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Belowground insights into nutrient limitation in northern hardwood forests

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Abstract Soil nutrient environments are changing in forests of the northeastern United States due to decades of anthropogenic nitrogen (N) emissions and acidic deposition, causing N enrichment and cation depletion, and possibly alleviating N limitation to forest growth. We asked whether biotic demand for phosphorus (P) or calcium (Ca) exceeded that for N and used an ingrowth core approach to test belowground responses to different nutrients. We tested fine root foraging for nutrients (N, P, or Ca) in three mid-age (26–30 years) and mature (≥100 years) northern hardwood forest stands in the Bartlett Experimental Forest (BEF), NH, and in one mature forest stand in the Hubbard Brook Experimental Forest (HBEF), NH, USA. Fine root colonization of cores responded clearly to Ca in mature forest at HBEF, responded to P in mid-age forests at BEF, and responded primarily to N in mature forests at BEF. Net N mineralization potential was higher in soils of mid-age than mature forests at BEF, with roots responding to N where N availability was low and to P or Ca where N availability was high. Nutrients elicited no responses from either fungi or phosphatase activity in mid-age forests, but in mature forests at BEF, N enhanced phosphatase activity. While no straightforward pattern emerged among the different mechanisms of nutrient acquisition that we tested, our results do suggest that P and Ca can be important limiting nutrients in these northern hardwood forests when N availability is relatively high. We hypothesize that the interacting effects of disturbance by forest harvest and N deposition can cause a transient P limitation to forest growth, and that other nutrients become more limiting as forests age.

Keywords Fine roots · Root foraging · Fungal hyphae · Phosphatase · Nutrient acquisition · Nutrient limitation · Northern hardwoods · Ingrowth

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Introduction

Anthropogenic pollution via nitrogen (N) deposition has changed soil nutrient regimes and may influence nutrient limitation of forest ecosystems in the northeastern US. Nitrogen generally is thought to limit plant productivity in temperate forest ecosystems because of its importance in photosynthesis and its mobility in soils (Aber et al. 1989; Vitousek and Howarth 1991). However, deposition of N from anthropogenic sources has altered soil properties and N cycling processes (Boxman et al. 1998; Fenn et al. 1998; Aber et al. 2003; Perakis and Hedin 2002), and

N enrichment of forest ecosystems may induce secondary limitations of plant productivity by other nutrients (Mohren et al. 1986; Reynolds et al. 1998; Prietzel et al. 2008; Hyvönen et al. 2008). At the same time, decades of acidic deposition have depleted calcium (Ca) and other base cations from soils in forest ecosystems of the northeastern US (Lawrence et al. 1995; Likens et al. 1998; Blum et al. 2002). This alteration of soil cation status can reduce soil pH, the activity of phosphorus (P) mineralizing enzymes, and ultimately P availability (Carreira et al. 2000; Fiorentino et al. 2003). Furthermore, low Ca supply can limit mycorrhizal colonization (St Clair and Lynch 2005; Juice et al. 2006), which could further impede P nutrition. The combination of relatively recent N enrichment, cation depletion, and possible changes in P availability justifies further examination of biotic responses to nutrients in temperate forests of the northeastern US.

Forest age should be considered in evaluating nutrient limitation to learn more about mechanisms of biogeochemical change over secondary succession in managed forests. The nature of nutrient limitation in these forests could change as trees age and the balance between supply and demand for different nutrients shifts. Nutrient accumulation in plant biomass is expected to be high in young and rapidly aggrading forests, and to decline with forest age (Woodwell and Whittaker 1968; Vitousek and Reiners 1975; Bormann and Likens 1979). It is likely that nutrient availability also changes as forest age. Nitrogen availability appears strongly buffered following disturbance (Fisk and Fahey 1990, 2001), and can decline as hardwood forests mature and microbial immobilization increases (McLauchlan et al. 2007; Fisk et al. 2002). However, consistent patterns of N availability in relation to forest age have not been found for north temperate forests (Ryan et al. 1997). Phosphorus and Ca are both ultimately derived from mineral sources such as apatite in northern hardwood forests but are removed from biotic recycling by very different processes, with P readily immobilized into very slowly available mineral forms and Ca easily lost by leaching (Yanai 1992; Likens et al. 1998) In more recently disturbed young stands, weathering patterns and hydrology could strongly influence localized immobilization and leaching processes, yielding different limiting nutrients among stands. Finally, the ability of trees to allocate C belowground to acquire limiting nutrients may change with forest age if allocation aboveground to stem and canopy growth constrains allocation belowground in young forests more so than in mature forests.

Patterns of belowground carbon (C) allocation can provide important insights into plant nutrient demand in forest ecosystems, especially those impacted by anthropogenic change (Franklin et al. 2009; Iverson and Norby 2008). Forest trees utilize a variety of different mechanisms to enhance acquisition of limiting nutrients. Allocation to enzyme production and also possibly fungal hyphae can increase the availability of nutrients for uptake (Sinsabaugh et al. 1993; Colpaert and Van Laere 1996; van Breeman et al. 2000; Landeweert et al. 2001). For example, soil P availability depends on activity of extracellular phosphatase enzymes that hydrolyze organic sources (Duff et al. 1994). Allocation to fine roots and to mycorrhizal hyphae can increase subsequent uptake of available nutrients. Because of the C costs associated with these and other mechanisms, it is logical to expect allocation to acquisition of the nutrients that are in highest demand and hence provide the most benefit to the plant (Chapin et al. 1986). For example, the balanced-growth hypothesis proposes that plants will preferentially allocate biomass to acquire the resource that most limits growth (Shipley and Meziane 2002), and supports the idea that nutrient demand by forest trees is indicated by allocation of C to belowground structures. Roots of many tree species proliferate in response to limiting nutrients (Mou et al. 1997; Huante et al. 1998; Bliss et al. 2002; Blair and Perfecto 2004) and foraging for nutrients has been directly linked to nutrient limitation in forest ecosystems using the root ingrowth technique (Raich et al. 1994; Gleeson and Good 2003). Similarly, fungal hyphae are known to respond to patches of limiting nutrients (Hagerberg et al. 2003). Biotic demand for P can also increase activity of the extracellular enzyme phosphatase (Dodd et al. 1987; Raghothama and Karthikeyan 2005), produced by decomposer microorganisms as well as plant roots and mycorrhizal fungi.

Our objective was to examine relative demand for N, P, and Ca in northern hardwood forests by testing belowground allocation to fine roots, fungal hyphae, and phosphatase enzymes in nutrient-amended ingrowth cores. Because of the possible combined effects of N enrichment, soil Ca depletion, and P

immobility, we hypothesized that fine roots and fungal hyphae would forage preferentially for P or Ca but not for N. We also hypothesized that soil phosphatase activity would be higher in forest stands where evidence of P foraging suggests P limitation, because production of phosphatase can be an important response to demand for P, especially where N is not limiting. To learn whether patterns of nutrient demand vary with ecosystem development following large-scale disturbance such as forest harvest, we also tested effects of forest age by comparing belowground responses between replicate mid-age (26–30 years) and mature (>100 years) forests.

Methods

Study sites and design

Study sites in the Bartlett Experimental Forest (BEF), NH, were in three replicate mid-age (26–30 years) and three replicate mature (≥116 years) forests, located between 250 and 400 m elevation. Mature forest composition is typical of northern hardwoods, with an overstory dominated by sugar maple (*Acer saccharum* Marsh), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britton). White ash (*Fraxinus americana* L.), white birch (*Betula papyrifera* Marsh) and red maple (*Acer rubrum* L.) are occasionally present (Table 1). Mid-age forests originated following clearcut harvest and are dominated by beech, yellow birch, and white birch, with pin cherry (*Prunus pensylvanica* L.f.), red maple, and aspen (*Populus grandidentata* Michx.) of secondary importance (Table 1). Sugar maple is generally a late-successional species in this region (Leak 1991) and occurs in relatively low abundance in early- to mid-successional stands. Soils are mostly spodosols overlying glacial till.

We studied an additional site at the Hubbard Brook Experimental Forest (HBEF), NH, located immediately west of Watershed 1 and the low-elevation hardwood study site used by Fisk et al. (2006), at 525 m elevation. The forest is secondary growth that arose following clearcut harvest between 1910 and 1920 (Likens et al. 1985). Sugar maple, American beech, and yellow birch dominate the overstory. Soils are mostly spodosols (typic and aquic haplorthods) overlying glacial till.

Ingrowth cores

We used a modified root ingrowth procedure (Vogt and Persson 1991) to test responses of fine roots, fungal hyphae, and phosphatase activity to different nutrient sources at the BEF and to test fine root responses at the HBEF. Ingrowth cores were installed at BEF in four 2 m × 2 m subplots within one 30 m × 30 m plot in each of our six study sites. We chose locations of subplots to minimize variation in

Table 1 Tree basal area (>2 cm DBH) and species composition in study plots at the Bartlett Experimental Forest (BEF; mid-age sites C4–C6 and mature sites C7–C9) and at the Hubbard Brook Experimental Forest (HBEF), NH

	Mid-age			Mature			
	C4	C5	C6	C7	C8	C9	HBEF
Percent of total basal area							
Acer pensylvanicum	0.0	5.7	3.0	0.0	0.0	0.0	0.8
A. rubrum	1.2	3.6	22.2	7.4	0.0	0.0	0.0
A. saccharum	0.0	2.6	2.4	33.0	33.2	38.2	36.5
Betula alleghaniensis	9.5	9.1	18.8	7.8	13.2	15.2	28.0
B. papyrifera	19.4	49.7	17.7	0.0	1.9	2.2	0.0
Fagus grandifolia	14.0	15.2	19.8	44.4	39.1	43.6	19.6
Fraxinus americana	0.0	0.0	0.0	0.0	6.7	0.0	15.1
Populus grandidentata	46.3	0.0	0.0	7.5	0.0	0.0	0.0
Prunus pensylvanica	5.3	14.1	11.6	0.0	0.0	0.0	0.0
Tilia americana	0.0	0.0	0.0	0.0	5.2	0.0	0.0
Tsuga canadensis	3.8	0.0	4.5	0.0	0.7	0.8	0.0
Total basal area (m^2/ha)	28.7	24.7	26.6	37.6	36.3	33.4	31.4

Data from the BEF are from the same 30 × 30 m plots used for ingrowth cores, and were provided by Hamburg et al. (unpublished)

plant species composition and microtopography (e.g. soil rock content, slope, and proximity to wet areas). At HBEF we tested root responses in six subplots (2 m × 2 m) distributed along a transect (~30 m) running up the slope. Tree basal area by species was quantified by measuring diameter at breast height (DBH) for all trees >2 cm DBH in a 10 m-wide belt along the transect (Table 1).

We installed 4 ingrowth cores per subplot at the HBEF in April 2004 and at the BEF in April 2005. Soil cores (5 cm diameter, 10 cm depth) were removed and the holes were refilled to approximately ½ cm below the forest floor surface with a uniform, root-free mixture of local soil from the B horizon (taken from approximately 20 cm depth). The same soil mixture was used for cores in both mid-age and mature forest to avoid biasing control nutrient availabilities. We added water several times as cores were filled to ensure contact with surrounding soil and to achieve a bulk density that was relatively similar to undisturbed soils. Completed ingrowth cores were covered with the Oi layer.

Nutrients were added in solid form at several times: one-third was mixed in the soil during ingrowth core installation, one-third was added to the surface of cores in late June, and one-third was added to the surface of cores in mid August. Soils in control cores received no added nutrients, N cores received a total of 20 g/m^2 of N as NH_4NO_3, and Ca cores received a total of 60 g/m^2 of Ca as $CaSiO_3$. These quantities were intended to roughly triple annual availability of N and Ca, based on our expectation that dissolution of $CaSiO_3$ would release approximately 20% of the total Ca over one growing season. Phosphorus cores received a total of 20 g/m^2 of P as NaH_2PO_4. This quantity more than triples annual P uptake by plants but was intended to allow for fixation of available P into mineral form.

The location of each core was marked with a one-cm wide strip of mesh nylon window screen, inserted around the circumference of the top of each core at the soil surface. Ingrowth cores were collected by re-coring (4.5 cm diameter, 10 cm depth; following Fahey and Hughes 1994) in October 2004 at the HBEF and in October 2005 at the BEF. Cores were refrigerated at 4°C and processed within 4 weeks of collection.

Fine roots (<1 mm diameter) were hand-sorted from each core and washed free of soil. We quantified fine root length using a line-intersect method (Tennet 1975) in which roots were cut into <1 cm pieces and their vertical and horizontal intersections were counted on a 1 cm grid. Root fragments were then dried at 60°C and weighed. Specific root length was calculated as root length/biomass. Following fine root removal, ingrowth core soil was homogenized and subsampled for later analysis of fungal hyphal length and phosphatase activity. Subsamples were stored at −20°C.

Fungal hyphae were extracted (BEF cores only) and quantified using a modification of the procedure of Miller et al. (1995). Soil subsamples (5 g) were soaked for 22 h in 0.35% Na-hexametaphosphate, sonicated at 43 W for 2 min, and mixed on a stir plate. The hyphal suspension was diluted and stained with 0.1% acid fuschin for 5 min and filtered through 0.4 μm ISOPORE^{TM} membrane filters. Air-dried filters were mounted in immersion oil on glass slides for later hyphal quantification.

Hyphal length was quantified using a line-intercept method (Tennet 1975). Two replicate filters of each sample were viewed at 400× magnification through a gridded ocular lens. Grid intersections were counted in 50 fields of view (FOV), resulting in 50–300 intersections per slide.

Phosphatase activity was quantified in 7 g soil subsamples shaken in 100 mL acetate buffer (50 mM, pH 5). Six 0.20 mL replicate aliquots of this solution were shaken for 1 h at 25°C with 5 mM *p*-nitrophenyl-phosphate (pNP) substrate. Aliquots were centrifuged and the reaction was stopped with 1.0 M NaOH. The optical density (OD) of pNP released after enzyme cleavage was quantified spectrophotometrically at 410 nm and results are expressed as change in OD g^{-1} h^{-1}.

Soil properties

We quantified soil properties, including nutrient availability, in three 30 m × 30 m plots (including the one used for ingrowth cores) in each forest stand at BEF. Distances between plots in a stand varied between 20 and 50 m. In early July, 2008, we collected approximately 30 two-cm diameter soil cores in each plot, separated Oe, Oa, and B (top 10 cm) horizons, and composited cores by horizon within each plot. Soils were refrigerated (4°C) for 4 days and gently homogenized. Subsamples of 4–5 g each for Oe, 7–8 g for Oa, and 10–12 g for

B horizons were used for analyses. Pairs of subsamples were used to estimate net N mineralization potential. We extracted NH_4^+ and NO_3^- from the first (initial) subsample of each pair by shaking for 1 h in 40 mL of 2 M KCl and after 18 h filtering through Whatman #1 paper. The second (final) subsample of each pair was incubated for 24 days at 20°C (± 2°C) and extracted in the same manner. We used a phenolate-hypochlorite method to quantify NH_4^+ (method 351.2, US EPA 1983) and a cadmium-reduction method to quantify NO_3^- (method 353.2, US EPA 1983) in extracts. Net N mineralization was estimated as the difference in $NH_4^+ + NO_3^-$ between final and initial subsamples.

We measured resin-available P by shaking soil subsamples for 18 h in 100 mL deionized water with nylon mesh bags containing bicarbonate-form anion-exchange resins (JT Baker Anion Exchange Resin, NA-38, OH- Form, Type I,16-50 Mesh) Resin bags were washed to remove all soil particles and inorganic P was extracted from resins by shaking for 1 h in 100 mL 0.5 M HCl. Extract P concentration was analyzed by the ammonium-molybdate-ascorbic acid method (Murphy and Riley 1962). We also measured bicarbonate-extractable P as an index of labile inorganic + organic P. Soils were extracted by shaking for 30 min in 40 mL of 0.5 M $NaHCO_3$. Extracts were filtered through Whatman # 2 paper and subjected to acid persulfate digestion. Inorganic P concentration in extracts was analyzed using the ammonium-molybdate-ascorbic acid method (Murphy and Riley 1962).

We extracted Ca by shaking subsamples for ½ h in 1 M NH_4Cl, after 18 h shaking briefly again, and after an additional 45 min filtering through Whatman #1 paper. Extract Ca concentration was analyzed using air/acetylene flame on a Varian SpectrAA (Palo Alto, CA) with lanthanum chloride (1%) to eliminate interferences. We quantified total C and N in pulverized subsamples using a Perkin Elmer series 2400 CHN analyzer (Perkin Elmer, Boston, MA). pH was measured in water with constant stirring, and percent sand, silt, and clay were quantified in B horizon soils using the hydrometer method (Sheldrick and Wang 1993).

Statistical analysis

Nutrient effects on fine roots at the HBEF were tested with one-way ANOVA and Tukey post hoc analysis. Effects of nutrients and forest age on fine roots, hyphal length, and phosphatase activity at the BEF were tested using split-plot ANOVA with nutrients and forest age as fixed effects and stands and plots within age as random effects. Plots were nested within stands, and stands were nested within forest age. We used the Glimmix procedure (PC SAS version 9.1.3, SAS Insitute, Cary NC 2006) with the Dunnett adjustment to compare multiple levels of treatments to the control level. Root length data were reported as absolute length (m/m^2). In order to minimize substantial variation among individual forest stands, root and hyphal length data in treatment cores were also expressed as percent of control cores at the subplot level. In this case the comparison of multiple levels of treatments to controls tested whether treatments differed from 100%.

Effects of forest age on soil properties at BEF were tested using a mixed-model ANOVA (PROC GLM) with stands nested as random effects within age.

Results

Fine root length was clearly elevated in +Ca compared to control cores at the HBEF ($P = 0.023$), and was similar among +N, +P, and control cores (Fig. 1). Nutrient treatment had a significant effect on fine root length at the BEF ($P = 0.036$). The pattern of fine root length response to individual nutrients differed between mid-age and mature stands (Fig. 2), and there was a marginally significant age by nutrient

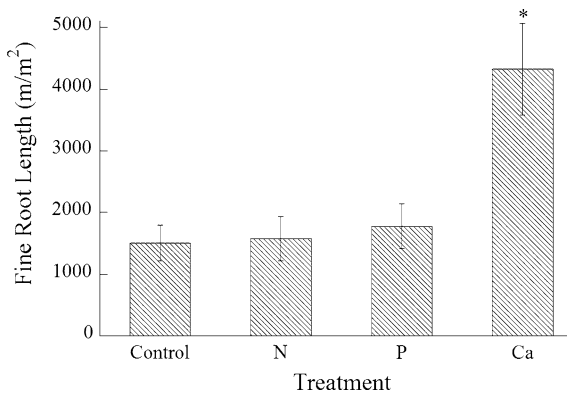

Fig. 1 Fine root length response to nutrient amendments in ingrowth cores in mature forest adjacent to Watershed 1 at the Hubbard Brook Experimental Forest, NH. *Error bars* indicate standard errors of the mean, $n = 6$. Significant differences from control are indicated by * $P < 0.05$

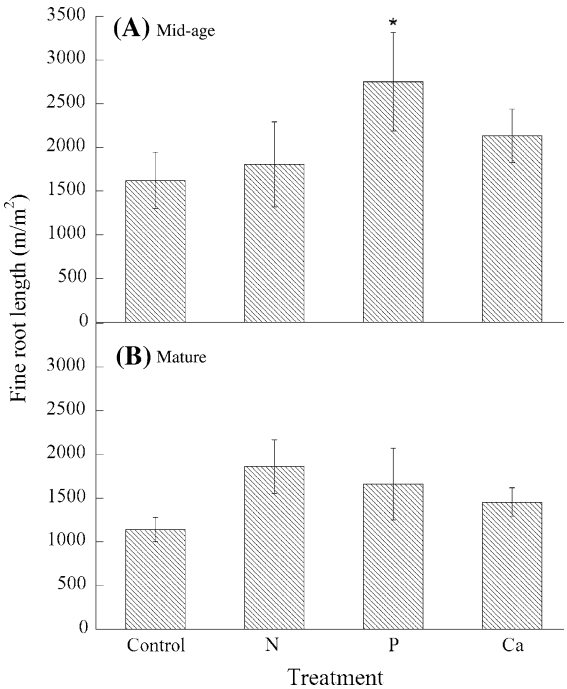

Fig. 2 Fine root length response to nutrient amendments in ingrowth cores in mid-age (**a**) and mature (**b**) forest stands at the Bartlett Experimental Forest, NH. *Error bars* indicate standard errors of the mean, $n = 12$. Significant differences from control are indicated by * $P < 0.05$

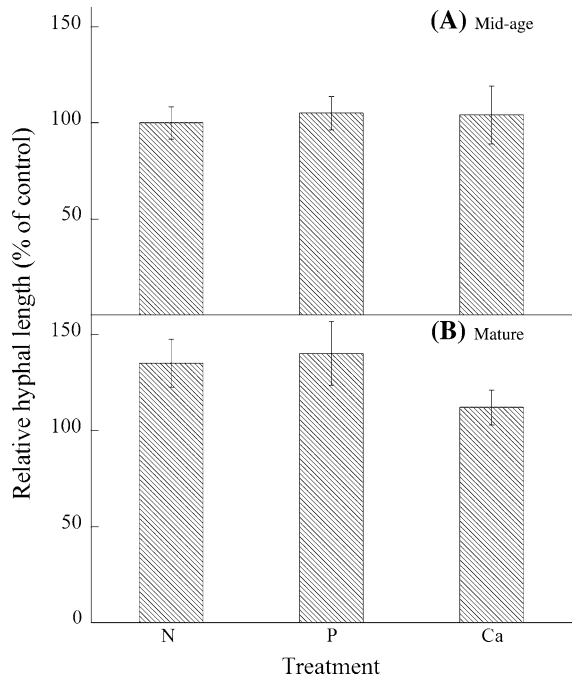

Fig. 3 Fungal hyphal length response to nutrient amendments in ingrowth cores, expressed relative to that in control cores (% of control in each subplot), in mid-age (**a**) and mature (**b**) forest stands at the Bartlett Experimental Forest, NH. *Error bars* indicate standard errors of the mean, $n = 12$. Significant differences from control are indicated by * $P < 0.05$

interaction ($P = 0.085$). In mid-age forest, fine root length was significantly higher in +P than in control cores ($P = 0.033$). No fine root response was found in +P cores in mature forest, however there was a trend toward greater fine root length in +N cores than in control cores ($P = 0.088$). Expression of fine root length as treatment length relative to control revealed a similar pattern. In mid-age forest stands fine root length in +P cores averaged 175% of controls ($P = 0.043$), while there was no significant response in +P cores in mature forest stands. In mature forest stands fine root length in +N cores averaged 185% of controls ($P = 0.022$). No significant treatment effects were detected for fine root biomass, which followed the same trends as length, or for specific root length (data not shown).

Fungal hyphal length in ingrowth cores at the BEF varied substantially among stands; more than two-fold among mid-age stands and almost three-fold among mature stands, precluding detection of nutrient effects. Expressing hyphal length in treatment cores as a percent of controls reduced this variation substantially and revealed a marginally significant trend toward higher relative length in +P than in control cores in mature forest ($P = 0.082$). Average hyphal response to N was similar to the response to P; however, not even a marginal effect was detected ($P > 0.10$) because of higher variation among stands (data not shown). There was clearly no response to any nutrient in mid-age forest stands (Fig. 3).

Phosphatase activity was higher in +N than in control cores in mature forest stands at the BEF ($P = 0.024$) and did not respond to any nutrient in mid-age forest stands (Fig. 4). There was a trend toward higher phosphatase activity overall in mid-age forests.

Net N mineralization potential in the Oe horizon was substantially higher in mid-age than mature forests ($P = 0.04$; Fig. 5). Net N mineralization was lower in Oa and B horizons compared to Oe, and was similar between mid-age and mature forests (Fig. 5). Resin-available P was similar between young and mature forests for all soil horizons, and also declined from Oe to Oa and B (Fig. 5). Bicarbonate-extractable

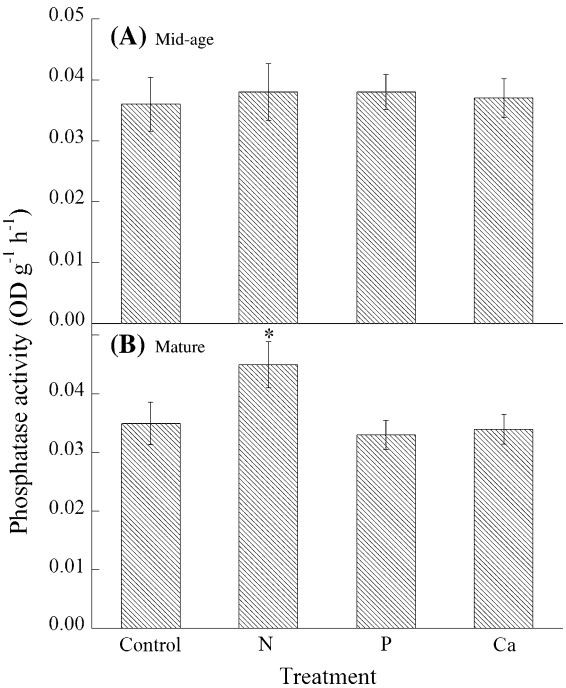

Fig. 4 Response of acid phosphatase activity to nutrient amendments in ingrowth cores in mid-age (**a**) and mature (**b**) forest stands at the Bartlett Experimental Forest, NH. *Error bars* indicate standard errors of the mean, $n = 12$. Significant differences from control are indicated by * $P < 0.05$

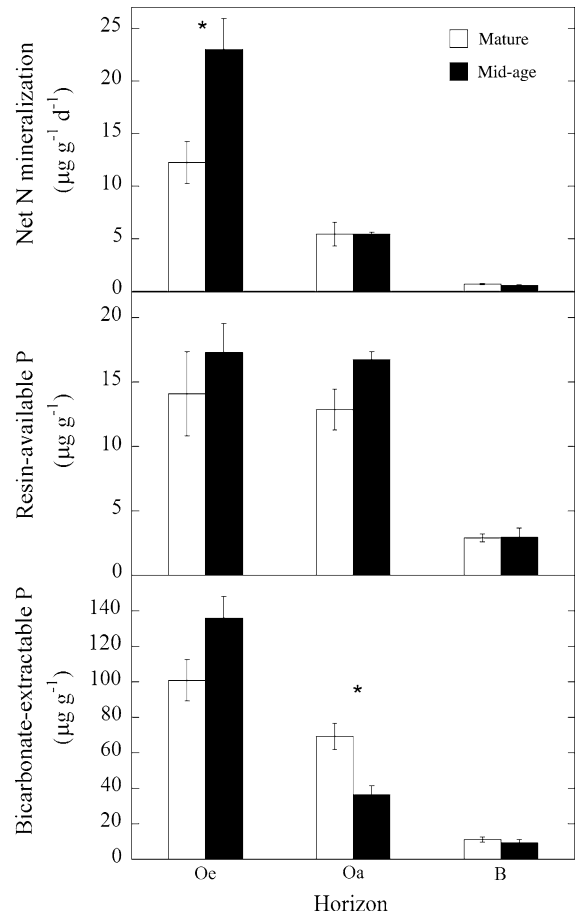

Fig. 5 Nutrient availability indices for soils collected in mid-July from mid-age and mature forest stands in the Bartlett Experimental Forest, NH. *Error bars* indicate standard errors of the mean, $n = 9$ except for bicarbonate-P in the Oe and Oa horizons, for which $n = 3$. Significant differences between young and mature are indicated by * $P < 0.05$

P was significantly lower in mid-age than mature forest in the Oa horizon (Fig. 5).

In forest stands with net N mineralization potential below about 20 μg N g^{-1} day^{-1}, fine root ingrowth response to N generally declined as N availability increased, whereas response to P increased at higher N availability (Fig. 6). These relationships fit second-order polynomial curves with $R = 0.99$ (root response to P) and $R = 0.94$ (root response to N). At the highest net N mineralization, in mid-age stand C6 (Table 1), fine root ingrowth did not respond to either N or P but instead responded to Ca (164% of controls).

Other forest floor and soil properties varied among forest stands (Table 2), as indicated by significant effects of stands nested within forest age [stand(age)]. Percent sand in B horizon soils differed among stands ($P = 0.04$), and soils in mid-age stand C6 and mature stand C8 were finer textured than the others (Table 2). In mid-age stand C6 this corresponded to higher extractable Ca (Table 2), and stand(age) effects were significant for Oa and B horizons ($P = 0.05$ for both). Other significant stand(age) effects were found in B horizon soils for organic C ($P = 0.05$) and pH ($P = 0.05$). Extractable Ca in the B horizon and C:N in Oa and B horizons were higher in mid-age than mature forest soils ($P = 0.05$ for Ca and 0.03 for C:N).

Discussion

Fine roots

Support for our hypothesis that fine root growth responds more to P or Ca than to N depended upon forest age and study site. While fine roots foraged most consistently for N in mature forest at BEF, this was not the case in mid-age forests at BEF where we

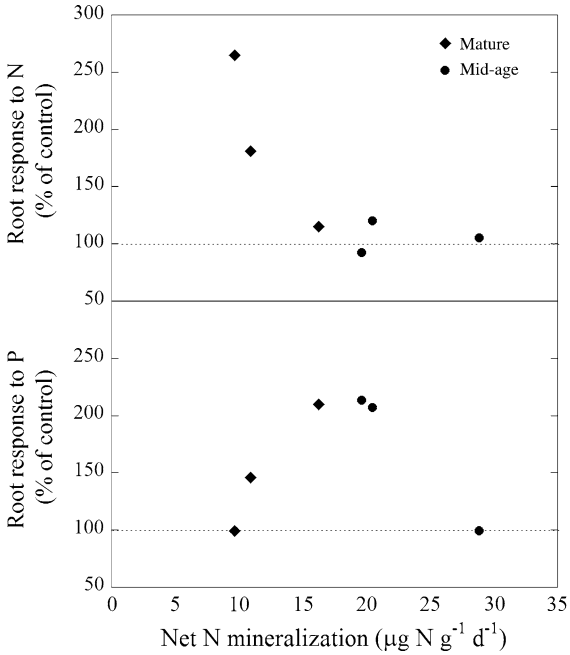

Fig. 6 Root ingrowth response to N and to P in relation to net N mineralization potential, in 3 mid-age and 3 mature hardwood forest stands in the Bartlett Experimental Forest, NH. Root ingrowth results are expressed as a percent of controls

found foraging primarily for P, or in mature forest at HBEF where roots clearly responded only to Ca. The clearest pattern to emerge from our data is that roots foraged for nutrients other than N where N availability was relatively high, even in mature forests. Fine roots foraged for N in the two mature forest stands where N mineralization was lowest, and foraged for P or Ca in stands with higher levels of N mineralization. The same trend extends to the HBEF where roots foraged for Ca. Net N mineralization potentials from Oe horizon of a nearby site were on the high end for mature forest (19.0 μg N g soil^{-1} day^{-1} ± 2.08 standard error of the mean) and mid-age forest (29.1 μg N g soil^{-1} day^{-1} ± 3.29; MC Fisk, unpublished data). While these data were not from our ingrowth study site, they were from nearby forest in HBEF that was similar in species composition, aspect, and elevation, and soils were collected and incubated in early July, concurrently with soils from BEF.

In addition to forest-age related differences in nutrient response, we found interesting variation in foraging for P vs Ca where N availability was high. Variation in metamorphic rock parent materials contributes to patterns of nutrient availability in this region (Hornbeck et al. 1997) that may influence differences in root foraging between BEF and HBEF. It is also well recognized that fertility varies at smaller scales, within the BEF, in relation to soil properties (Leak 1982). This could contribute, for example, to the difference between mid-age stand C6 and the other mid-age stands. However, the combination of finer-textured soils, high extractable Ca, and high net N mineralization in soils of mid-age C6 do not provide any obvious explanation consistent with a foraging response to Ca rather than to P. Mature stand C8 shared similarly fine-textured soils and high extractable Ca (Table 2), but in this site N availability was low and fine roots foraged exclusively for N (data not shown). Stand C6 also differed notably in species composition (Table 1), and it is possible that red maple roots are especially effective at foraging for Ca. Factors such as soil mineralogy, site hydrology, and plant species composition should be further examined to better understand patterns of plant nutrient demand in these northern hardwood forest ecosystems.

Our root foraging results provide evidence that nutrients other than N can be limiting in these northern hardwood forest ecosystems, if we assume that trees allocate C preferentially to acquisition of the nutrient that most limits their growth. This interpretation relies on plasticity in fine root growth and is consistent with the expectation that plants optimize use of resources to acquire what most limits their growth (Bloom et al. 1985; Chapin et al. 1986; Shipley and Meziane 2002). This interpretation also is supported by studies in which the ingrowth method successfully predicted limiting nutrients, as demonstrated by aboveground production response to fertilization, in Hawaiian forest (Raich et al. 1994) and in temperate pine forest (Gleeson and Good 2003). The possibility of P limitation in mid-age forests in this study adds to a growing number of studies in eastern hardwood forests indicating that trees are not always responsive only to N. High demand for P in our early-successional stands concurs with findings in hardwood forests in West Virginia, where root ingrowth and root-associated P uptake enzymes both suggested P limitation (Gress et al. 2007). Our results in the northern hardwoods of central New Hampshire also are consistent with foliar chemistry response to fertilization in early successional stands of the same region (Fahey et al. 1998), and growth of young sugar maple in relation to soil

Table 2 Soil properties for Oe, Oa, and B (surface 10 cm) horizons of individual study sites in the Bartlett Experimental Forest

Soil horizon	Bartlett site	Organic C (mg/g)	C:N	Extractable Ca (mg/g)	pH	Sand (%)	Silt (%)	Clay (%)
Oe								
	Mid-C4	407 (16.5)	23 (0.1)	2.64 (0.012)	4.75 (0.117)			
	Mid-C5	374 (67.1)	19 (1.9)	2.58 (0.532)	4.63 (0.199)			
	Mid-C6	436 (20.7)	21 (0.9)	2.72 (0.383)	4.74 (0.049)			
	Mature-C7	397 (44.4)	20 (1.4)	2.35 (0.290)	4.76 (0.220)			
	Mature-C8	400 (30.5)	18 (0.6)	2.66 (0.332)	4.72 (0.178)			
	Mature-C9	424 (23.8)	25 (1.0)	2.18 (0.347)	4.67 (0.105)			
	Mean Mid-age	363 (46.4)	19 (2.0)	2.32 (0.352)	4.70 (0.071)			
	Mean Mature	365 (42.9)	18 (1.5)	2.10 (0.350)	4.71 (0.088)			
Oa								
	Mid-C4	229 (30.2)	25 (1.1)	0.95 (0.045)	4.54 (0.207)			
	Mid-C5	250 (36.9)	24 (2.4)	0.94 (0.106)	4.77 (0.189)			
	Mid-C6	325 (27.4)	23 (1.1)	1.42 (0.039)	4.15 (0.097)			
	Mature-C7	207 (20.1)	22 (1.0)	0.76 (0.153)	4.53 (0.191)			
	Mature-C8	215 (28.5)	21 (1.1)	1.04 (0.229)	4.54 (0.257)			
	Mature-C9	220 (25.6)	20 (2.6)	0.65 (0.107)	4.65 (0.072)			
	Mean Mid-age	268 (19.7)	24 (0.6)	1.10 (0.086)	4.49 (0.124)			
	Mean Mature	214 (8.8)	21 (0.7)	0.82 (0.103)	4.57 (0.100)			
B								
	Mid-C4	39 (0.4)	26 (1.4)	0.08 (0.010)	4.58 (0.058)	70 (3.1)	8 (1.2)	22 (3.7)
	Mid-C5	39 (1.7)	22 (0.9)	0.11 (0.016)	4.85 (0.069)	71 (1.7)	9 (1.3)	20 (0.5)
	Mid-C6	50 (6.2)	22 (0.6)	0.11 (0.012)	4.62 (0.057)	55 (1.5)	10 (1.0)	35 (1.0)
	Mature-C7	33 (4.2)	20 (0.9)	0.08 (0.030)	4.76 (0.095)	62 (1.9)	7 (0.4)	31 (1.9)
	Mature-C8	42 (1.8)	20 (0.5)	0.07 (0.012)	4.81 (0.023)	55 (3.3)	9 (0.8)	36 (2.8)
	Mature-C9	41 (1.9)	17 (0.6)	0.08 (0.018)	4.90 (0.040)	61 (0.5)	10 (0.5)	29 (0.6)
	Mean Mid-age	43 (2.6)	23 (0.8)	0.11 (0.008)	4.69 (0.054)	65 (2.9)	9 (0.7)	26 (2.6)
	Mean Mature	39 (2.0)	19 (0.6)	0.07 (0.011)	4.82 (0.037)	59 (2.6)	9 (0.4)	32 (2.6)

Sand, silt, and clay were not measured for Oe and Oa horizons. Mid refers to mid-age sites, with trees 24–30 years old. Mature refers to sites with trees over 100 years old. Standard errors in parentheses, n = three 30 m × 30 m plots for site means and $n = 3$ sites for each forest age

chemistry and fertilization in Ontario (Gradowski and Thomas 2006; 2008). In contrast, the lack of response to P and the trend toward foraging by fine roots for N in the mature forest at BEF is more consistent with the idea of N limitation in temperate forest ecosystems. This response to N agrees with recent findings by Finzi (2009) in southern New England, whereas the response to Ca at HBEF agrees with observations that forest growth improved following Ca addition to Watershed 1 at HBEF (Juice et al. 2006).

An important alternative to consider is that the fine root responses that we found are a consequence of species-specific traits in our mixed-species stands, rather than an indication of ecosystem-level nutrient limitation. Species of fine roots of the small size classes that we studied, even those of different mycorrhizal type, cannot be easily distinguished visually in these forests (Yanai et al. 2008), and so we cannot directly answer that question in our study. It is likely that we detected responses by some species but not others, as the ability of plant roots to proliferate in response to nutrients, though widespread, is not a universal trait (Robinson 1994, George et al. 1997). We might expect roots of faster growing, early-successional species to be more responsive to limiting nutrients than those of more

slowly growing species. However, fine root foraging ability does not always differ clearly in relation to tree successional status (Blair and Perfecto 2004) and this should be evaluated on a species by species basis. It is also possible that fine root response to different nutrient availabilities is genetically constrained rather than phenotypically plastic. This could contribute to the pattern we observed if, for instance, fine roots of important early successional species such as white birch are generally more responsive to P than to N. While little is known about fine root growth or plasticity in relation to P supply in northern hardwood species, response by white birch, red maple, and sugar maple to NO_3^- in other studies (Gloser et al. 2008; van Vuuren et al. 2003; Pregitzer et al. 1993) is not consistent with a genetic adaptation to forage only for P. Furthermore, within mature forests the remarkably consistent species composition argues against an effect of different species' traits. With the exception of red maple in mid-age stand C6, variations in species composition within mid-age forests do not correspond in any obvious way to the nutrient responses that we observed. Hence, it is unlikely that species effects are the primary cause of the nutrient foraging patterns found here. However, we do suggest that further research distinguishing between contributions of genetic adaptation and nutrient limitation would be useful for resolving these questions more clearly to learn more about potential species effects on ecosystem biogeochemistry.

It might be unexpected for P to be limiting over the long-term in north-temperate forests, where primary mineral P is still available (Blum et al. 2002). However, we suggest that P limitation in mid-age forests is a shorter-term consequence of relatively high N availability interacting with successional P dynamics and compounded by the lack of mobility of P compared to N. Young forests with rapid biomass accumulation rates may be especially responsive to relatively high net N mineralization, and to N enrichment from deposition. If relatively high N availability enhances productivity in these rapidly aggrading forests, which may have a high P requirement (Sterner and Elser 2002), then this could induce a shortage of P. Forest harvest is also expected to reduce biologically available P early in succession by removing biomass P and also by disrupting nutrient uptake processes that conserve P in surface soil horizons of undisturbed forests, minimizing its movement into less available mineral forms (Wood et al. 1984; Hornbeck et al. 1987; Yanai 1992; 1998). While we did not find that patterns of labile P corresponded to those of fine root foraging, there is good evidence that labile P pools do not necessarily reveal differences in P uptake and recycling, and that depletion from more slowly recycling pools should be taken into account (Richter et al. 2006). Our data suggest that labile P has been depleted from the Oa horizon in mid-age stands while recycling processes have maintained labile P in the Oe hoizon, and more information on slowly recycling P is needed to clarify whether P has been mobilized into recycling pools as forests age.

Fungal hyphae and phosphatase activity

Hyphal lengths presented here provide a simple ecosystem-level assessment of total fungal response to different nutrients, and suggest little to no response in terms of C allocation to forage for nutrients. This clearly did not correspond to patterns of fine root response, and in mid-age forests the lack of response to P by hyphae was somewhat surprising. The faster growth rates of hyphae compared to fine roots should mean that they can respond more rapidly to a nutrient that is in high demand. Furthermore, finer-scale soil exploitation by hyphae compared to roots should be an advantage for acquiring P because of the lack of P mobility in soil. However, fungi are not necessarily limited by the same nutrients as plants, especially if they are able to access different forms such as organic P. It is also possible that increases in some types of fungi are not detected because of corresponding decreases in other types of fungi. Mycorrhizal, rather than saprotrophic, fungi probably account for most of the fungi detected in this study based on vertical distributions documented by Lindahl et al. (2007) and Hendricks et al. (2006), and on sequence data from the ingrowth cores revealing >90% ectomycorrhizal (ECM) species (MC Fisk, unpublished data). Individual mycorrhizal species can differ in their resource acquisition traits, including the nutrients to which they respond (Tuckwell et al. 2006; Wallander et al. 2003; Perez-Moreno and Read 2000; Wallander 2000; Bending and Read 1995), and dominant fungi may change as forests mature. Further exploration of foraging at more detailed taxonomic resolution would be useful to resolve whether an increase in fungal foraging can occur without an increase in total hyphae.

Responses of phosphatase activity to nutrients appear consistent with N limitation or N–P colimitation in mature forests, but are more difficult to interpret in mid-age forests. The lack of phosphatase response to P addition in this study is not consistent with findings of other studies, in which excess P suppressed phosphatase activity and available nutrient concentrations were inversely related to extracellular enzyme activity (Clarholm 1993; Allison and Vitousek 2005). Enzyme activity may have decreased over time during soil storage in our samples, so that we detected only the most marked responses. Treseder and Vitousek (2001) proposed that production of N-rich enzymes can be limited by N availability. Thus, it is also possible that phosphatase production is low overall in our sites, because of low N supply in mature forests or high N demand in rapidly aggrading mid-age forests, and therefore is decreased no further by excess P. While this explanation does not seem consistent with the fine root response to P in mid-age sites, it does concur with phosphatase response to N in mature forests.

Conclusions

The interesting contrasts between mid-age and mature forests shown here illustrate the complexity of belowground processes that mediate responsiveness to nutrient environments. We found no simple or uniform response among the different mechanisms of nutrient acquisition that we tested; responses to nutrients in mid-age forests were restricted to fine roots, whereas fungal hyphae and phosphatase activity were also responsive in mature forests. We also found notable variation among study sites in the nutrients to which fine roots responded. Nevertheless, our fine root responses are good evidence for P limitation in some mid-age forests, an especially interesting idea because limitation by P is generally unexpected in forests such as these in which weatherable forms of primary mineral P are still available. We hypothesize that P limitation in mid-age forests is a transient effect of the coinciding disturbances by forest harvest and N deposition, and that other nutrients become more limiting as forests age. This hypothesized change in limitation may be associated with changes in tree species composition that take place following forest regeneration but also should be further evaluated in concert with the possibility of genetic differences in fine root traits among tree species and functional differences among fungal species.

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