, mass per unit volume, and specific root length. Depth was represented as a continuous variable using the midpoint of each of the constructed soil horizons from the surface of the forest floor (2.5, 10, 20 cm) as a covariate in analysis of covariance (ANCOVA). Predictor variables were N addition, P addition, soil depth, and all two- and three-way interactions among fertilization treatments and depth.

Residuals met assumptions of normality for all statistical tests. All analyses were conducted using PROC GLM in SAS 9.4 (SAS Institute, Cary, NC).

RESULTS

Fine Root Biomass

After 5 years of N and P addition in full factorial combination, fine root biomass in the upper 30 cm soil did not respond significantly to N ($P = 0.61$ for the main effect of N in ANOVA), P ($P = 0.62$ for the main effect of P) or their interaction ($P = 0.50$ for the interaction of N and P) (Figure 1). Fine root biomass was similar among the three stands ($P = 0.16$ for the main effect of stand), averaging 591 ± 60 g/m^2 in C7, 571 ± 66 g/m^2 in C8, and 683 ± 24 g/m^2 in C9. Neither N ($P = 0.11$) nor P ($P = 0.35$) affected root depth distribution, described by the ratio of roots in the 0–10 and 10–30 cm depth interval; addition of N + P together resulted in a more shallow distribution of fine root biomass than predicted by the main effects of N and P (Figure 2; $P = 0.03$ for the interaction).

Fine Root Ingrowth

Fine root ingrowth length in each stand was consistently greater in plots receiving N alone compared with other plots. Averaged across stands, ingrowth length in N-addition plots was 40% greater than in control or P-addition plots, and 35% greater than in N + P plots (Figure 3A). We detected main effects of N addition ($P = 0.04$), but not P addition ($P = 0.17$) and we did not detect an N × P interaction ($P = 0.18$). Fine root ingrowth length differed among stands ($P = 0.04$) and tended to be greatest in the 0–10 cm mineral soil ($P = 0.01$ for the main effect of depth; Figure 3A). We did
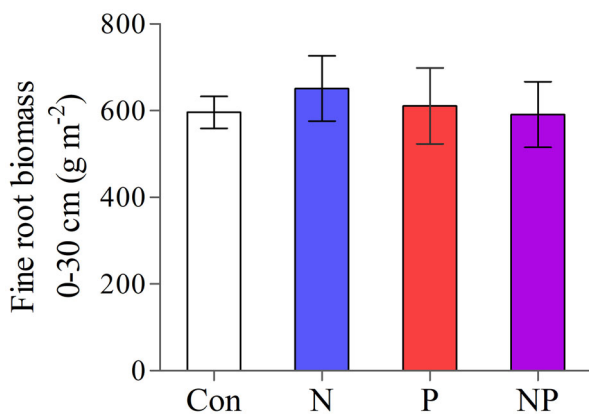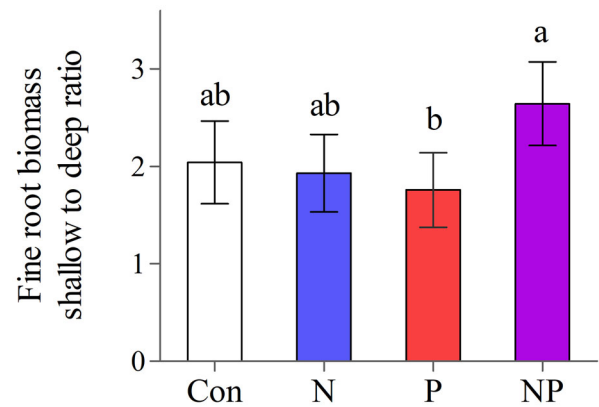

Figure 2. Ratio of fine root biomass in shallow (0–10 cm) versus deep (10–30 cm) soils in three mature northern hardwood forest stands. Values are means of the three replicate stands, and error bars are standard errors of the means.

not detect interactions of N × depth ($P = 0.57$) or P × depth ($P = 0.10$).

Fine root ingrowth mass was also greatest in plots receiving N alone in each of the 3 forest stands. The effect of P addition ($P = 0.06$ for the main effect of P) was more consistent than the effect of N addition ($P = 0.16$ for the main effect of N); the most striking effect was lower ingrowth mass in N + P plots ($P = 0.01$ for the N × P interaction) than expected from the main effects of N and P. Ingrowth mass in plots receiving N alone was 36% higher than controls ($P = 0.04$) and 42% higher than in N + P plots ($P = 0.02$, Figure 3B). Ingrowth mass, like root length, was greatest in the 0–10 cm mineral soil ($P = 0.002$ main effect of depth for the main effect of depth) (Figure 3B). We did not detect an interaction of N × depth ($P = 0.51$), but there was a marginal interaction of P × depth ($P = 0.09$).

Ingrowth root length density declined from an average of 6.8 ± 0.9 cm/cm^3 soil in the forest floor, to 4.9 ± 0.4 cm/cm^3 at 0–10 cm depth in the mineral soil, to 3.7 ± 0.4 cm/cm^3 at 10–20 cm depth (Figure 4A). Treatments affected the vertical distribution of ingrowth length: length density shifted toward deeper soil in response to N ($P = 0.08$ for depth × N interaction) and toward the surface in response to P ($P = 0.04$ for depth × P; Figure 4A). Fine root mass density, like length density, shifted toward the surface in response to P ($P = 0.05$ for the depth × P interaction, Figure 4B). Although the depth × N interaction was not significant for mass density ($P = 0.25$), the lack of a decline with depth in response to N contrasts with the other treatments (Figure 4B). Specific root length differed among stands ($P = 0.001$) and

Figure 1. Fine root biomass (0–1 mm diameter) in the top 30 cm of soil in three mature northern hardwood forest stands in response to four fertilization treatments: control (white), N (blue), P (red) and N + P (purple). Values are means of the three replicate stands, and error bars are standard errors of the means.
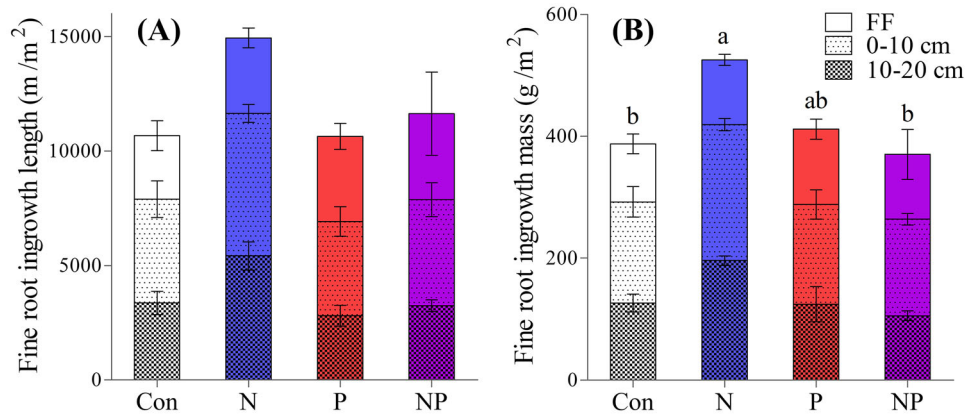
Figure 3. Fine root ingrowth (**A**) length and (**B**) biomass in response to fertilization treatments: control (Con), N, P, and N + P. Stacked bars represent values for the three soil horizons. Values are means from the three replicate stands, and error bars are standard errors of the means of each horizon. Treatments sharing a lowercase letter do not differ at $\alpha = 0.10$.
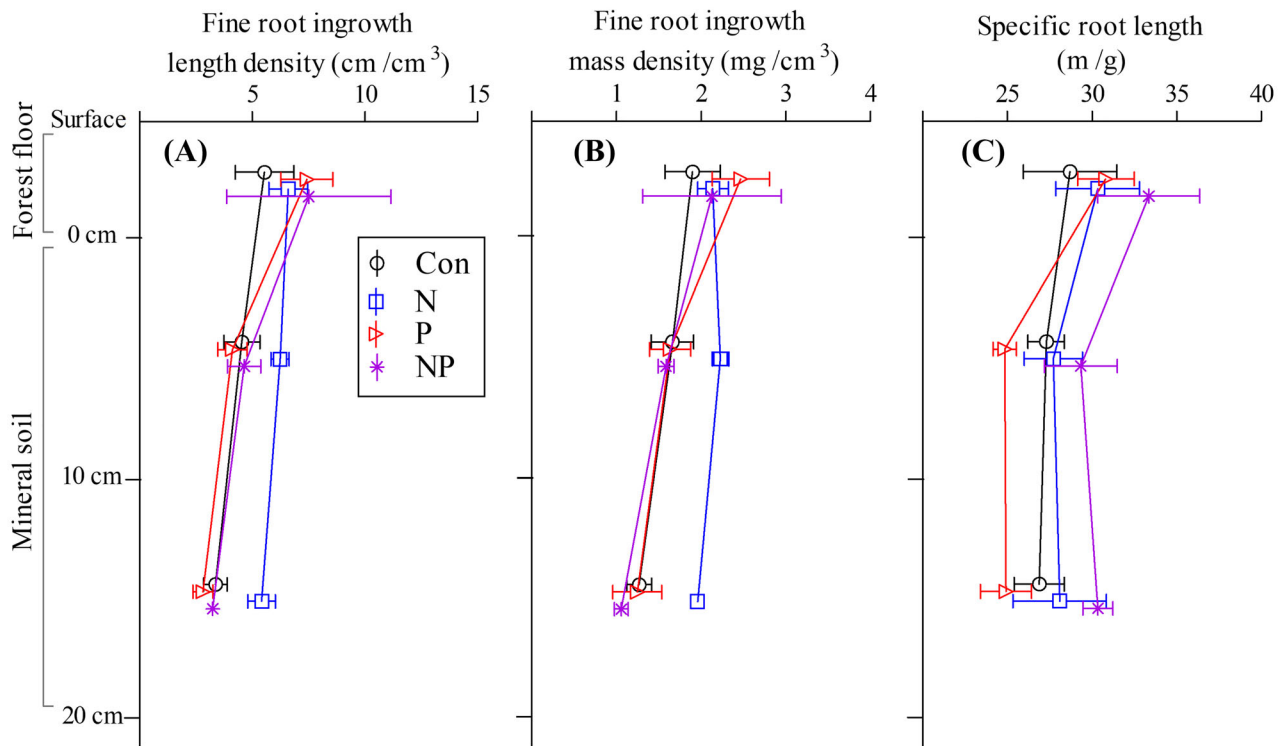

Figure 4. Vertical distribution (forest floor (FF), 0–10 cm, and 10–20 cm depths) of fine root ingrowth (**A**) length density, (**B**) mass density and (**C**) specific root length in response to fertilization treatments: control (Con), N, P, and N + P. Values are means from the three replicate stands, and error bars are standard errors of the means.

decreased with depth ($P = 0.01$, Figure 4C; 30.8 ± 1.2 m/g for forest floor, 27.3 ± 0.8 m/g for 0–10 cm, and 27.6 ± 1.0 m/g for 10–20 cm). There were no treatment × depth interactions ($P = 0.46$ for depth × N, and $P = 0.34$ for depth × P).

DISCUSSION

In these northern hardwood forests, in the same time frame that aboveground diameter growth responded primarily to P addition (Goswami and others 2018), we found that addition of P or N, separately or in combination, did not reduce either fine root biomass estimated by soil coring (Fig-

ure 1) or total fine root growth (forest floor to 20 cm depth in mineral soil) in ingrowth cores (Figures 3 and 4). These findings do not support the most straightforward prediction of allocation theory that alleviating aboveground nutrient limitation (single or co-limitation) reduces root growth. Instead, we found that total fine root growth increased markedly in response to N addition, especially in mineral soil, and only in the absence of added P (Figures 3 and 4). Such a response to N could indicate direct nutrient limitation of root growth. However, if that were the case we would expect at least as great a growth response to N and P together as to N alone. Instead, we found reduced root growth with N and P, compared to P alone. This result is consistent with the hypothesis that N addition intensified P limitation, promoting root growth in response to elevated N as a mechanism to increase P acquisition. Our results illustrate how multiple nutrient elements can mediate C allocation in these forests. Control of C allocation by different resources above vs belowground has been shown in grassland ecosystems (Cleland and others 2019) and tropical forest (Wright and others 2011; Waring and others 2019); ours is the first study to test this possibility in a temperate forest.

The interpretation that roots grew more with added N because of greater demand for P is supported by lower resin-available P and litter P concentrations in N-addition plots (Table 1). These results suggest greater P uptake by plants or soil microorganisms and/or greater P retranslocation by trees (Gonazles and Yanai 2019). The shift in depth distribution in response to N addition also is consistent with greater demand for P. Nitrogen addition stimulated more roots to grow in deeper soils, where more weatherable P was located (Blum and others 2002; Schaller and others 2010). Examination of trace elements weathered from apatite in ingrowth cores could provide a test of this proposed mechanism for P acquisition at different depths (manuscript in preparation).

Support for the idea that higher N availability increases allocation to roots in response to P demand does not eliminate the possibility that root growth is also directly responsive to N availability: greater soil N availability may simultaneously increase P demand and alleviate direct constraints on growth. The high growth-related nutrient demands of roots can surpass uptake at growing tips, requiring recycling from storage via phloem transport (Marschner and others 1997; Millard 1996). High N requirement for root growth could also contribute to P limitation if it limits the growth of absorptive roots. Whether nutrient availability di-

rectly limits root growth deserves further attention to best interpret the balance of above- and belowground controls of C allocation to growth (Simon and others 2017).

The possibility that production above- and belowground responds to different elements calls into question the use of root foraging for nutrients as an indicator of whole-plant limitation. Fine root foraging (that is, proliferation in response to small patches of elevated nutrient availability) has been widely used to identify growth-limiting nutrients (Raich and others 1994; Gleason and Good 2003). In the mature forest stands used in this study, prior to fertilization, we observed that roots foraged primarily for N, especially in stands with low soil N availability; we suggested that this indicated N limitation of aboveground growth (Naples and Fisk 2010). Inferring limitation from root ingrowth responses depends on the assumption that above- and belowground growth will respond to the same nutrient, which is challenged by our current finding that root growth was greater in plots receiving N in a system where tree diameter growth was mostly greater in plots receiving P.

Increased root growth in response to N addition (Figure 3) without a detectable increase in root biomass stocks (Figure 1) is consistent with increased fine root turnover under high nutrient availability (Reich 2014). The ratio of fine root ingrowth to fine root biomass, which provides an indication of root turnover, was higher in the N treatment (0.67 ± 0.09) than control (0.53 ± 0.05), P (0.56 ± 0.08), or N + P (0.51 ± 0.01). Fine root turnover increased in response to N addition in Michigan northern hardwoods (Burton and others 2012) and in response to potassium addition in lowland tropical forest in Panama (Wright and others 2011; Yavitt and others 2011). Plasticity in root turnover within ecosystems complements the idea of a root economics spectrum in which root turnover increases with soil fertility (Wright and others 2004; Reich 2014; Prieto and others 2015). The validity of a root economics spectrum remains debated (Kramer-Walter and others 2016; Enrique and others 2018), largely because effects of mycorrhizal associations must be considered (Bergmann and others 2020; Vleminckx and others 2021). Nevertheless, the possibility that fertility controls root turnover has important implications for C cycling in forest ecosystems, and our results add to evidence of ecosystem-level responses to nutrient availability.

Our findings depart from the prediction of resource allocation theory that alleviating a nutrient limitation should shift biomass allocation from

belowground to aboveground growth (Hermans and others 2006). That fine root growth did not decline does not eliminate the possibility that total belowground C allocation decreased in response to P addition. However, soil respiration did not decline with P addition (Mann 2021), as would be expected if belowground C allocation had decreased (Raich and Nadelhoffer 1989; Haynes and Gower 1995), other things (aboveground litter inputs and soil C storage) being equal. Allocation to mycorrhizal associations generally declines in response to nutrient enrichment, although responses to elevated N and P are not uniform by either AM or EM associations (Treseder 2004) and in some cases depend on whether mycorrhizal and plant growth are limited by different nutrients (Johnson and others 2003; Li and others 2019). In our study sites, AM colonization of the dominant species, sugar maple, did not respond to P addition (our unpublished data). Further work including ectomycorrhizae is needed to more comprehensively interpret belowground responses (Köhler and others 2018; Meeds and others 2021), as ectomycorrhizal tree species (*Betula spp, Fagus grandifolia*) constitute a large proportion of the basal area in these stands (Goswami and others 2018).

The interaction of above- and belowground controls of allocation that we found here, along with other results from the MELNHE experiment, provides evidence of multiple element limitation, in which the effort expended to acquire resources reflects the relative availability of each resource relative to demand for that resource (Rastetter and others 2013). For example, the activity of P-mineralizing phosphatase enzymes was positively related to N availability across the MELNHE stands prior to fertilization treatments (Ratliff and Fisk 2016). Similarly, foliar resorption, an important mechanism of nutrient conservation, should reflect the availability not just of the nutrient resorbed but of the other co-limiting nutrients. Indeed, we observed greater foliar P resorption where soil N was high in six MELNHE stands prior to treatment (See and others 2015), indicating greater demand for P; foliar P resorption was not sensitive to soil P. Four years post-treatment, foliar P resorption was increased in response to N addition, and vice versa, in three young MELNHE stands (Gonzales and Yanai 2019), which is consistent with lower litter P concentrations under N addition in the mature MELNHE stands of the current study (Table 1). Similarly, lower soil P availability in response to N addition (Table 1) suggests greater plant or microbial uptake of P. These responses are consistent with the increased availability of one nutrient promoting allocation to acquisition of another (Marklein and Houlton 2012; Allison and Vitousek 2005).

In conclusion, aboveground growth in response to P addition and belowground growth in response to N addition suggest multiple-element control of C allocation in northern hardwood forests. Even though N-P co-limitation of aboveground growth was not indicated by a greater tree diameter response to N + P than to P alone in our experiment in these mature northern hardwood forests (Goswami and others 2018), our results indicate a different form of N-P co-limitation in plant resource acquisition, in which root growth in response to N addition reduces P limitation status by improving P acquisition. When responses to nutrient addition differ above- and belowground, detecting nutrient co-limitation may not be as simple as observing greater aboveground growth in response to two nutrients in combination than in response to either nutrient alone (Arrigo 2005). Our findings show the need to refine the traditional concept of resource allocation in plant nutrient dynamics to account for multiple element interactions.

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Declarations

Conflict of interest   The authors declare that they have no conflict of interest.

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