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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 the mature northern hardwood forest stands where aboveground growth increased primarily in response to $P$ addition. We did not detect a $Q$ 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 ( $P$ = 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 $N+P$ than predicted from the main effects of $N$ and $P(P=0.01$ for the interaction of $N \times P$ ). The response of or growth to N availability diversal in detectable responses in fine root biomass ( $P=0.61$ ), which is consistent with increased root turnover with N addition. We prove that the differential growth response to fertilization between above- and belowground components is a mechanism by which trees enhand acquisition in response to increasing N availability. illustrating how both elements co-limit northern hardwood forest production	
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# Fine Root Growth Increases in Response to Nitrogen Addition in Phosphorus-limited Northern Hardwood Forests

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#### 16 Abstract

17 Resource allocation theory posits that increased soil 18 nutrient availability results in decreased plant 19 investment in nutrient acquisition. We evaluated 20 this theory by quantifying fine root biomass and 21 growth in a long term, nitrogen  $(N) \times$  phosphorus 22 (P) fertilization study in three mature northern 23 hardwood forest stands where aboveground 24 growth increased primarily in response to P addi-25 tion. We did not detect a decline in fine root bio-26 mass or growth in response to either N or P. 27 Instead, fine root growth increased in response to 28 *N*, by 40% for length (P = 0.04 for the main effect 29 of *N* in ANOVA), and by 36% for mass, relative to 30 controls. Fine root mass growth was lower in re-31 sponse to N + P addition than predicted from the

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**Author's Contribution:** MCF, RDY, TJF, and SS designed the study. SS, HD, MCF, and TJF collected the data. SS performed analyses and drafted the manuscript, and MCF, RDY, and TJF contributed substantially to writing and revisions.

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main effects of *N* and *P* (P = 0.01 for the interaction 32 of  $N \times P$ ). The response of root growth to N 33 34 availability did not result in detectable responses in fine root biomass (P = 0.61), which is consistent 35 with increased root turnover with N addition. We 36 propose that the differential growth response to 37 38 fertilization between above- and belowground 39 components is a mechanism by which trees en-40 hance *P* acquisition in response to increasing N availability, illustrating how both elements co-limit 41 northern hardwood forest production. 42

**Key words:** allocation; co-limitation; fine root; 43 nitrogen; northern hardwood forest; phosphorus. 44

#### **H**IGHLIGHTS

47 48

- Neither biomass nor growth of fine roots 49 declined in response to nutrient addition 50
- Fine root growth increased in response to added 51 N unless *P* was also added 52
- Co-limitation may be mediated by interacting 53 above- and belowground limitations 54



#### 55 **INTRODUCTION**

56 Plants are expected to respond to variation in soil 57 nutrient availability by adjusting biomass allocation 58 to maximize resource uptake and growth, accord-59 ing to the economic theory of resource acquisition (Bloom and others 1985; Tilman 1988; Thornley 60 61 1991). This theory predicts that nutrient limitation should stimulate plants to increase carbon (C) 62 allocation belowground (Tilman 1988; Poorter and 63 64 Nagel 2000), whereas increased nutrient availabil-65 ity should allow C allocation to shift aboveground, 66 promoting effective competition for light (Giardina 67 and others 2003). However, the response by roots to altered nutrient availability is not always con-68 69 sistent with this expected C allocation response: 70 Root biomass in forests has shown negative (Fahey 71 and others 1998), neutral (Lee and Jose 2003; 72 Burton and others 2012), positive (Li and others 73 2015), and mixed (Phillips and Fahey 2007; Peng 74 and others 2017) responses to nutrient additions in different experiments. 75

76 One possible explanation is that interactive ef-77 fects among multiple limiting nutrients contribute 78 to variable belowground responses to nutrient 79 enrichment. Expectations of allocation response to 80 resource limitation have traditionally been framed 81 in terms of single-nutrient limitation (Hermans and 82 others 2006; Eyles and others 2009; De Parseval 83 and others 2016); however, two commonly limiting macronutrients, nitrogen (N) and phosphorus 84 85 (P), are known to interactively influence plant 86 growth (Harpole and others 2011). Synergistic ef-87 fects of N and P addition on net primary produc-88 tivity suggest a dynamic balance in plant demand 89 for the two nutrients, such that the addition of one 90 could induce greater limitation by the other (Har-91 pole and others 2011; Rastetter and others 2013). If 92 alleviating limitation by one nutrient increases 93 limitation by another, allocation to root growth 94 would not be expected to decline, unless the other 95 nutrient also was added.

96 Root growth could also be constrained by low 97 nutrient availability and thus increase in response 98 to nutrient addition. The absorptive roots, in the 99 first several root orders (Guo and others 2008a), 100 have high proportions of metabolically active cortex, high N requirements to support nutrient up-101 102 take and metabolism (Pregitzer and others 2002; 103 Lux and others 2004; Guo and others 2008b; Ya-104 hara and others 2019), and form mycorrhizal 105 associations with fungi that also require nutrients 106 (Johnson 2010; Nasholm and others 2013; Has-107 selquist and others 2016). As a consequence, allo-108 cation of C to support root growth can be

constrained by limited supplies of nutrients needed 109 for construction and metabolism (Hagedorn and 110 others 2016; Simon and others 2017). Growth of 111 new roots could be limited by the same nutrient 112 that limits aboveground growth (whole-plant sin-113 gle-element limitation) or by a different nutrient 114 (whole-plant multi-element limitation). Root 115 growth has responded to the addition of different 116 nutrients than aboveground growth in lowland 117 tropical ecosystems (Wright and others 2011; 118 Wurzburger and others 2015; Waring and others 119 2019) and wetlands (Darby and Turner 2008). This 120 whole-plant multi-element limitation would be 121 consistent with the varied stoichiometry of plant 122 tissues (He and others 2015) which reflects the 123 different functions of foliage and absorptive roots 124 (Gargallo-Garriga and others 2014; Schreeg and 125 others 2014). 126

There have been few tests of multiple element 127 limitation in temperate forests. We established a 128 study of Multiple Element Limitation in Northern 129 Hardwood Ecosystems (MELNHE) in 13 stands of 130 three age classes distributed across three sites in 131 central New Hampshire, the USA, in which *N* and *P* 132 have been added in full factorial combination since 133 2011. These treatments were effective; by 2015, 134 both soil (Fisk and others 2014; Goswami and 135 others 2018) and foliar N and P concentrations 136 (Gonzales and Yanai 2019; Hong 2019) reflected 137 addition of the respective nutrient. We found that 138 tree diameter growth responded primarily (but not 139 uniformly) to *P* addition (Goswami and others 140 2018; reporting tree growth as of 2015). This may 141 be surprising considering that hardwood forests on 142 glacially derived soils have been assumed to be N143 limited (Walker and Syers 1976; Vitousek 2004); 144 however, historical anthropogenic N enrichment 145 may have shifted the ecosystem toward *P* limitation 146 by increasing the supply of *N* relative to *P* (Vitousek 147 and others 2010). Phosphorus limitation was also 148 indicated by responses of foliar nutrient concen-149 tration and resorption in three of the young 150 MELNHE stands in 2014 (Gonzales and Yanai 151 2019) and by foliar *N* and *P* concentrations across 152 ten of the stands in 2015–16 (Hong 2019). By 2015, 153 *P* addition had alleviated *P* limitation enough that 154 resin-available P remained elevated (Goswami and 155 others 2018), soil P- and N-acquiring enzymes 156 activity shifted from *P*- toward *N*-acquisition (Shan 157 2020), and foliar N:P ratios declined to 14.7, com-158 pared to 21.5 in the controls (Hong 2019). 159

To evaluate and refine the traditional concept 160 that C allocation to root growth decreases in response to alleviation of nutrient limitation of 162 aboveground growth, we measured fine root bio-163

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164 mass and growth in three mature northern hard-165 wood stands in the MELNHE experiment, 5 years after the onset of fertilization. We tested the alter-166 167 native hypotheses that (1) aboveground nutrient 168 limitation controls allocation to root growth or (2) 169 belowground nutrient limitation controls allocation 170 to root growth. The aboveground limitation 171 hypothesis predicts that root growth will decline in 172 response to addition of one or more limiting 173 nutrients. It is also possible that root growth will 174 increase in response to one nutrient if adding that 175 nutrient increases the demand for co-limiting 176 nutrients, in which case that increase will be ne-177 gated by adding the other co-limiting nutrients. 178 Alternatively, the belowground limitation hypoth-179 esis predicts that root growth will increase in re-180 sponse to adding one or more limiting nutrients; 181 adding the second nutrient will not negate the ef-182 fects of the first.

#### 183 MATERIALS AND METHODS

#### 184 Site Description and Nutrient Treatments

Our study sites were three forest stands (designated 185 186 C7, C8, and C9) in the Bartlett Experimental For-187 est, White Mountain National Forest, NH, which 188 form part of a larger study of Multiple Element 189 Limitation in Northern Hardwood Ecosystems 190 (MELNHE). These stands are located at elevations 191 ranging from 330 to 590 m, with slopes of 5 - 30%. 192 The mean annual temperature is 6 °C, and the 193 mean annual precipitation is 1400 mm. These 194 stands are typical mature (> 100 years), secondgrowth northern hardwood forests in the north-195 196 eastern United States that originated following 197 forest harvest in the late nineteenth and early 198 twentieth century. The overstory is dominated by 199 sugar maple (Acer saccharum Marsh), American 200 beech (Fagus grandifolia Ehrh.), and yellow birch 201 (Betula alleghaniensis Britton), along with some white ash (Fraxinus americana L.) and white birch 202 203 (Betula papyrifera Marsh) (Goswami and others 204 2018). Soils are predominantly Spodosols (Typic 205 and Aquic Haplorthods) derived from glacial drift with well-developed surface organic horizons 4-206 207 8 cm thick (Yanai and others 2006; Vadeboncoeur 208 and others 2012). Soil characteristics are described 209 in more detail by Vadeboncoeur and others (2012), 210 See and others (2015), and Ratliff and Fisk (2016).

211 In each stand, we delineated four  $50 \times 50$  m 212 treatment plots and randomly assigned treatments 213 to each plot. The plots were fertilized annually with 214 N (30 kg N ha<sup>-1</sup> y<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>), P (10 kg P ha<sup>-1</sup> 215  ${}^{1}y^{-1}$  as NaH<sub>2</sub>PO<sub>4</sub>), N + P (at the same rates), or nothing (control) beginning in June 2011. A 10-m216wide treated buffer surrounded an inner217 $30 \times 30$  m measurement area in each plot.218

Phosphorus concentrations of leaf litter, collected 219 in 5 baskets per plot in autumn of 2016, were 220 elevated by *P* addition and depressed by *N* addition 221 (Table 1). Soil *P* availability, captured by three-222 week incubation of resin strips in the organic 223 horizon in 2014, 2015, and 2016, was elevated by P 224 addition and depressed by N addition, and resin-225 available N and extractable inorganic N were ele-226 vated by N addition (Table 1). Potential N miner-227 alization in 2-week laboratory incubations of 228 organic and mineral horizons in 2014 was not 229 influenced by either N or P addition and effects of 230 nutrient addition on extractable inorganic N and N231 mineralization did not differ by depth (Table 1). 232 The methods used to quantify resin-available 233 nutrients were described by Fisk and others (2014), 234 and the methods for N mineralization were de-235 scribed by Ratliff and Fisk (2016). 236

#### Fine Root Biomass

Fine root biomass was measured in each plot in late 238 August 2015 by soil coring and manual dry sorting 239 of live roots from soil. Two soil cores were collected 240 near the four corners and the center of the mea-241 surement area of each plot (n = 10 cores per plot)242 using 5-cm diameter split-PVC pipe corers ham-243 mered into the soil with a rubber mallet. To avoid 244 large rocks, locations were first probed to avoid 245 obstruction in the surface soil. The nominal depth 246 of sampling was 30 cm but because of obstructions, 247 the actual depth of sampling averaged 27 cm. Each 248 core was divided in the field into two depth 249 increments, 0-10 cm (including Oe and Oa hori-250 zons) and 10-30 cm. Samples were transported to 251 the laboratory for storage at -20 °C until laboratory 252 processing. 253

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Live fine roots of 0-1 mm diameter were hand 254 sorted from each sample; dead roots were distin-255 guished by their color and low tensile strength. The 256 sorting procedure differed between 0-10 cm and 257 10-30 cm depths because of the time required for 258 many of the organic matter-rich surface cores (of-259 ten exceeding 3 h). For 0–10 cm samples that were 260 expected to take more than 1 h to sort, a timed 261 picking approach was employed (Metcalfe and 262 others 2007). For this temporal prediction method, 263 fine root biomass was estimated from the dimin-264 ishing root mass recovered during sequential, 265 timed picking intervals. We used four 10-min 266 intervals and estimated total mass by extrapolation 267

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	$(\mu g d^{-1})$	$(\mu g \ d^{-1})$	(%)	(%)		$N \ (\mathrm{mg} \ \mathrm{m}^{-2})$	0	mineraliza (mg m <sup>-2</sup> d	tion ( $\mu g$ <sup>-1</sup> )	$N g^{-1}$ )		2
	~					FF	Min	FF M	in FF	0-1	10 cm	10- 20 cm
Control		1.4 (0.7)	4.1 (1.4)	0.035		(0.008)	1.031				0.047)	149
315 (128)	44 (8)	32 (18)	32.2	2.4 (1.1)	4.7 (2.5)							(71)
Ν		0.4 (0.1)	27.7 27.7	0.027		(0.004)	1.037			1)	0.049)	281
(104)	498 (88)	49 (4)	(0.1) 28 (4)	35.0	6.9 (3.4)	3.1 (0.5)						
Ь		7.2 (3.7)	3.55	(c. <del>1</del> 4.) 0.074		(0.011)	1.014		(0.	.04) 143	3 (59)	260
43 (13) $N + P$	27 (9)	23.8 (16.8) 1.4 (0.4)	7.8 (4.1) 19.4	6.7 (4.9) 0.047		(0.005)	1.074				0.045)	377
-			(4.5)							-		
(120)	454 (65)	56 (11)	24 (9)	33.1	9.1 (3.4)	6.0 (2.2)						
ANOVA res	ults: df, F, significan	ce (* < 0.05, ** < 0	.01, *** < 0	0.001, ****	< 0.0001)	ns not significé	ant					
Ν		1,6, 8.07, *	1,6, 44.83.	1,6, 12.29.	1,6, 3.65.	1,14, 38.2, **	**	1,14, 0.16,	ns 1,24, 1.6	, j 3.		
			***	*	su				ns			
P		1,6, 7.71, *	1,6, 1.91,	1,6,	1,6,	1,14, 0.00, ns	(	1,14, 0.01,	ns 1,24,	.		
			ns	30.03, **	0.32, ns		4		0 ns	,c/		
$N \times P$		1,6, 0.12, ns	1,6, 0.10,	1,6, 2 25	1,6, 738	1,14, 0.87, ns		1,14, 0.10,	1,24, n 2 4, 0 2	, 0		
			SII	ردد.د ns	,0C.2 NS				u ns	۶ <b>0</b> ,		
Depth						1,14,23.08,	Y	1,14, 8.20,	*	2,2	4,	
						< < <	7			<b>I</b> *	.4.6U, ***	
Depth $\times N$						1,14, 0.01,		1,14, 0.77,	ns	2,2.	4, 0.24,	
Denth < D						ns 111275		1 14 0 24	34	ц с с	1S 1 0 03	
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to 98% of the asymptote. For 10–30 cm samples, allroots were hand sorted from each core.

270 Sorted roots were washed free of adhering soil on 271 a 0.4 mm sieve, divided by size class, dried to 272 constant mass at 70 °C, and weighed to 0.1 mg. Ash content was quantified by drying and ashing 273 274 about 100 root samples at 450 °C for 4 h; ash 275 fraction did not differ systematically by stand or 276 treatment. Fine root biomass was expressed on a 277 unit area basis (to the depth of sampling) corrected 278 for the measured area of rocks, boulders and root 279 crowns where fine root biomass was assumed to be 280 zero (Bae 2013, Bae and others 2015, Fahey and 281 others 2017). The fine root biomass distribution 282 between 0-10 cm versus 10-30 cm was compared 283 by calculating a shallow-to-deep mass ratio (root 284 biomass of 0–10 cm divided by that of 10–30 cm).

#### 285 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).

292 In June 2013, we prepared ingrowth cores in 293 eight systematically located positions in each 294 treatment plot. At each location, we collected soil 295 with a corer 5 cm in diameter and 25 cm in length. 296 Soil was separated into three layers: forest floor 297 (FF, Oe + Oa), 0-10 cm (mineral) and 10- 20 cm 298 (mineral). Soil was prepared for filling each layer of 299 the core holes in a plot by combining the 8 cores of 300 the same layer with extra soil from that layer in the 301 same plot, homogenizing, and gently passing 302 through 5 mm mesh hardware cloth to remove 303 roots and rocks. We carefully refilled each core hole 304 with 10 cm of each of the two mineral layers and 305 5 cm of homogenized forest floor. We inserted a 5cm diameter ring of hardware cloth in the top to 306 307 mark the core location. Installation was completed 308 in early July 2013.

309 All ingrowth cores were incubated in situ for 310 2 years to allow complete root colonization. During this time, the cores were fertilized individually with 311 equal amounts of N or P per  $m^2$  as the surrounding 312 313 plot. In August 2015, all cores were extracted by re-314 coring the marked locations with a smaller diame-315 ter (4.5 cm) corer to a depth of 20 cm in the min-316 eral soil. The cores were separated into three layers 317 (forest floor, 0 - 10 cm, and 10 - 20 cm), trans-318 ported to the laboratory and stored at 4 °C for up to 319 2 weeks prior to further processing. Fine root 320 (< 1 mm) diameter was sorted from soils by hand, washed with tap water, and scanned. Fine root 321 length was quantified with the Analyze Skeleton 322 plugin (Niemisto and others 2005) in ImageJ 323 (Schneider and others 2012). Dry root mass was 324 measured after oven drying at 60 °C. 325

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#### Data Analysis

For fine root biomass, the response variables were 327 the sum of root biomass per unit area in the 0-328 10 cm and 10–30 cm depths and the ratio of root 329 biomass in two depths (the shallow-to-deep mass 330 ratio). Biomass and shallow-to-deep mass ratio 331 from the replicate cores within each plot was 332 averaged before analysis. Analysis of variance was 333 conducted with N (present or absent), P (present or 334 absent), their interaction, and forest stand as fixed 335 effects. The same analysis was conducted for resin-336 available *N* and *P*, using plot-level values averaged 337 over the 3 years of measurement. 338

Ingrowth response variables were fine root in-339 growth length and biomass per unit area and 340 specific root length. Analysis of variance was con-341 ducted with N, P, soil depth, and all two- and three-342 way interactions among fertilization treatments 343 and depth. Depth was a categorical variable with 344 three levels: forest floor, and 0-10 cm and 10-345 20 cm mineral soil. Extractable inorganic *N* and *N* 346 mineralization rates were analyzed using the same 347 model, except that the depths were forest floor and 348 0-10 cm mineral soil. Tukey's post hoc test was 349 performed for the pairwise comparison among the 350 four fertilization treatments (control, N, P, and 351 N + P) when a significant  $N \times P$  interaction was 352 found. 353

To describe patterns of ingrowth with depth, the 354 response variables were densities of fine root in-355 growth length, mass per unit volume, and specific 356 root length. Depth was represented as a continuous 357 variable using the midpoint of each of the con-358 structed soil horizons, from the surface of the forest 359 floor (2.5, 10, 20 cm) as a covariate in analysis of 360 covariance (ANCOVA). Predictor variables were N361 addition, *P* addition, soil depth, and all two- and 362 three-way interactions among fertilization treat-363 ments and depth. 364

Residuals met assumptions of normality for all365statistical tests. All analyses were conducted using366PROC GLM in SAS 9.4 (SAS Institute, Cary, NC).367

#### RESULTS

#### **Fine Root Biomass**

After 5 years of N and P addition in full factorial370combination, fine root biomass in the upper 30 cm371



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372 soil did not respond significantly to N (P = 0.61 for 373 the main effect of *N* in ANOVA), P(P = 0.62 for the 374 main effect of P) or their interaction (P = 0.50 for)375 the interaction of N and P) (Figure 1). Fine root 376 biomass was similar among the three stands (P = 0.16 for the main effect of stand), averaging 377  $591 \pm 60 \text{ g m}^{-2}$  in C7,  $571 \pm 66 \text{ g m}^{-2}$  in C8, and  $683 \pm 24 \text{ g m}^{-2}$  in C9. Neither *N* (*P* = 0.11) nor *P* 378 379 (P = 0.35) affected root depth distribution, de-380 381 scribed by the ratio of roots in the 0-10 and 10-382 30 cm depth interval; addition of N + P together 383 resulted in a more shallow distribution of fine root 384 biomass than predicted by the main effects of *N* and 385 *P* (Figure 2; P = 0.03 for the interaction).

#### 386 Fine Root Ingrowth

387 Fine root ingrowth length in each stand was con-388 sistently greater in plots receiving N alone com-389 pared with other plots. Averaged across stands, 390 ingrowth length in N-addition plots was 40% 391 greater than in control or *P*-addition plots, and 392 35% greater than in N + P plots (Figure 3A). We 393 detected main effects of *N* addition (P = 0.04), but 394 not *P* addition (P = 0.17) and we did not detect an 395  $N \times P$  interaction (P = 0.18). Fine root ingrowth 396 length differed among stands (P = 0.04) and tended 397 to be greatest in the 0–10 cm mineral soil (P = 0.01398 for the main effect of depth; Figure 3A). We did 399 not detect interactions of N  $\times$  depth (P = 0.57) or 400  $P \times depth \ (P = 0.10).$ 

401 Fine root ingrowth mass was also greatest in plots 402 receiving *N* alone in each of the 3 forest stands. The 403 effect of *P* addition (P = 0.06 for the main effect of 404 *P*) was more consistent than the effect of *N* addition



**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 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.

(P = 0.16 for the main effect of N); the most strik-405 ing effect was lower ingrowth mass in N + P plots 406  $(P = 0.01 \text{ for the } N \times P \text{ interaction})$  than expected 407 from the main effects of *N* and *P*. Ingrowth mass in 408 plots receiving *N* alone was 36% higher than con-409 trols (P = 0.04) and 42% higher than in N + P plots 410 (P = 0.02, Figure 3B). Ingrowth mass, like root 411 length, was greatest in the 0-10 cm mineral soil 412 (P = 0.002 main effect of depth for the main effect)413 of depth) (Figure 3B). We did not detect an 414 interaction of  $N \times \text{depth} (P = 0.51)$ , but there was 415 a marginal interaction of  $P \times \text{depth}$  (P = 0.09). 416

Ingrowth root length density declined from an 417 average of  $6.8 \pm 0.9$  cm cm<sup>-3</sup> soil in the forest 418 floor, to  $4.9 \pm 0.4$  cm cm<sup>-3</sup> at 0–10 cm depth in 419 the mineral soil, to  $3.7 \pm 0.4$  cm cm<sup>-3</sup> at 10–20 cm 420 depth (Figure 4A). Treatments affected the vertical 421 422 distribution of ingrowth length: Length density shifted toward deeper soil in response to N423  $(P = 0.08 \text{ for depth} \times \text{N interaction})$  and toward 424 the surface in response to P (P = 0.04 for depth  $\times$ 425 P; Figure 4A). Fine root mass density, like length 426 density, shifted toward the surface in response to P 427  $(P = 0.05 \text{ for the depth} \times P \text{ interaction, Fig-}$ 428 ure 4B). Although the depth  $\times N$  interaction was 429 not significant for mass density (P = 0.25), the lack 430 of a decline with depth in response to N contrasts 431 432 with the other treatments (Figure 4B). Specific root length differed among stands (P = 0.001) and 433 decreased with depth (P = 0.01, Figure 434 **4**C;  $30.8 \pm 1.2$  m/g for forest floor,  $27.3 \pm 0.8$  m/g for 435 0-10 cm, and  $27.6 \pm 1.0$  m/g for 10-20 cm). There 436 were no treatment  $\times$  depth interactions (P = 0.46437 for depth  $\times N$ , and P = 0.34 for depth  $\times P$ ). 438

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**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.

#### 439 **DISCUSSION**

440 In these northern hardwood forests, in the same
441 time frame that above ground growth responded
442 primarily to *P* addition (Goswami and others 2018),
443 we found that addition of *P* or *N*, separately or in
444 combination, did not reduce either fine root bio445 mass estimated by soil coring (Figure 1) or total

fine root growth (forest floor to 20 cm depth in 446 mineral soil) in ingrowth cores (Figs. 3 and 4). 447 These findings do not support the most straight-448 forward prediction of allocation theory that allevi-449 ating aboveground nutrient limitation (single or 450 co-limitation) reduces root growth. Instead, we 451 found that total fine root growth increased mark-452 edly in response to *N* addition, especially in mineral 453

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454 soil, and only in the absence of added *P* (Figs. 3 and 455 4). Such a response to N could indicate direct 456 nutrient limitation of root growth. However, if that 457 were the case we would expect at least as great a 458 growth response to *N* and *P* together as to *N* alone. 459 Instead, we found reduced root growth with *N* and 460 *P*, compared to *P* alone. This result is consistent 461 with the hypothesis that N addition intensified P462 limitation, promoting root growth in response to 463 elevated N as a mechanism to increase P acquisi-464 tion. Our results illustrate how multiple nutrient 465 elements can mediate C allocation in these forests. 466 Control of C allocation by different resources above 467 vs belowground has been shown in grassland 468 ecosystems (Cleland and others 2019) and tropical 469 forest (Wright and others 2011; Waring and others 470 2019); ours is the first study to test this possibility 471 in a temperate forest.

472 The interpretation that roots grew more with 473 added N because of greater demand for P is supported by lower resin-available *P* and litter *P* con-474 475 centrations in N-addition plots (Table 1). These 476 results suggest greater *P* uptake by plants or soil 477 microorganisms and/or greater *P* retranslocation by 478 trees (Gonazles and Yanai 2018). The shift in depth distribution in response to N addition also is con-479 480 sistent with greater demand for P. N addition 481 stimulated more roots to grow in deeper soils, 482 where more weatherable *P* was located (Blum and 483 others 2002; Schaller and others 2010; Figure 4). 484 Examination of trace elements weathered from 485 apatite in ingrowth cores could provide a test of this 486 proposed mechanism for *P* acquisition at different 487 depths (manuscript in preparation). Support for the 488 idea that higher *N* availability increases allocation 489 to roots in response to *P* demand does not eliminate 490 the possibility that root growth in unfertilized soils 491 is also directly responsive to N availability: Greater 492 soil N availability may simultaneously increase P 493 demand and alleviate direct constraints on growth. 494 The high growth-related nutrient demands of roots 495 can surpass uptake at growing tips, requiring 496 recycling from storage via phloem transport (Marschner and others 1997; Millard 1996). High N 497 498 requirement for root growth could also contribute 499 to *P* limitation in our unfertilized plots if it limits 500 the growth of absorptive roots. Whether nutrient 501 availability directly limits root growth deserves 502 further attention to best interpret the balance of 503 above- and belowground controls of C allocation to 504 growth (Simon and others 2017).

The possibility that production above- and
belowground respond 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 509 patches of elevated nutrient availability) has been 510 widely used to identify growth-limiting nutrients 511 (Raich and others 1994; Gleason and Good 2003). 512 In the mature forest stands used in this study, prior 513 to fertilization, we observed that roots foraged 514 primarily for N, especially in stands with low soil N515 availability; we suggested that this indicated N 516 limitation of aboveground growth (Naples and Fisk 517 2010). Inferring limitation from root ingrowth re-518 sponses depends on the assumption that above-519 and belowground growth will respond to the same 520 nutrient, which is challenged by our current find-521 522 ing that root growth was greater in plots receiving *N* in a system where aboveground growth was 523 mostly greater in plots receiving *P*. 524

Increased root growth in response to N addition 525 (Figure 3) without a detectable increase in root 526 biomass stocks (Figure 1) is consistent with in-527 creased fine root turnover under high nutrient 528 availability (Reich 2014). The ratio of fine root in-529 growth to fine root biomass, which provides an 530 indication of root turnover, was higher in the N531  $(0.67 \pm 0.09)$ 532 treatment than control  $(0.53 \pm 0.05),$ P $(0.56 \pm 0.08),$ 533 or N + P $(0.51 \pm 0.01)$ . Fine root turnover increased in re-534 sponse to N addition in Michigan northern hard-535 woods (Burton and others 2012) and in response to 536 potassium addition in lowland tropical forest in 537 Panama (Wright and others 2011; Yavitt and others 538 539 **2011**). Plasticity in root turnover within ecosystems complements the idea of a root economics spec-540 trum in which root turnover increases with soil 541 fertility (Wright and others 2004; Reich 2014; Pri-542 eto and others 2015). The validity of a root eco-543 nomics spectrum remains debated (Kramer-Walter 544 545 and others 2016; Enrique and others 2018), largely because effects of mycorrhizal associations must be 546 considered (Bergmann and others 2020; Vleminckx 547 and others 2021). Nevertheless, the possibility that 548 fertility controls root turnover has important 549 implications for carbon cycling in forest ecosys-550 tems, and our results add to evidence of ecosystem-551 level responses to nutrient availability. 552

Our findings depart from the prediction of re-553 source allocation theory that alleviating a nutrient 554 limitation should shift biomass allocation from 555 belowground to aboveground growth (Hermans 556 and others 2006). That fine root growth did not 557 decline does not eliminate the possibility that total 558 belowground carbon allocation decreased in re-559 sponse to *P* addition. However, soil respiration did 560 not decline with *P* addition (Mann 2021), as would 561 562 be expected if belowground C allocation had decreased (Raich and Nadelhoffer 1989; Haynes and 563

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564 Gower 1995), other things (aboveground litter in-565 puts and soil C storage) being equal. Allocation to 566 mycorrhizal associations generally declines in re-567 sponse to nutrient enrichment, although responses to elevated *N* and *P* are not uniform by either AM 568 569 or EM associations (Treseder 2004) and in some 570 cases depend on whether mycorrhizal and plant 571 growth are limited by different nutrients (Johnson 572 and others 2003; Li and others 2019). In our study 573 sites, AM colonization of the dominant species, 574 sugar maple, did not respond to *P* addition (our 575 unpublished data). Further work including ecto-576 mycorrhizae is needed to more comprehensively 577 interpret belowground responses (Köhler and oth-578 ers 2018; Meeds and others 2021), as ectomycor-579 rhizal tree species (Betula spp, Fagus grandifolia) 580 constitute a large proportion of the basal area in 581 these stands (Goswami and others 2018).

582 The interaction of above- and belowground 583 controls of allocation that we find here, along with other results from the MELNHE experiment, pro-584 585 vides evidence of multiple element limitation, in 586 which the effort expended to acquire resources 587 reflects the relative availability of each resource 588 relative to demand for that resource (Rastetter and 589 others 2013). For example, the activity of P-min-590 eralizing phosphatase enzymes was positively re-591 lated to N availability across the MELNHE stands 592 prior to fertilization treatments (Ratliff and Fisk 593 2016). Similarly, foliar resorption, an important 594 mechanism of nutrient conservation, should reflect 595 the availability not just of the nutrient resorbed but 596 of the other co-limiting nutrients. Indeed, we ob-597 served greater foliar *P* resorption where soil *N* was 598 high in six MELNHE stands prior to treatment (See 599 and others 2015), indicating greater demand for P; 600 foliar *P* resorption was not sensitive to soil P. Four years post-treatment, foliar P resorption was in-601 602 creased in response to N addition, and vice versa, in 603 three young MELNHE stands (Gonzales and Yanai 604 2019), which is consistent with lower litter P con-605 centrations under N addition in the mature 606 MELNHE stands of the current study (Table 1). 607 Similarly, lower soil P availability in response to Naddition (Table 1) suggests greater plant or micro-608 609 bial uptake of P. These responses are consistent 610 with the increased availability of one nutrient promoting allocation to acquisition of another 611 612 (Marklein and Houlton 2012; Allison and Vitousek 2005). 613

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 response to N + P619 than to *P* alone in our experiment in these mature 620 northern hardwood forests (Goswami and others 621 2018), our results indicate a different form of *N-P* 622 co-limitation in plant resource acquisition, in 623 which root growth in response to N addition re-624 duces *P* limitation status by improving *P* acquisi-625 tion. When responses to nutrient addition differ 626 above- and belowground, detecting nutrient co-627 limitation may not be as simple as observing greater 628 aboveground growth in response to two nutrients 629 in combination than in response to either nutrient 630 alone (Arrigo 2005). Our findings show the need to 631 refine the traditional concept of resource allocation 632 in plant nutrient dynamics to account for multiple 633 element interactions. 634

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#### Declarations

Conflict of interestThe authors declare654that they have no conflict of interest.655

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