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The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization

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Abstract Northern hardwood forests in the eastern US exhibit species-specific influences on nitrogen (N) cycling, suggesting that their phosphorus (P) cycling characteristics may also vary by species. These characteristics are increasingly important to understand in light of evidence suggesting that atmospheric N deposition has increased N availability in the region, potentially leading to phosphorus limitation. We examined how P characteristics differ among tree species and whether these characteristics respond to simulated N deposition (fertilization). We added NH_4NO_3 fertilizer (50 kg ha^{-1} $year^{-1}$) to single-species plots of red oak (*Quercus rubra* L.), sugar maple (*Acer saccharum* Marsh.), eastern hemlock (*Tsuga canadensis* (L.) Carr.), American beech (*Fagus grandifolia* Ehrh.), and yellow birch (*Betula alleghaniensis* Britt.), in the Catskill Mountains, New York from 1997 to 2007. Species differences were observed in foliar, litter and root P concentrations, but all were unaffected by a cumulative N fertilization of 550 kg/ha. Similarly, measures of soil P availability and biotic P sufficiency differed by species but were unaffected by N fertilization. Results suggest species exhibit unique relationships to P as well as N cycles. We found little evidence that N fertilization leads to increased P limitation in these northern hardwood forests. However, species such as sugar maple and red oak may be sufficient in P, whereas beech and hemlock may be less sufficient and therefore potentially more sensitive to future N-stimulated P limitation.

Keywords Catskill Mountains · Nitrogen · Nutrient limitation · Temperate forest

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Introduction

The influence of forest tree species on nutrient cycling is important for understanding how ecosystems will respond as forest composition changes. Many studies have shown that tree species can influence the cycling of carbon (C), nitrogen (N) and other nutrients in the soils beneath their canopies (Binkley 1995), that these influences may occur over short time periods (Gower and Son 1992), and that they may affect ecosystem-scale processes (Lovett and Rueth 1999; Lovett et al. 2002). However, while some aspects of tree species' effects on forest nutrient cycles are well studied, others are poorly characterized.

Effects of tree species on N cycling have been well studied due to concerns about atmospheric N deposition. Among the many effects of atmospheric N

deposition on forests, including soil acidification, cation leaching, and tree mortality (e.g. Fenn et al. 1998, Aber et al. 2003, Wallace et al. 2007), cycling and retention of N by forested watersheds is a critical human health concern, especially where terrestrial ecosystem processes affect downstream acidification, eutrophication, and water quality. Tree species composition within watersheds may play a key role. Research has shown considerable variation in N cycling processes that influence watershed N retention, depending upon forest composition (Lovett and Rueth 1999; Lovett et al. 2002; Fabio et al. 2009). For example, within the northern hardwood ecosystem, species are known to vary in their influence on N cycling. Interspecific differences in litter chemical quality are an important controller of N cycling rates and ecosystem N retention. Variation in litter C:N ratios, lignin:N ratios, or tannins result in different rates of decomposition and concomitant N mineralization and nitrification (Finzi et al. 1998; Lovett et al. 2004; Templer 2005). In particular, sugar maple (*Acer saccharum*) leaf litter has a lower C:N ratio compared to red oak (*Quercus rubra*) litter, and forest floors beneath sugar maple have higher rates of N mineralization and nitrification compared to those beneath red oak (Finzi et al. 1998). Because ammonium (NH_4) is held on soil exchange sites but nitrate (NO_3) is easily leached, the effect of litter quality on N mineralization and nitrification indirectly influences the retention or loss of N within the soil.

In contrast to N, less is known about the influence of tree species on phosphorus (P) cycling. Tree species are thought to influence P cycling through interspecific differences in root distribution, indirect pH effects on P solubility, phosphatase activity, and production of organic acids that chelate P-binding metals (e.g., aluminum) (reviewed by Binkley 1995). Tree species may also influence P cycling through ecto- (but not endo-) mycorrhizae that directly access mineral forms of P like apatite, though this is poorly understood (Blum et al. 2002; Wallander et al. 2005). Effects of tree species on P cycling have been observed in both tropical and temperate forests dominated by dinitrogen fixers (Zou et al. 1995), in pine stands of the southeastern US (Polyakova and Billor 2007) and subtropical evergreen forests (Kamei et al. 2009). Within the northern hardwood ecosystem, little is known regarding the P status of different species or their influence on P cycling; however, evidence suggests that species differences in P status exist. In a mixed-species forest in Ohio, Boerner and Koslowsky (1989) found greater inorganic P in soils beneath white ash (*Fraxinus americana*) compared to sugar maple (*Acer saccharum*) or American beech (*Fagus grandifolia*). Similarly, Finzi (2009) reported greater inorganic P in forests soils dominated by northern red oak (*Quercus rubra*), eastern hemlock (*Tsuga canadensis*), and beech compared to a forest dominated by sugar maple and white ash.

The P status of northern hardwoods, as well as the linkages between biogeochemical cycles of N and P, are increasingly important to understand in light of evidence that terrestrial ecosystems may frequently be co-limited by N and P (Elser et al. 2007), and that atmospheric N deposition may induce phosphorus (P) limitation (Mohren et al. 1986; Tessier and Raynal 2003; Gress et al. 2007). Added inputs from atmospheric N deposition have the potential to alter the nutrient status of receiving ecosystems (Aber et al. 1989; Dise and Wright 1995; Galloway et al. 1995), leading to altered nutrient limitation, and changes in stoichiometry (Sterner and Elser 2002). While northern hardwood forests have historically been considered N-limited systems, atmospheric N deposition has increased N availability in many areas (Aber et al. 2003). With increased N availability, nutrient limitation by cations such as calcium (Ca) may occur (Juice et al. 2006). However, P limitation could also arise if: (1) N deposition increases N availability, stimulating primary production and therefore biotic P demand, or (2) deposition-related acidity mobilizes soil aluminum (Al) and iron (Fe) and therefore reduces available P, through increased P sorption and decreased mineralization of organic matter (Carreira et al. 2000; Norton et al. 2004).

If continued N deposition results in P limitation, then understanding the P status of tree species, and how their P status changes in response to N additions, will be important for predicting future ecosystem function. Species N cycling characteristics vary in response to N additions (Templer et al. 2005); therefore species may vary in their sensitivity to N-induced P limitation. Sensitivity in this case is defined as the degree to which a change in the input of one nutrient causes change in another nutrient's indicators of availability and demand. Indicators of P status are described below.

We hypothesized that northern hardwood species known to differ in N cycling characteristics would differ both in P cycling characteristics, and in how those P characteristics respond to N additions. We hypothesized that stands dominated by species such as sugar maple and red oak would have a richer P status than stands dominated by hemlock, a species frequently found on nutrient poor sites. Rich P status would be indicated by relatively greater P concentrations in plant tissues and soil microbes (increased microbial P per microbial biomass C), lower activity of extracellular soil enzymes involved in P acquisition (phosphatases), and/or increased availability of P in soil (both extractable inorganic and organic P). We also hypothesized that if N additions cause increased N availability, then indicators of P limitation would increase, and that the response would be tree species-specific. For example, in response to N additions, increased P limitation would be indicated by declining P concentrations in plant tissues and soil microbes, increased phosphatase activity, and/or reduced availability of P in soil. Based on previous reports from forests with high N deposition loads (Pare and Bernier 1989b; Gradowski and Thomas 2006), we hypothesized that sugar maple stands would be most sensitive to N-induced P limitation and that hemlock stands would be least sensitive. To address these hypotheses we examined indicators of P status in single-species plots, with and without N fertilizer.

Methods

Site description

We studied forests in the Catskill Mountains, an area of 5000 km^2 in southeastern New York. The bedrock in this region consists of flat-lying sandstones, shales and conglomerates of Devonian age, overlain by glacial till of variable depth (Rich 1934; Stoddard and Murdoch 1991). The soils are thin Inceptisols (Stoddard and Murdoch 1991) with pH ranging from 3 to 4 (Lovett et al. 2004). The climate is characterized by cool summers and cold winters. Mean annual temperature is 4.3°C and mean annual precipitation is 153 cm (Lovett and Rueth 1999).

We studied the five dominant tree species in the Northern Hardwood forest association of the Catskill region (Braun 1950; McIntosh 1972): sugar maple, American beech, yellow birch, eastern hemlock and red oak. Hereafter we refer to these species as Northern Hardwoods. For each species, pairs of monospecific plots were established in the central Catskills. Monospecific plots were 6 m in radius with the inner 3 m radius of the plot containing three canopy dominant trees of the target species (Lovett et al. 2004). Paired plots were located within 20 m of each other, and pairs were replicated 6 times in at least three different watersheds to encompass spatial variation. Within each pair, one plot remained a control plot, and the other was fertilized with N. There were 60 plots in total: 5 species $\times$ 2 N treatments $\times$ 6 replicates. From 1997 to 2007, granular NH_4NO_3 fertilizer was added to one plot of each pair. Fertilizer was applied four times per year (June, July, August and November) for an annual dose of 50 kg N ha^{-1} $year^{-1}$ and a cumulative fertilization of 550 kg ha^{-1} over the duration of the study. Over the term of the fertilization treatment, there have been no significant increases in productivity (e.g. net primary productivity, basal area increments) in the fertilized plots (G. Lovett et al. unpublished). Total (wet + dry) atmospheric N deposition in the Catskill Mountains varies across the landscape up to 4-fold (Weathers et al. 2000), but ambient N deposition in this area is roughly 11 kg N ha^{-1} $year^{-1}$ (NADP http://nadp.sws.uiuc.edu; CASTNET http://www.epa.gov/castnet).

Field sampling

Plant tissues were sampled and P concentrations used as measures of P nutritional status. Foliage was sampled in late July and early August 1997, 2002 and 2006 by shooting foliage from the mature canopy trees in each plot with a shotgun using steel shot. Three samples of sunlit leaves near the tops of the trees were collected per plot. In 1997 and 2002, aboveground litterfall was collected in plastic baskets (0.23 m^2 area) in which fiberglass screen was suspended to trap litter. Litter was collected approximately bi-weekly from late August through November using three baskets per plot. Litter was composited over the collection period and then sorted by species. Foliage and litter were dried in a 60°C oven, and ground in a Spex CertiPrep 8000

Mixer/Mill (Metuchen, NJ) prior to analysis. Fine roots (< 2 mm diameter) were collected from 15 × 15 cm forest floor blocks in 2006. Roots were separated from bulk soil and gently cleaned using brushes until 5-6 g of tissue was obtained per plot.

In 2007, organic and mineral soil layers were sampled from 20 of the 60 plots. Because soil samples were analyzed for temporally sensitive biological characteristics, the subset of plots were chosen so that all species were represented, but all plots could be sampled within 2 days. Additionally, we excluded plots with advanced stages of beech bark disease (Griffin et al. 2003). The subset of plots was sampled on May 16, May 30, and June 15 (hereafter referred to as mid-May, late-May and mid-June, respectively), for indicators of P supply and biotic demand. Prior to May 16 and May 30 sampling dates, fertilizer had not been applied since November 2006. Fertilizer was applied to the plots immediately following the late-May sampling. To avoid any short-term "pulse" effects of N fertilizer, the last soil sampling date of June 15 was chosen to occur more than 2 weeks after the fertilizer had been applied. On each date, a 2 cm diameter soil corer was used to collect the organic (Oe and Oa) and top 5 cm of mineral horizons. Three to five cores were taken per plot until approximately 50 g of each horizon was collected. Soils were separated by horizon, bulked by plot, sieved to pass 2 mm mesh and immediately stored at 4°C until analysis.

Lab methods

Foliar, litter, and root tissues were ashed in a muffle furnace at 550°C and dissolved in 6 M nitric acid. Following digestion, foliar and litter P concentrations were determined colorimetrically on an autoanalyzer (Technicon System 2, Tarrytown NY) at the University of Kentucky (Fiske and Subbarow 1925). Resorption was calculated for 1997 and 2002 as the percentage of foliar P not found in litter [(foliar P – litter P)/foliar P × 100)]. Because only upper-canopy foliage was sampled, calculated resorption is interpreted as an index of true resorption. Root P concentrations were measured colorimetrically by a modified malachite green assay conducted in 96-well microplates and read on a microplate spectrophotometer (Molecular Devices VERSAmax, Sunnyvale CA) (D'Angelo et al. 2001; Jeannotte et al. 2004). Foliar N concentrations were determined by combustion in a C:N analyzer (Leco CN 2000, Leco Corp., St. Joseph MN).

For analyses of soil extractable P, microbial biomass P, and phosphatase activity, soil samples were removed from refrigeration and analyzed at field moisture content. A subsample was oven dried at 60°C to determine gravimetric moisture content (Templer & Dawson 2004) so concentrations could be expressed on a dry weight basis. The standard method for soil gravimetric moisture determination is typically drying soil at 105°C. However, we dried soils at 60°C because volatilization of N can occur at higher temperatures. Laboratory tests showed that drying these organic and mineral soils at 60 and 105°C resulted in a minor change in mass of 0.01–0.03% suggesting little residual water remaining in these soils. Subsamples of mineral soils were air dried prior to pH and P fractionation, and concentrations are reported on an air-dried weight basis.

On each 2007 sampling date, extractable soil P and microbial biomass P were measured in organic and mineral horizons using anion exchange resin strips (Myers et al. 2005). Microbial biomass P (P_{mic}) was measured by capturing P released upon application of a biocide to soil (Myers et al. 1999). From each field sample, two 3 g sub-samples of fresh soil were shaken for 24 h in bottles containing 50 mL H_2O to which two 1 × 4 cm anion exchange resin strips were added. The resin strips were previously treated by shaking them in 0.5 M $NaHCO_3$. One of the sub-samples had 2.5 ml of 1-Hexanol added to it as a biocide and the other sub-sample was a control without 1-Hexanol. Following the 24 h shaking period, resin strips were rinsed in de-ionized water, shaken dry, placed in 50 ml of 0.5 M HCl and shaken at 120 RPM for 45 min. Resin strips were then removed and HCl extracts analyzed for inorganic P (P_i) using the malachite green colorimetric assay cited above. To create compatible acidity for the malachite green assay, HCl extracts were acidified to 1.26 N using sulfuric acid. Inorganic P from control samples was considered to be plant-available. A 5–10 ml aliquot of the HCl extract was digested in tubes containing 2.44 ml of sulfuric acid (37% acid V/V, containing no P), 0.3 g of K_2SO_4 and two selenium (Se) granules. The tubes were heated to 160°C for 1.5 h and then 220°C for 1 h, to remove water and HCl, while leaving concentrated sulfuric

acid in place. The tubes, containing the concentrated sulfuric acid, were covered with Teflon balls (to maintain constant acid concentration) and digested at 360°C for 1 h. Following digestion the remaining liquid in the tubes was diluted to 25 ml with water (creating a 1.26 N solution), and analyzed for total P(P_t), also by the malachite green assay. The digested samples had an increase in colorimetrically reactive phosphate (i.e., P_t was greater than Pi). The increase in phosphate came from the hydrolysis of organic P (P_o) that was previously recovered from the resins. Therefore P_o was calculated as P_t minus P_i in the control samples (Rubaek and Sibbesen 1993). We acknowledge that because we used anion resins, any positively charged organic P would not be captured by this method. P_{Mic} was calculated as the difference in P_t between control and biocide samples. Due to sample contamination with microbial growth in a separate set of extractions for microbial C determination, we were unable to determine ratios of P_{Mic} to microbial biomass C.

The activity of acid phosphatase enzymes in the soils was used as an index of biotic P demand in excess of P supply. Phosphatases are produced by plant roots and microbes but production and activity is down-regulated when inorganic P is abundant (Spiers and McGill 1979). On each 2007 sampling date, sub-samples of 0.35 g organic or 0.50 g mineral soil were weighed into bottles to which 50 mM pH 5.0 acetate buffer was added to make a 125 ml soil slurry. Each bottle was shaken vigorously by hand for 1 min and then sonicated for 30 s to disperse soil particles. Two replicate samples were assayed per plot per horizon. Assays were conducted in 96-well microplates using methylumberiferol-linked phosphate substrate following the methods of Saiya-Cork (2002). Assay plates were incubated in the dark at 22°C for 1–2.5 h and fluorescence (emission wavelength was 450 nm) was read on a fluorescence spectrophotometer equipped with a plate-reader (Perkin Elmer LS50B). Organic horizon assays were completed within 2–3 days of sampling. Mineral horizon assays were completed within 4–5 days. To compare enzyme patterns across sampling dates, relative enzyme activities were calculated for each plot ($n = 20$) as a percentage of the mean activity on each date. Relative activities for each plot were then averaged across all three dates. A sub-sample was analyzed for percent moisture by drying at 60°C. Another subsample was air dried and analyzed for P fractionation, as described below, and pH. Soil pH was measured using a 1:10 soil: water ratio for organic horizons and a 1:2 ratio for mineral horizons (Hendershot et al. 1993).

We further characterized soil P availability via sequential soil fractionation. These techniques rely on operationally defined fractions to approximate biologically available P pools (Cross and Schlesinger 1995). However, different methods result in different conclusions regarding the size of plant available P pools (Johnson et al. 2003). The fractionation method was modified for use with forest mineral soils (Psenner et al. 1988; I. Fernandez, personal communication Sept. 2007). The principal modification was the use of 0.1 M NaOH rather than 1 M NaOH. The procedure extracts fractions of P that approximate the following pools: (A) ion-exchangeable P, (B) reducible metal-hydroxide P, (C) organically bound P and labile Al- or Fe-bound P, and (D) crystalline or calcium-bound P. Because P may precipitate or adsorb with aluminum (Al) and iron (Fe) in acid soils, we also measured these metals in each extract by inductively coupled plasma (ICP).

Each fraction was determined by shaking 1 g air-dried soil in a 50 ml centrifuge tube to which a sequence of extractants was added. Following shaking, the extractant was separated from residual soil by centrifugation, and the resulting supernatant was vacuum filtered from the tube and saved for analysis. The addition of extractant, shaking, centrifugation and filtration was then repeated with a 1 min shaking time before proceeding to the next extract in the sequence. Ion-exchangeable P was determined by shaking each sample in 8 ml 1 M NH_4Cl for 24 h at 25°C. Reducible metal-hydroxide P was determined by shaking samples in 25 ml 0.11 M $NaHCO_3$ and 0.11 M $Na_2S_2O_4$ for 1 h at 40°C. Organically bound and labile Al- or Fe-bound P was determined by shaking samples in 0.1 M NaOH for 16 h at 25°C. Crystalline P was determined by shaking samples in 0.5 M HCl for 16 h at 25°C. Aliquots of each extract (8 ml of NH_4Cl extracts, 25 ml of all others) were added to vessels containing 1 ml de-ionized water, 4.5 ml concentrated nitric acid, and 1.5 ml concentrated hydrochloric acid, and then microwave digested (EPA Method 3051). While EPA Method 3051 was not originally designed for P analysis, comparisons show that this method provides better

recovery of P than conventional soil digests (Dancer et al. 1998). Digests were analyzed for P, Al, and Fe by ICP. An aliquot of the NaOH extract was analyzed for inorganic P (NaOH-Pi) prior to digestion by the malachite-green colorimetric method and the organic P fraction (NaOH-Po) was calculated as the difference of total P (NaOH-P) and inorganic NaOH-Pi. Total soil P, Al, and Fe were determined from 0.25 g soil using the same microwave procedure. A final residual fraction was determined as the difference between the total and the sum of all previous fractions. In all procedures where P concentrations were determined, we ran 10% of the samples for QA/QC (replicates) and used NIST Apple Leaves (SRM 1515) as tissue standards. All P concentrations from QA/QC runs had coefficients of variation less than 5% and P concentrations from tissue standards were within the certified ranges. Unless otherwise noted, all concentrations are reported as means ± 1 standard error.

Statistical analysis

All measurements were averaged for each plot. Plot means were analyzed in a mixed model ANOVA (SAS Proc Mixed) using species and fertilization as main fixed effects and site (each plot-pair within a watershed) as a random effect. Due to the paired plot design, site was nested within species. When the same measurement was made on a plot over time, a repeated measures mixed model was used with species and fertilization as fixed effects and site as a random effect (after Templer et al. 2005). Again, site was nested within species. When main effects were significant, pair-wise post-hoc comparisons of sub-group means were made using the Student–Newman–Keuls procedure. When interactions were significant, differences in simple effects were examined using F tests on adjusted least squares means. Least-squares regressions were used to test the strength of linear relationships between variables and correlations were tested using Pearson correlation coefficients (SAS Proc Corr). We compared foliar P concentrations in this study with data from the Foliar Chemistry Database of the Northeastern Ecosystem Research Cooperative (FCD-NERC, http://www.folchem.sr.unh.edu/index.html). We accessed the database on November 23–30, 2008 using the following search criteria for each of the five tree species. Trees were located within Massachusetts, Maine, New York, New Hampshire, Pennsylvania, Vermont or West Virginia and between 380 and 944 m in elevation (similar to our study sites). This search resulted in foliar P measures from 1324 trees (from 191 plots). We excluded the Buttermilk Falls sites in New York (32 trees from 6 plots) from the results because foliar P concentrations for all species in these sites were 2–5 times higher than any other site. We contacted several researchers familiar with the data but found no explanation for this difference. We compared each species' foliar P concentrations and foliar N:P from control plots to FCD-NERC data using Wilcoxon two-sample tests. All statistical analyses were conducted using SAS software (Version 9.1, SAS Institute, Inc. 2006).

Results

Plant tissue

Overall, P concentrations in foliage, litter and fine roots differed by species, but were unaffected by N fertilization. While litter P concentrations and P resorption varied between years ($p < 0.0001$ for both), foliar P concentrations did not ($p = 0.30$, Table 1). Repeated sampling of foliage in 1997, 2002, and 2006 showed that foliar P concentrations differed by species ($F = 3.14$, $p = 0.03$), but N fertilization had no effect ($F = 0.99$, $p = 0.32$). Foliar P concentrations ranged from 0.78 to 2.04 mg g^{-1} and in all years hemlock foliage was lower in P compared to red oak and yellow birch foliage. Red oak foliage had more P than any other species in both 2002 and 2006. Beech foliage declined in P from 1.45 mg g^{-1} in 1997 to 1.25 mg g^{-1} in 2006. Although this decline was not statistically significant ($p = 0.14$), beech was the only species in which foliar P concentrations declined over time and many of the sampled beech trees showed signs of beech bark disease. Wilcoxon two-sample tests comparing foliar P from this study to FCD-NERC data showed that for all species except maple there were no significant differences between our and others' data sets. Maple foliar P from our plots averaged 1.25 ± 0.05 mg g^{-1} ($n = 36$) and was significantly greater ($W = 8846$, $p = 0.03$) than

Table 1 Phosphorus concentrations in foliage, leaf litter and fine roots and percent foliar resorption from control and nitrogen fertilized plots in the Catskill Mountains, NY

	Year	American beech		Eastern hemlock		Sugar maple		Red oak		Yellow birch	
		Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized
Foliage (mg g^{-1})	1997	1.49 ± 0.12	1.44 ± 0.15 a	0.99 ± 0.06	1.05 ± 0.08 b	1.18 ± 0.15	1.34 ± 0.13 a	1.42 ± 0.10	1.41 ± 0.13 a	1.41 ± 0.10	1.50 ± 0.12 a
	2002	1.35 ± 0.07	1.25 ± 0.12	1.23 ± 0.17	1.14 ± 0.12	1.30 ± 0.13	1.29 ± 0.12	1.55 ± 0.05	1.50 ± 0.06	1.51 ± 0.08	1.38 ± 0.08
	2006	1.27 ± 0.06	1.24 ± 0.09 b	1.19 ± 0.05	1.09 ± 0.03 b	1.18 ± 0.07	1.21 ± 0.05 b	1.52 ± 0.05	1.44 ± 0.06 a	1.46 ± 0.06	1.40 ± 0.04 a
Litter (mg g^{-1})	1997	0.43 ± 0.07	0.41 ± 0.08 a	0.37 ± 0.03	0.35 ± 0.04 a	0.36 ± 0.11	0.39 ± 0.09 a	0.92 ± 0.17	0.88 ± 0.12 b	0.50 ± 0.04	0.51 ± 0.07 a
	2002	0.81 ± 0.10	0.84 ± 0.12 b	0.38 ± 0.02	0.38 ± 0.04 c	0.62 ± 0.12	0.60 ± 0.14 bc	1.14 ± 0.16	1.13 ± 0.20 a	0.87 ± 0.10	0.87 ± 0.09 b
Resorption (%)	1997	71.7 ± 4.1	72.2 ± 3.9 a	62.7 ± 2.0	65.6 ± 4.8 a	71.5 ± 5.7	71.7 ± 4.2 a	36.5 ± 10.2	36.4 ± 10.1 b	64.1 ± 2.1	66.8 ± 2.5 a
	2002	41.0 ± 5.5	34.2 ± 3.6 b	66.9 ± 3.8	66.0 ± 1.9 a	54.2 ± 4.5	56.0 ± 6.0 a	26.7 ± 10.2	24.9 ± 12.1 b	42.0 ± 6.7	37.0 ± 5.6 b
Fine roots (mg g^{-1})	2006	0.85 ± 0.12	0.77 ± 0.11 bc	0.61 ± 0.03	0.66 ± 0.04 c	1.28 ± 0.22	1.11 ± 0.11 a	1.04 ± 0.09	0.88 ± 0.12 b	0.57 ± 0.04	0.69 ± 0.07

Plots were dominated by either American beech, eastern hemlock, sugar maple, northern red oak, or yellow birch. Within each row significant differences among species, when present, are indicated in lower case letters. No fertilization effects were significant

mean maple foliar P in the FCD-NERC data (mean 1.15 ± 0.01 mg g^{-1}, $n = 387$).

Foliar N:P ratios in 2006 varied significantly among species in 2006 ($F = 4.0$, $p = 0.01$) but not 1997 ($p = 0.45$, Table 2). Foliar N:P ratios were significantly greater in yellow birch than in hemlock. In 2006 after 9 years of fertilization, foliar N:P was significantly greater in fertilized trees compared to controls (Table 2; $F = 10.3$, $p = 0.004$). This difference was attributable to significantly greater ($F = 9.9$, $p = 0.004$) foliar N concentrations (foliar N data not shown) in fertilized trees rather than decreases in foliar P. For all species except beech, there was no significant difference between foliar N:P ratios in control plots and those found within the FCD-NERC dataset. Beech foliage in our study had slightly but significantly lower foliar N:P than that found in the FCD-NERC dataset ($Z = -2.07$, $p = 0.04$; Table 2).

Litter P ranged from 0.14 to 1.69 mg g^{-1}. In both 1997 and 2002 oak litter P concentrations were almost twice those of sugar maple or hemlock. Across all species, mean P resorption was greater in 1997 (62%) compared to 2002 (45%), the only years in which we had foliage and litter samples. In both years, oak had the lowest resorption of P (mean of 32%) while hemlock had the highest (mean 65%). Variation in P resorption between years was lower for red oak, hemlock and sugar maple and higher for beech and yellow birch. Concentrations of P in roots (P_{Root}) differed by species ($F = 5.9$, $p = 0.002$) but not fertilization ($p = 0.28$). P_{Root} ranged from 0.40 to 2.17 mg g^{-1} and was greatest in maple plots and lowest in hemlock and birch plots. P_{Root} was positively correlated with foliar P concentrations for beech ($r^2 = 0.77$, $p < 0.001$) and maple ($r^2 = 0.48$, $p = 0.01$) but not other species.

Soil moisture and pH

Over the three sampling dates in 2007, gravimetric soil moisture in organic and mineral horizons did not differ by species or fertilization. Organic soils ranged from 42 to 70% moisture and differed between sampling dates ($F = 26.02$, $p = <0.0001$). Organic soils decreased from a mean of 59 ± 0.01% moisture in mid-May, to 54 ± 0.02% moisture in late-May, and then rose to 59 ± 0.01% moisture in mid-June. Mineral soils ranged from 15 to 37% moisture and

Table 2 Comparisons of mass-based N:P ratios in foliage of northern hardwood tree species for this study (after 9 years of fertilization), FCD-NERC, and Finzi (2009)

Species	This study (2006 only)		FCD-NERC	Finzi (2009)
	Control	Fertilized		
Sugar maple	15.5 ± 1.1	16.7 ± 1.0 A	17.0 ± 0.31	16.7 ± 0.34
Yellow birch	17.7 ± 1.2	19.3 ± 0.4 B	17.1 ± 0.29	na
American beech	17.1 ± 1.5	18.1 ± 1.9 AB	18.8 ± 0.38	16.8 ± 0.46
Red oak	15.4 ± 0.7	16.5 ± 1.4 AB	15.9 ± 0.78	15.3 ± 0.50
Eastern hemlock	12.4 ± 0.8	13.6 ± 0.6 A	13.4 ± 0.70	8.7 ± 0.77

Capital letters indicate significant differences among species

like organic soils, differed, albeit slightly, among sampling dates ($F = 3.76$, $p = 0.04$). Mineral soils averaged 25 ± 0.01% moisture in both mid-May and late-May while in mid-June soils averaged 26 ± 0.01% moisture.

Soil pH in the organic horizon ranged from 3.04 to 4.31 and decreased from a mean of 3.63 ± 0.06 in mid-May to 3.35 ± 0.05 in mid-June ($F = 45.68$, $p < 0.0001$; statistical tests done on H^+ concentrations, Table 3). Organic horizons in fertilized plots were significantly more acidic than in control plots ($F = 20.63$, $p = 0.0001$, Table 3), except for horizons under beech (species × fertilizer interaction $F = 5.13$, $p = 0.004$). Organic soil acidity also differed by species ($F = 8.0$, $p = 0.02$). Oak organic soils (mean pH 3.86 ± 0.07) were significantly less acidic than beech (3.25 ± 0.04) and maple soils (3.32 ± 0.05). In mineral horizons, soil pH ranged from 2.82 to 3.59 and increased slightly, but not significantly ($p = 0.17$), from a mean of 3.11 ± 0.05 in mid-May to 3.15 ± 0.04 in mid-June. Like organic soils, mineral soils were consistently more acidic in fertilized plots compared to controls ($F = 8.09$, $p = 0.009$). Mineral soil pH differed by species ($F = 8.22$, $p = 0.02$), and species interacted with date ($F = 7.36$, $p < 0.0001$). Across all dates, mineral soils beneath oak were significantly less acidic (mean pH 3.46 ± 0.03) compared to beech (2.94 ± 0.03) or maple soils (3.08 ± 0.05).

Extractable inorganic and organic P

Extractable inorganic P (P_i) was used as a measure of available P in organic ($P_{i\text{-}Org}$) and mineral ($P_{i\text{-}Min}$) horizons. In organic horizons, $P_{i\text{-}Org}$ ranged from 0.02 to 20.34 μg g^{-1} and varied by sampling date ($F = 6.11$, $p = 0.01$), declining from a mean of 5.79 μg g^{-1} in mid-May to 3.01 μg g^{-1} by mid-June (Table 3). Though not statistically significant ($F = 4.99$, $p = 0.054$), species differences in $P_{i\text{-}Org}$ may be ecologically significant. Across all sampling dates there was a trend toward greater $P_{i\text{-}Org}$ under oak and lower $P_{i\text{-}Org}$ under beech and hemlock. In mid-June $P_{i\text{-}Org}$ was undetectable in hemlock plots. $P_{i\text{-}Org}$ was not significantly affected by fertilization ($p = 0.36$) but in both late-May and mid-June $P_{i\text{-}Org}$ tended to be greater in fertilized plots of birch, maple, and oak, compared to controls. $P_{i\text{-}Org}$ declined linearly as soil pH decreased (Fig. 1c—$r^2 = 0.37$, $F = 18.82$, $p = 0.0002$). Beech and maple had more acidic organic horizons and lower $P_{i\text{-}Org}$ while oak plots had the least acidic conditions and the greatest $P_{i\text{-}Org}$ (Table 3). Organic soil moisture was not linearly related to $P_{i\text{-}Org}$ ($p = 0.37$).

In mineral horizons, $P_{i\text{-}Min}$ ranged from 0.09 to 2.40 μg g^{-1} and was on average six times lower than $P_{i\text{-}Org}$. $P_{i\text{-}Min}$ was unaffected by species or fertilization ($p = 0.51$ and $p = 0.68$, respectively; Table 3). Like $P_{i\text{-}Org}$, $P_{i\text{-}Min}$ varied by date ($F = 40.33$, $p < 0.0001$), and was lowest in mid-June. Across all dates, there was a trend toward greater $P_{i\text{-}Min}$ in oak and birch soils and lower $P_{i\text{-}Min}$ in hemlock soils. In mid-June $P_{i\text{-}Min}$ was undetectable in fertilized hemlock plots. Unlike $P_{i\text{-}Org}$, there was no significant relationship between $P_{i\text{-}Min}$ and soil pH. In late-May, the driest sampling date, there was a strong positive correlation between $P_{i\text{-}Min}$ and mineral soil moisture ($r^2 = 0.57$, $F = 25.96$, $p < 0.0001$). On all other dates this correlation was weak.

Table 3 Soil parameters measured on three dates in 2007 in control and nitrogen fertilized plots in the Catskill Mountains, NY

	Horizon	Date	American beech		Eastern hemlock		Sugar maple		Red oak		Yellow birch		Species, Fertilizer and Date Effects
			Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized	
Pi ($\mu g\ g^{-1}$)	Organic	Mid-May A	1.49 ± 0.17	1.46 ± 0.12	0.71 ± 0.08	0.98 ± 0.05	9.12 ± 2.06	3.32 ± 1.57	11.72 ± 5.55	10.77 ± 1.46	8.12 ± 3.92	10.22 ± 4.54	D
		Late-May B	1.62 ± 0.09	0.73 ± 0.40	0.40 ± 0.05	0.75 ± 0.21	1.85 ± 0.57	3.33 ± 1.65	8.32 ± 0.25	13.98 ± 6.36	2.04 ± 1.64	5.24 ± 4.67	
		Mid-June B	1.07 ± 0.18	0.04 ± 0.03	ND	ND	2.41 ± 0.25	3.36 ± 1.11	6.57 ± 1.93	12.08 ± 5.33	0.93 ± 0.65	3.69 ± 3.55	
	Mineral	Mid-May A	0.71 ± 0.14	0.37 ± 0.02	0.19 ± 0.08	0.21 ± 0.01	0.49 ± 0.19	0.76 ± 0.07	1.40 ± 0.99	0.45 ± 0.16	0.62 ± 0.34	1.18 ± 0.74	D
		Late-May B	1.29 ± 0.57	0.80 ± 0.07	0.56 ± 0.29	0.56 ± 0.12	1.18 ± 0.39	0.93 ± 0.45	1.37 ± 0.42	1.21 ± 0.11	1.25 ± 0.25	1.39 ± 0.77	
		Mid-June C	0.16 ± 0.04	0.13 ± 0.02	0.11 ± 0.01	ND	0.18 ± 0.04	0.25 ± 0.11	0.47 ± 0.29	0.19 ± 0.10	0.28 ± 0.11	0.91 ± 0.77	
Po ($\mu g\ g^{-1}$)	Organic	Mid-May A	0.59 ± 0.13	0.64 ± 0.03	0.64 ± 0.01	0.78 ± 0.41	0.52 ± 0.12	0.67 ± 0.03	0.56 ± 0.13	0.83 ± 0.05	0.91 ± 0.00	0.62 ± 0.38	D, F
		Late-May B	0.19 ± 0.08	0.21 ± 0.03	0.05 ± 0.00	0.10 ± 0.01	0.37 ± 0.34	0.66 ± 0.58	0.30 ± 0.00	0.31 ± 0.04	0.30 ± 0.20	0.74 ± 0.03	
		Mid-June B	0.30 ± 0.10	0.40 ± 0.07	0.12 ± 0.08	0.37 ± 0.18	0.35 ± 0.05	0.72 ± 0.19	0.36 ± 0.12	0.35 ± 0.17	0.42 ± 0.06	0.51 ± 0.08	
	Mineral	Mid-May A	0.32 ± 0.11	0.30 ± 0.11	0.32 ± 0.13	0.26 ± 0.00	0.33 ± 0.01	0.30 ± 0.01	0.27 ± 0.05	0.43 ± 0.11	0.31 ± 0.03	0.29 ± 0.07	D, S x F
		Late-May B	0.61 ± 0.11	0.49 ± 0.02	0.51 ± 0.06	0.41 ± 0.02	0.67 ± 0.01	0.49 ± 0.02	0.52 ± 0.11	0.66 ± 0.24	0.80 ± 0.09	0.70 ± 0.06	
		Mid-June C	0.51 ± 0.07	0.42 ± 0.07	0.30 ± 0.01	0.41 ± 0.05	0.44 ± 0.01	0.39 ± 0.00	0.35 ± 0.07	0.45 ± 0.00	0.47 ± 0.11	0.37 ± 0.12	
pH	Organic	Mid-May A	3.35 ± 0.16	3.29 ± 0.00	3.76 ± 0.02	3.63 ± 0.18	3.52 ± 0.05	3.58 ± 0.27	4.12 ± 0.18	3.75 ± 0.10	3.79 ± 0.16	3.60 ± 0.08	D, S, F, S x F
		Late-May B	3.24 ± 0.06	3.34 ± 0.02	3.50 ± 0.03	3.35 ± 0.02	3.34 ± 0.04	3.51 ± 0.06	4.15 ± 0.03	3.70 ± 0.01	3.72 ± 0.11	3.45 ± 0.21	
		Mid-June C	3.11 ± 0.07	3.18 ± 0.02	3.38 ± 0.03	3.28 ± 0.07	3.14 ± 0.04	3.19 ± 0.14	3.80 ± 0.12	3.63 ± 0.04	3.51 ± 0.17	3.32 ± 0.12	
	Mineral	Mid-May	3.01 ± 0.13	2.96 ± 0.02	3.11 ± 0.01	3.10 ± 0.03	2.93 ± 0.06	2.96 ± 0.14 A	3.54 ± 0.02	3.48 ± 0.10	3.02 ± 0.07	2.99 ± 0.06 A	S, F, S x D
		Late-May	2.88 ± 0.04	2.87 ± 0.05	3.07 ± 0.02	3.02 ± 0.03	3.19 ± 0.11	3.16 ± 0.18 B	3.51 ± 0.03	3.36 ± 0.14	3.24 ± 0.01	3.11 ± 0.05 B	
		Mid-June	3.02 ± 0.07	2.90 ± 0.06	3.17 ± 0.00	3.04 ± 0.06	3.14 ± 0.16	3.07 ± 0.09 AB	3.47 ± 0.09	3.37 ± 0.03	3.19 ± 0.12	3.12 ± 0.02 AB	
Phosphatase activity (nmol $h^{-1}\ g^{-1}$)	Organic	Mid-May A	3317 ± 118	3250 ± 131	4649 ± 427	3623 ± 19	2083 ± 150	2846 ± 1136	1759 ± 215	2058 ± 128	3156 ± 44	2580 ± 411	D, S x F x D
		Late-May A	2912 ± 61	3101 ± 99	3417 ± 489	3244 ± 114	1646 ± 120	2579 ± 796	1866 ± 133	2165 ± 168	2908 ± 213	2783 ± 442	
		Mid-June B	3141 ± 2	4148 ± 510	5277 ± 949	3711 ± 74	2562 ± 56	3446 ± 899	2463 ± 262	3246 ± 436	3344 ± 296	3886 ± 503	
	Mineral	Mid-May A	562 ± 39	502 ± 70 A	1072 ± 609	829 ± 90	734 ± 206	611 ± 37	551 ± 225	330 ± 5 A	450 ± 28	568 ± 35 A	D, S x D, F x D
		Late-May A	753 ± 198	1118 ± 137 AB	1221 ± 578	1000 ± 39	674 ± 213	751 ± 129	828 ± 30	960 ± 159 B	733 ± 88	726 ± 100 AB	
		Mid-June B	835 ± 121	1186 ± 278 B	1039 ± 534	1097 ± 162	833 ± 168	929 ± 111	653 ± 139	713 ± 117 AB	864 ± 69	855 ± 39 B	

Table 3 continued

	Horizon	Date	American beech		Eastern hemlock		Sugar maple		Red oak		Yellow birch		Species, Fertilizer and Date Effects
			Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized	Control	Fertilized	
PMic ($\mu g\ g^{-1}$)	Organic	Mid-May A	93.7 ± 14.6	109.0 ± 16.3	145.7 ± 0.3	133.7 ± 20.0	184.3 ± 5.4	214.9 ± 83.4 A	128.1 ± 9.7	153.3 ± 0.2 AB	213.9 ± 32.4	171.7 ± 5.8	D, S x D
		Late-May B	112.1 ± 33.5	115.5 ± 16.9	127.3 ± 1.4	127.2 ± 11.2	93.8 ± 10.6	146.0 ± 57.2 B	90.7 ± 15.8	134.7 ± 9.7 A	178.4 ± 33.4	149.6 ± 8.7	
		Mid-June B	100.2 ± 26.5	119.2 ± 15.5	123.4 ± 12.8	108.6 ± 14.1	130.7 ± 24.1	137.7 ± 47.5 B	132.9 ± 0.8	173.1 ± 10.0 B	158.4 ± 3.2	156.8 ± 10.5	
	Mineral	Mid-May A	13.1 ± 1.5	9.7 ± 1.3	10.2 ± 5.7	8.0 ± 0.7	18.2 ± 1.3	18.4 ± 0.7	9.9 ± 4.1	6.4 ± 0.6	11.8 ± 0.2	15.4 ± 0.6	D
		Late-May B	17.6 ± 5.7	15.2 ± 0.6	9.9 ± 5.2	12.8 ± 3.7	15.1 ± 0.3	15.1 ± 0.3	12.0 ± 1.6	11.2 ± 1.9	14.3 ± 2.3	14.2 ± 0.4	
		Mid-June B	11.9 ± 1.5	12.5 ± 0.8	6.8 ± 4.0	8.6 ± 2.6	20.5 ± 2.5	16.9 ± 5.5	8.5 ± 2.2	7.7 ± 1.2	16.5 ± 1.1	15.9 ± 0.2	

Plots were dominated by either American beech, eastern hemlock, sugar maple, red oak, or yellow birch. A subset of 20 plots was sampled from a total of 60 plots. Letters in the Date column indicate significant differences among sampling dates across all species. Within each species, differences among sampling dates are shown to the right of the species

P_i Inorganic phosphorus, P_o organic phosphorus, P_{Mic} microbial biomass phosphorus, *ND* not detectable, i.e. below 0.02 ppm detection limit, *ns* not significant

Species, fertilizer, and date effects column summarizes significant differences, for example, *F* fertilizer effect, *S* species effect, S × D = species and date interaction. P_o was calculated as the difference of total extractable phosphorus (P_t—not shown) and P_i

Extractable organic P in organic horizons ($P_{o\text{-}Org}$) ranged from 0.03 to 1.24 μg g^{-1}, representing 3–88% of total extractable P ($P_{t\text{-}Org}$), and declined from a mean of 0.68 ± 0.05 μg g^{-1} in mid-May to 0.32 ± 0.07 μg g^{-1} in late-May and then increased to 0.39 ± 0.04 μg g^{-1} in mid-June (Table 3). $P_{o\text{-}Org}$ was unaffected by species but was significantly greater in fertilized plots compared to controls ($F = 4.35$, $p = 0.047$). In mid-May and mid-June, the proportion of $P_{o\text{-}Org}$ to $P_{t\text{-}Org}$ tended to be greater in hemlock plots followed by beech plots. On all dates the proportion of $P_{o\text{-}Org}$ to $P_{t\text{-}Org}$ was lowest in oak plots. Extractable organic P in mineral horizons ($P_{o\text{-}Min}$) ranged from 0.19 to 0.90 μg g^{-1} representing 34–84% of total extractable P, and species differences interacted with fertilizer ($F = 3.13$, $p = 0.03$). Oak fertilized plots were significantly greater in $P_{o\text{-}Min}$ than controls ($F = 6.67$, $p = 0.02$) while beech, maple, and birch all had lower $P_{o\text{-}Min}$ in fertilized plots compared to controls.

Enzyme activity

Across all sampling dates, phosphatase activity in organic horizons ($Phos_O$) declined linearly ($r^2 = 0.34$, $F = 15.80$, $p = 0.0004$) with increasing $P_{i\text{-}Org}$ (Fig. 1a). Compared to oak and maple plots, where $P_{i\text{-}Org}$ was higher and $Phos_O$ was lower, hemlock and beech plots had lower $P_{i\text{-}Org}$ and greater $Phos_O$. When relative phosphatase activities were compared (adjusted for differences among sampling dates), $Phos_O$ declined linearly ($r^2 = 0.58$, $F = 24.81$, $p < 0.0001$) with increasing P_{Root} (Fig. 1b). Oak and maple plots tended to have greater P_{Root} concentrations and generally lower $Phos_O$ activities. Conversely hemlock, beech, and birch plots tended to have lower P_{Root} and greater $Phos_O$ activity. $Phos_O$ ranged from 1526 to 6226 nmol h^{-1} g^{-1} and varied by date ($F = 47.86$, $p < 0.0001$) with activities increasing from a mean of 2932 nmol h^{-1} g^{-1} in mid-May to 3522 nmol h^{-1} g^{-1} by mid-June. Organic horizon soil moisture was positively correlated with $Phos_O$ in both mid-May ($r^2 = 0.54$, $p = 0.0001$), and mid-June ($r^2 = 0.48$, $p = 0.0005$), but not in late-May ($p = 0.08$), the driest sampling date. There was an overall weakly negative correlation between organic soil pH and $Phos_O$ ($r^2 = 0.15$, $p = 0.03$). While neither species nor fertilizer differences were significant

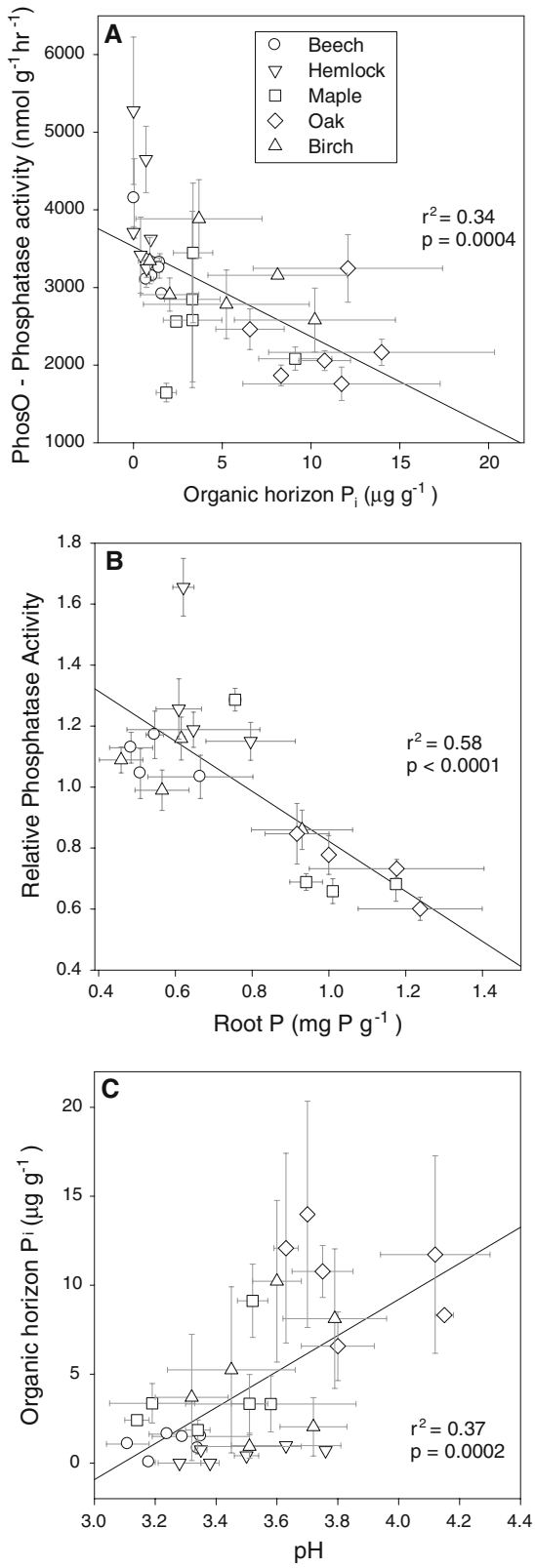

Fig. 1 Soil and fine root measurements within the organic horizons of single-species plots in the Catskill Mountains, NY. Plots were dominated by either American beech, eastern hemlock, sugar maple, red oak, or yellow birch. Phosphatase activity ($Phos_O$) and resin-extractable inorganic phosphorus (P_i) were measured on three dates in 2007: mid-May, late-May, and mid-June. **a** P_i and phosphatase activity ($Phos_O$). *Bars* represent one standard error for two sample replications. **b** Root phosphorus concentration and relative phosphatase activity. Root phosphorus was measured on samples taken in summer 2006. For each plot, a relative phosphatase activity was calculated on each sampling date as a percentage of mean activity in all plots on that date (1.0 represents the mean activity). Relative activities for each plot were then averaged across the three sampling dates. *Bars* represent one standard error, for relative activity across the three sampling dates (*y*-axis), and for two sample replications (*x*-axis). **c** Soil pH and P_i. *Bars* represent one standard error for two sample replications

($p = 0.054$ and $p = 0.53$, respectively), $Phos_O$ tended to be greater in hemlock and beech soils and lower in maple and oak soils. Species × fertilizer × date interactions were significant ($F = 2.88$, $p = 0.02$), and the species × fertilizer interaction was significant in late-May ($F = 3.59$, $p = 0.006$) and mid-June ($F = 3.7$, $p = 0.005$). Within maple and oak plots, on all three dates mean $Phos_O$ was greater in fertilized plots compared to controls (Fig. 2). Conversely, $Phos_O$ was lower in fertilized hemlock plots compared to controls. Activities in beech and birch soils were inconsistent across sampling dates; however in mid-June fertilized plots of both species had greater $Phos_O$ compared to controls. Phosphatase activity in mineral horizons ($Phos_M$) ranged from 325 to 1799 nmol h^{-1} g^{-1} and varied by date ($F = 33.26$, $p < 0.0001$). In contrast to organic horizons, $Phos_M$ was not correlated with mineral soil moisture on any date ($p = 0.25$). Mineral soil pH was negatively correlated with $Phos_M$ in mid-June ($r^2 = -0.47$, $p = 0.03$), but not on other dates. While no significant differences in $Phos_M$ were found with regard to species, species interacted with date ($F = 3.34$, $p = 0.01$), and patterns in $Phos_M$ reflected those of the overlying organic horizon. For example, when averaged across all dates $Phos_M$ was greatest in hemlock plots and lowest in oak plots. $Phos_M$ was unaffected by fertilization ($p = 0.83$). Fertilization and date had interactive ($F = 4.58$, $p = 0.02$) effects on $Phos_M$. However within species, $Phos_M$ responses to fertilization were inconsistent.

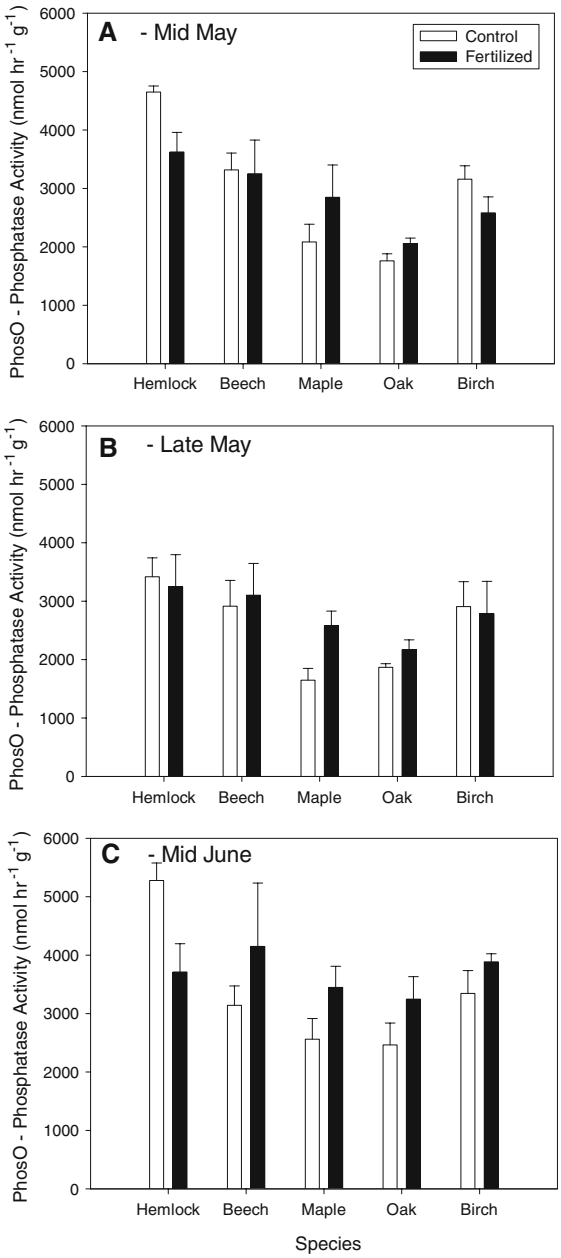

Fig. 2 Phosphatase activity ($Phos_O$) within the organic soil horizons of single-species plots in the Catskill Mountains, NY. Plots were dominated by either American beech, eastern hemlock, sugar maple, red oak, or yellow birch. Activity was measured on three dates in 2007, mid-May (**a**), late-May (**b**), and mid-June (**c**)

Microbial biomass P

P_{Mic} ranged from 74 to 298 μg g^{-1} in organic horizons and from 2 to 23 μg g^{-1} in mineral horizons (Table 3). P_{Mic} was unaffected by species or fertilizer treatment in both horizons, though there was a significant interaction of species and sampling date ($F = 8.96$, $p < 0.0001$).Without correction for differences in microbial biomass (see methods), we cannot infer more from the P_{Mic} data.

P fractionation

Sequential fractionation of mineral soils for P, Fe and Al showed few significant differences among species, and no differences due to N fertilization (Table 4). Among P fractions, ion-exchangeable P (NH_4Cl-P) represented only 2% of total soil P and had an average concentration of 6.12 mg P g^{-1}. Phosphorous in organic matter or adsorbed to labile Al- or Fe-hydroxides (NaOH-P) represented the largest fraction of total soil P (60%), and had a mean concentration of 194 mg P g^{-1}. Oak soils had the greatest total P concentrations and had greater NaOH-P compared to other species. Within the NaOH-P fraction, oak soils had significantly greater inorganic P concentrations (phosphate—Pi) than beech, hemlock or birch ($F = 8.4$, $p = 0.019$).

Patterns of Fe concentrations in soil fractions were similar to those of Al concentrations. Total soil Fe and Al tended to be greater in beech and oak soils and lower in hemlock soils. Mean Fe and Al concentrations were greatest in the residual fraction (9065 and 9319 μg g^{-1} respectively), and this fraction represented the majority of total soil concentrations (62 and 74%, respectively). Hemlock soils tended to be lowest in both residual and total soil Fe and Al. The only significant differences between species occurred in the NaOH-extractable fraction, where oak soils had greater Fe concentrations than hemlock, maple and birch soils ($F = 6.85$, $p = 0.03$). Oak soils also tended to have greater Al in the NaOH-extractable fraction. Strong positive correlations between P, Fe, and Al concentrations were observed within many of the soil fractions (Table 5). Within the NaOH-extractable fraction, Fe and Al concentrations were well correlated with P. Fe and Al in the HCl-extractable fraction (HCl–Fe, HCl–Al) were also well correlated with NaOH-extractable P.

Species profiles

To summarize species differences in P, we created "species profiles" (after Lovett et al. 2004). The

Table 4 Phosphorus (P), iron (Fe), and aluminum (Al) concentrations in sequential fractions of mineral soils from control and nitrogen fertilized plots in the Catskill Mountains, NY

Species treatment	1 M NH_4Cl	0.11 BD	0.1 M NaOH		0.5 M HCl	Residual	Total
				P μg g^{-1}			
American beech			P_i	P_o			
Control	7.64 ± 2.22	31.4 ± 1.0	82 ± 27 A	130 ± 71	2.7 ± 1.0	68 ± 30	322 ± 132
Fertilized	6.00 ± 0.22	35.0 ± 4.8	67 ± 3	102 ± 22	2.0 ± 0.1	59 ± 8	271 ± 13
Eastern hemlock							
Control	8.36 ± 4.58	31.4 ± 16.3	55 ± 29 A	98 ± 80	2.5 ± 1.1	35 ± 16	202 ± 119
Fertilized	6.60 ± 1.63	31.5 ± 1.8	37 ± 1	62 ± 18	2.1 ± 0.4	30 ± 14	169 ± 37
Sugar maple							
Control	7.31 ± 1.62	30.7 ± 6.9	99 ± 75 AB	107 ± 67	2.2 ± 0.8	113 ± 72	359 ± 224
Fertilized	6.80 ± 1.11	37.8 ± 11.6	84 ± 18	82 ± 2	1.5 ± 0.0	118 ± 13	329 ± 45
Red oak							
Control	4.27 ± 0.09	29.3 ± 2.9	236 ± 16 B	113 ± 51	6.5 ± 1.3	166 ± 35	559 ± 73
Fertilized	5.10 ± 0.67	46.1 ± 1.7	232 ± 30	113 ± 94	1.8 ± 0.4	153 ± 9	551 ± 114
Yellow birch							
Control	4.19 ± 0.50	28.5 ± 6.3	50 ± 25 A	62 ± 4	1.5 ± 0.0	59 ± 10	206 ± 46
Fertilized	4.94 ± 0.21	27.7 ± 6.9	60 ± 33	68 ± 28	5.9 ± 4.5	96 ± 26	262 ± 98
				Fe μg g^{-1}			
American beech							
Control	107 ± 1	2556 ± 111	942 ± 204 AB		1749 ± 891	13851 ± 7874	19205 ± 8856
Fertilized	146 ± 6	3314 ± 634	1089 ± 43		1715 ± 280	13632 ± 6568	19895 ± 6165
Eastern hemlock							
Control	134 ± 98	953 ± 83	558 ± 168 A		767 ± 397	4852 ± 62	7264 ± 683
Fertilized	93 ± 1	1836 ± 665	533 ± 116		754 ± 58	4925 ± 344	8141 ± 1068
Sugar maple							
Control	58 ± 13	3399 ± 1243	557 ± 309 A		2616 ± 1984	8899 ± 3400	15529 ± 6949
Fertilized	100 ± 74	3190 ± 1224	546 ± 153		1994 ± 385	9467 ± 2163	15297 ± 4000
Red oak							
Control	24 ± 10	2846 ± 898	1384 ± 186 B		5300 ± 1645	9054 ± 685	18608 ± 1237
Fertilized	75 ± 17	4443 ± 426	1538 ± 503		3655 ± 1749	8436 ± 186	18147 ± 2847
Yellow birch							
Control	81 ± 8	840 ± 617	418 ± 42 A		1080 ± 43	8316 ± 2340	10736 ± 1715
Fertilized	64 ± 2	769 ± 558	571 ± 140		2188 ± 1449	9217 ± 1517	12809 ± 632
				Al μg g^{-1}			
American beech							
Control	643 ± 339	86 ± 10	1880 ± 967		1496 ± 754	11897 ± 6669	16002 ± 8718
Fertilized	675 ± 207	106 ± 34	1777 ± 272		1503 ± 424	11222 ± 5408	15283 ± 6276
Eastern hemlock							
Control	877 ± 746	69 ± 27	1275 ± 846		1734 ± 1489	4687 ± 2895	8641 ± 6002
Fertilized	392 ± 127	85 ± 38	745 ± 17		1034 ± 423	5207 ± 2560	7463 ± 3055
Sugar maple							
Control	335 ± 171	58 ± 13	968 ± 572		1330 ± 528	8907 ± 4185	11597 ± 5469
Fertilized	423 ± 230	67 ± 37	782 ± 131		1172 ± 356	10783 ± 1001	13227 ± 1754

Table 4 continued

Species treatment	1 M NH_4Cl	0.11 BD	0.1 M NaOH	0.5 M HCl	Residual	Total
Red oak						
Control	389 ± 34	62 ± 11	2755 ± 124	1892 ± 327	12535 ± 1371	17632 ± 1777
Fertilized	562 ± 19	95 ± 15	2242 ± 818	1353 ± 452	11292 ± 706	15544 ± 1972
Yellow birch						
Control	347 ± 29	35 ± 3	538 ± 49	1093 ± 154	8294 ± 1374	10308 ± 1512
Fertilized	307 ± 78	39 ± 16	630 ± 214	1115 ± 298	8362 ± 2004	10453 ± 2610

Plots were dominated by either American beech, eastern hemlock, sugar maple, red oak, or yellow birch

BD Bicarbonate-dithionite (0.11 M $NaHCO_3$ and 0.11 M $Na_2S_2O_4$). For each element, letters indicate significant differences between species within each column

profiles (Fig. 3) were created by calculating the percent difference between a species mean and the overall (all species) mean for each variable measured (foliar P, litter P, resorption, root P, inorganic P, and phosphatase activity). The profiles are presented for each species, ordered from those species with indicators of poor P status (e.g., low P supply) to those with increasingly rich P status (e.g., high P supply) (Fig. 3). Hemlock had relatively lower foliar P concentrations (mean 1.12 ± 0.04 mg g^{-1}, $n = 36$) than all other species (in FCD-NERC data mean hemlock foliar P was 1.10 ± 0.04 mg g^{-1}, $n = 68$) and had relatively low P concentrations in litter, roots, and soils. Oak had the highest foliar P (in our study oak mean foliar P was 1.48 ± 0.03 mg g^{-1}, $n = 35$ while it was 1.48 ± 0.03 mg g^{-1}, $n = 37$ in the FCD-NERC data). Oak also had the highest litter P and available soil P, while having the lowest resorption and phosphatase activity. Beech was near average in tissue P concentrations, low in P_i and slightly above average in phosphatase activity. Beech foliar P was slightly (but not significantly) greater (mean 1.34 ± 0.05 mg g^{-1}, $n = 36$) compared to the FCD-NERC data (1.29 ± 0.01 mg g^{-1}, $n = 445$), and as a result, beech N:P ratios were slightly but significantly smaller (Table 2).

Birch was above average in P_i, and near or below average in phosphatase activity. Birch foliar P (and foliar N:P) was similar in this study (1.44 ± 0.03 mg g^{-1}, $n = 36$) and in FCD-NERC data (mean 1.42 ± 0.02 mg g^{-1}, $n = 387$). Maple was relatively high in P resorption and root P but slightly below average in P_i and phosphatase activity. Maple foliar P was greater in this study (mean 1.25 ± 0.05 mg g^{-1}, $n = 36$) than in the FCD-NERC data (1.15 ± 0.01 mg g^{-1}, $n = 387$), and therefore maple N:P tended to be lower (Table 2).

Discussion

Species' P profiles and comparison to N profiles

We interpreted the nutrient status of each species, using the species profiles and comparisons with FCD-NERC data. The profiles (Fig. 3), containing several indicators, are potentially more robust than any single indicator. For example, a single measure such as foliar P concentration may be influenced by both nutrient demand and availability; therefore its interpretation is difficult without the context of additional indicators.

Species differed in the P indicators we measured. For example, compared to other species hemlock is P-poor. Hemlock foliar P concentrations in this study were not atypical (they were similar to those found in the FCD-NERC data) and were lower than all other species. Additionally, P tissue concentrations in hemlock litter and roots were low and soils were low in P_i indicating that P supplies may be small. We also compared the species' P profiles with their N profiles (Lovett et al. 2004). This comparison revealed that some species may be nutritionally similar with respect to both N and P, while other species affect N or P differentially. For example, hemlock's P profile is somewhat analogous to its N profile: as with P, its needles are N poor and available N is low (Lovett et al. 2004). These profiles suggest

Table 5 Pearson correlation coefficients between phosphorus (P) concentrations and iron (Fe) and aluminum (Al) concentrations in sequential fractions of forest mineral soils from plots within the Catskill Mountains, NY

P fraction	Fe						Al					
	NH_4Cl	BD	NaOH	HCl	RES	TOT	NH_4Cl	BD	NaOH	HCl	RES	TOT
NH_4Cl	0.68 **	0.10	0.02	0.00	0.21	0.19	0.83 ***	0.28	0.30	0.74 ***	0.31	0.42
BD	0.63 **	0.64 **	0.45 *	0.26	0.02	0.27	0.61 **	0.64 **	0.38	0.51 *	0.34	0.43
NaOH-P	−0.04	0.57 **	0.84 ***	0.88 ***	0.34	0.69 ***	0.42	0.29	0.89 ***	0.71 ***	0.71 ***	0.80 ***
NaOH-Pi	−0.22	0.65 **	0.84 ***	0.84 ***	0.16	0.56 *	0.17	0.25	0.78 ***	0.43	0.56 **	0.62 **
NaOH-Po	0.22	0.29	0.56 **	0.66 **	0.47 *	0.64 **	0.61 **	0.25	0.76 ***	0.84 ***	0.68 ***	0.78 ***
HCl	−0.20	−0.08	0.28	0.62 **	0.06	0.22	0.10	−0.08	0.38	0.42	0.34	0.38
RES	−0.27	0.54 *	0.55 *	0.86 ***	0.26	0.59 **	0.05	0.02	0.54 *	0.38	0.64 **	0.62 **
TOT	−0.12	0.62 **	0.79 ***	0.92 ***	0.34	0.70 ***	0.29	0.23	0.81 ***	0.61 **	0.74 ***	0.78 ***

Fractions are in sequence: NH_4Cl—ion-exchangeable, BD—reducible metal hydroxides, NaOH—organically bound and labile Al- and Fe-bound (P, inorganic P_i, and organic P_o), HCl—crystalline or Ca-bound, RES—residual, TOT—total

Asterisk indicate statistical significance * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

that hemlock nutrient concentrations are low, and thus nutrient turnover may be slow compared to the other species examined. In contrast to hemlock, oak appears P rich. Among the five species, oak had the highest foliar P in this study (and in the FCD-NERC data). Oak also had the highest indicators of P supply (litter P and available soil P), while having the lowest indicators of demand in excess of supply (lowest resorption and phosphatase activity). Interestingly, oak's P profile contrasts its N profile. While oak apparently has a rich P supply, its soils are low in extractable N and display relatively low rates of N cycling (Lovett et al. 2004).

The other species occupy a middle ground in the spectrum of P status. Beech was near average in tissue P concentrations (it is also near average in foliar N), low in P_i and slightly above average in phosphatase activity. From 1997 to 2006 beech was the only species with a consistent decrease in foliar P in both control and fertilized plots. This decrease may be a result of declines in tree health due to beech bark disease (Griffin et al. 2003). Maple and birch appear sufficient in P, though not as strongly as oak. Comparisons with N characteristics suggest that birch may be relatively nutrient rich compared to the other species. Aside from oak, birch had the greatest foliar and litter P (this study), and had the greatest foliar and litter N among all the species (Lovett et al. 2004). Maple strongly resorbs N (Lovett et al. 2004) as well as P (this study), and therefore may efficiently recycle accumulated internal nutrients rather than relying heavily on uptake. In contrast to maple, oak's low P resorption and relatively high P_i suggests that oak may be less reliant on internally recycled P and more dependent on uptake. Thus, in comparison to N cycling where oak soils display slow rates of N mineralization (Finzi et al. 1998) and nitrification (Lovett et al. 2004), P turnover in oak soils may be relatively rapid as suggested by the high concentrations of P_i and proportion of P bound in organic fractions.

We can also interpret the comparative P profiles as an indirect indicator of potential P limitation. However, we do this cautiously for three reasons. First, we did not apply a P fertilizer and therefore could only speculate about how species would respond to added P. Second, we cannot assume that N limitation was relieved by the N fertilizer. Foliar N:P ratios were greater in fertilized plots (but still agreed well with

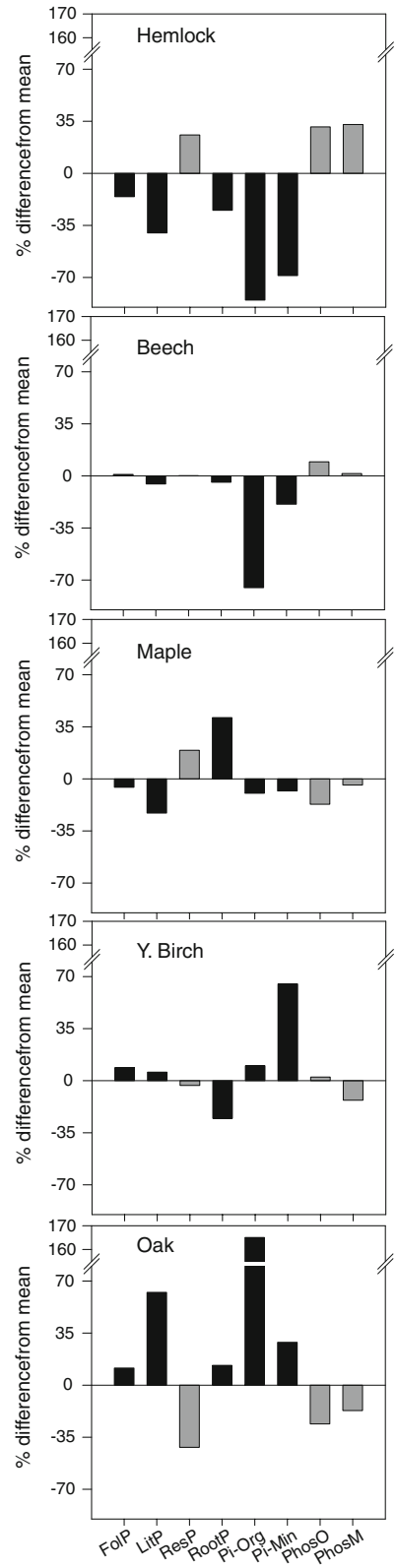

Fig. 3 Mean differences in indicators of P supply and demand for tree species growing in the Catskill Mountains, NY. Species means for each indicator were determined relative to the mean across all species. Indicators of supply are shaded *black* and indicators of demand are shaded *grey*. Abbreviations for each indicator are: *FolP* foliar P (mg g^{-1}), *LitP* litter P (mg g^{-1}), *ResP* percent resorption efficiency, *RootP* root P (mg g^{-1}), *Pi-Org* organic horizon inorganic P (μg g^{-1}), *Pi-Min* mineral horizon inorganic P (μg g^{-1}), $Phos_O$ organic horizon phosphatase enzyme activity (nmol h^{-1} g^{-1}), $Phos_M$ mineral horizon phosphatase enzyme activity (nmol h^{-1} g^{-1})

those in the FCD-NERC dataset) and this was due to increased N concentrations, not decreases in P concentrations. The increase in foliar N with fertilization suggests that N limitation may still be occurring. As mentioned previously, there were no changes in productivity due to the N fertilizer treatment (G. Lovett et al. unpublished), so we cannot conclusively determine whether limitation by N, P, Ca, or another nutrient is occurring. Third, there are many indicators that could be used to assess P status, and we acknowledge that we have not attempted to capture them all here. For example, while we measured available P in the soil, tree species are known to differ in their mycorrhizae, which in turn may affect the sources of P available to them. In particular, trees with endomycorrhizal associations, like sugar maple, may have limited ability to access mineral P compared to the other species in this study, all of which are ectomycorrhizal. If some ectomycorrhizae provide trees with P directly from mineral sources (Wallander et al. 2005) or organic sources (Dighton 1983), then available soil P may underestimate the tree's actual P supply. Similarly phosphatase activity would overestimate actual demand.

Bearing these considerations in mind, we infer the potential for P limitation based on our indicators of P supply and demand, and through comparison with other studies. For example, hemlock appears the most susceptible to P limitation. P resorption and phosphatase activity were high indicating that hemlock's biotic P requirement may be greater than P supply. Hemlock weakly resorbs N (Lovett et al. 2004), suggesting that P could be a more limiting nutrient than N. In support of this suggestion, Finzi (2009) found that hemlock had slightly greater basal area increments in response to P additions compared to N additions (although it responded most strongly to

N + P additions). Beech, maple, and birch, occupying the middle of the P status spectrum, are interpreted to be progressively less sensitive to P limitation, while oak appears to have the least potential for P limitation. Oak indicators of P demand are low while indicators of supply are high. Among the species examined, oak poorly resorbs P but moderately resorbs N (Lovett et al. 2004), suggesting that for oaks, N might be in shorter supply than P. In support of this suggestion, Finzi (2009) showed that oaks fertilized with N had greater basal area increments than those fertilized with P.

In contrast to studies occurring where the ambient N deposition rate is greater than in our study sites, our indicators of P status do not suggest that sugar maple is P limited. In Ontario sugar maple stands, Gradowski and Thomas (2006) linked sugar maple diameter growth to P availability and suggested that P limitation was the result of increased biotic demand under N-sufficient conditions. Those stands receive N inputs of 20 kg N ha^{-1} $year^{-1}$, greater than the ambient deposition rate in our study sites (~11 kg N ha^{-1} $year^{-1}$), although local landscape features may result in deposition rates in excess of 40 kg N ha^{-1} $year^{-1}$ within the Catskill Mountains (Weathers et al. 2000). Where P limitation has been suggested as a cause of poor sugar maple regeneration (Pare and Bernier 1989a), foliar P concentrations were on average lower (1.00 mg g^{-1}) than those found here (1.25 mg g^{-1}) or in the FCD-NERC data (1.15 mg g^{-1}). However, we sampled sunlit upper-canopy leaves, which may have greater nutrient concentrations than mid-canopy leaves sampled in other studies (Pare and Bernier 1989a). Of the variables we examined, only above-average P resorption might suggest P limitation in sugar maple (among all species mean P resorption was 53%, while in sugar maple it was 64%). Phosphorus resorption rates for sugar maple have been reported to range from 24% in the central Appalachians, to more than 80% in New Hampshire (Eickmeier 1982; Fiorentino et al. 2003). However, these rates may not be directly comparable because our index of resorption is based on nutrient concentration per leaf mass, rather than per leaf area (van Heerwaarden et al. 2003).

Resorption and plant nutritional status

Whether foliar resorption efficiency is a good indicator of plant nutritional status is subject to debate due to the number of non-nutritional factors that may influence resorption (Aerts 1996; Killingbeck 1996). It is also debatable whether high resorption efficiency is a sign of nutrient limitation that is not apparent elsewhere in the ecosystem. For example, because sugar maple stands display low soil C:N ratios, high nitrification rates, and retain little NO_3 (Finzi et al. 1998; Lovett et al. 2004), they could be assumed to be N saturated and low N resorption would be expected. The sugar maple stands we studied display these symptoms of N saturation, but have high foliar N resorption (Lovett et al. 2004). One explanation for high N resorption is that sugar maple may prefer NH_4 over NO_3 (Templer and Dawson 2004). Therefore sugar maple's biotic N demand may not be met, even with abundant NO_3. Alternatively, sugar maple may have strong resorption in general. In our study sugar maple had relatively high P resorption, and other studies suggest it has greater P use efficiency than beech, hemlock, or oak (Finzi 2009). So, if resorption is an indicator of limitation, then these sugar maple stands may be co-limited by both N and P. However, other indicators did not suggest P limitation in sugar maple (it was near average in foliar P, and had lower than average phosphatase activities) and increases in sugar maple foliar P concentrations have followed increased P resorption (Fiorentino et al. 2003), suggesting that resorption may not be a good measure of nutrient limitation. Ultimately, an increase in productivity in response to an added nutrient is the best indicator of nutrient limitation.

Links among biogeochemical cycles with N fertilization

In soil, N availability is thought to influence P availability via the N-rich phosphatase enzymes produced by plants and microbes when N availability is sufficient. Biota can invest N into the production of enzymes like phosphatase for acquiring other nutrients, such as P (Houlton et al. 2008). Nitrogen additions have resulted in increased phosphatase activity within tropical forests (Olander and Vitousek 2000), and grasslands (Johnson et al. 1998; Phoenix et al. 2004). Studies conducted in mixed hardwood stands of maple and oak have generally shown stimulation of enzymes involved in the mineralization of labile C and N (e.g. β-glucosidase) but only moderate increases in phosphatase activity in

response to long-term N fertilization (Saiya-Cork et al. 2002; DeForest et al. 2004; Sinsabaugh et al. 2005). We found that fertilization alone had no simple effect on phosphatase activity, but interacted with species and date. These species are known to vary in their ability to retain added N (Templer et al. 2005) so their ability to use added N for phosphatase production may also vary. Further, fertilization had no effect on P_i, our measure of available P. Thus we found only weak evidence linking N and P availability via phosphatase. These findings suggest either that the N additions were not great enough, that they did not simulate actual atmospheric deposition closely enough to significantly increase phosphatase activity or phosphatase production (and activity), or that the stands may not be N-limited.

If the Northern Hardwood forests we studied are N saturated, then P availability rather than N availability may be a more proximal control on phosphatase activity. The negative correlations between phosphatase activity and both P_i, and root P (Fig. 1a, b) suggest that species differences contributing to P availability are largely responsible for determining phosphatase activity, i.e., litter chemistry may influence soil properties which then control P and phosphatase. For example, soil pH influences the adsorption and precipitation of P as well as the activities of many enzymes including phosphatases (Sinsabaugh et al. 2008). We found that soil pH differed among species and was more strongly correlated with available P (Fig. 1c) than with phosphatase activity. These results suggest that individual species effects on soil chemistry influence soil pH, and therefore available P, which in turn influences phosphatase production and activity. Future work is needed to examine the relative strength of both the biotic (e.g. organic forms of P), and abiotic influences (e.g. soil pH, exchangeable Al) on phosphatase activity.

In the forest stands we studied, the stimulating effects of N fertilizer on phosphatase activity appear weak in comparison to the influence of species type. However, because we did not apply a P fertilizer, our study cannot separate the influence of soil P status from the influence of species. A two-way fertilization experiment with N and P in different forest types (e.g., under different species) could elucidate the strength of these influences (site fertility and species effects), both of which may be affecting enzyme activities in the forest stands we studied. The strongest differences between control and fertilized plots occurred in mid-June 2007, 2 weeks after a fertilization event (prior to the May 2007 sampling dates plots had not been fertilized since November 2006). Therefore, there could be a short-term enzymatic response to N fertilizer in addition to the long-term trends observed in other studies (Sinsabaugh et al. 2005). Enzyme assays conducted with greater frequency before and after fertilization events could further investigate this result.

Differences in soil P due to species and fertilization

We observed trends suggesting species differences in P_i, our measure of plant-available P, and propose that these differences are attributable to differences in organic matter quality. As argued by Lovett et al. (2004) with regard to N cycling, the data from this study support the interpretation that differences in P availability are due to species effects on site conditions, rather than inherent site differences determining the occurrence of species. However, N cycling is largely under biotic control, whereas P cycling is controlled by both biotic (species) and geochemical processes, e.g., sorption, chemical reactions, and precipitation. For example, we have no way of conclusively determining whether differences in P_i are a result of species rather than site effects (Table 3). However, since P_i differences among species were strongest in organic horizons and no differences were observed in mineral horizons, we interpret these differences as largely species effects rather than site effects. The sequential fractionation of mineral soils also supports the conclusion that differences in soil P chemistry are due to species effects. For example, while total concentrations of Al and Fe (metals capable of adsorbing P), were similar beneath oak and beech (Table 4), oak soils had greater concentrations of Pi, Al, and Fe in the organic fraction extractant (NaOH), and greater total P. We speculate that species differences in decomposition products, root exudates and other organic compounds lead to differential weathering or leaching of Al, Fe, and P from organic fractions of the mineral soil. The weathering or leaching of organic matter fractions may be especially important for P bioavailability. Where Al and Fe remain in organic fractions, they

may bind P in a form that is relatively accessible to microbes (compared to P that has been sorbed or precipitated with Al or Fe minerals). Loss or absence of Al and Fe from organic matter may cause subsequent decreases in P bioavailability as these P binding sites decrease. If any P is lost from organic and upper mineral horizons it may become relatively inaccessible due to increased binding in lower mineral horizons (Wood 1980).

Contrary to our expectations, N fertilization had no effect on P_i. Soil acidity was increased in the fertilized plots compared to controls (Table 3), and we expected that this acidity would decrease P_i via increased sorption and precipitation of P with Al and Fe (Note that P_i and soil acidity were negatively related across *all* plots (Fig. 1c). One explanation is that these very acidic soils may have already been close to their maximum capacity to adsorb and precipitate P. The soils' capacity to bind P may be small or nearly saturated if minerals that bind P (e.g., secondary Al minerals) are scarce. This capacity may not have changed with fertilization despite further acidification. Another explanation is that changes in P_i due to fertilization may have been obscured by shifts in P cycling or biotic P uptake, i.e., if N fertilization simultaneously stimulates mineralization processes and increases biotic P demand, then assimilation by plants and microbes could result in only small changes to extractable P. Plant production and phosphorus concentrations in plant tissues were not increased by fertilization, suggesting that plant demand for P did not increase. However microbes may be immobilizing any P mineralized via biotic processes. In general, P is thought to be efficiently cycled from organic matter to biota within organic horizons (Wood et al. 1984) and microbes in organic horizons may be strong competitors for inorganic P, assimilating up to 90% of newly available P (Walbridge et al. 1991). That we found higher $P_{i\text{-}Org}$ and slightly higher microbial biomass P (albeit uncorrected for microbial C) in fertilized organic horizons in late-May and mid-June supports the view that microbes act as strong sinks for P.

Ecosystem losses of unavailable soil P (e.g., dissolved organic P—DOP) have been predicted to occur in response to long-term N additions (Perring et al. 2008). However, DOP has been observed to vary with soil pH (Vaz et al. 1993), and we expected decreased P_o in fertilized plots due to the acidifying effects of N fertilizer. Counter to our expectation, P_o was consistently higher in organic horizons of fertilized plots. This is surprising because increased acidity should decrease the amount of SOM released to solution (i.e., the opposite response to liming treatments). Soil acidification results in greater positive charges on organic matter and decreased solubility, thereby lowering the exposure of SOM to biochemical decomposition. The changes in soil acidity due to fertilization were small, suggesting that other factors may influence P_o. One potential explanation for increases in P_o could be rapid turnover of the microbial biomass as has been reported for other N fertilization studies (Fisk and Fahey 2001). Long-term N fertilization is thought to suppress the activity of many microbes, resulting in a smaller active biomass with faster turnover time. Regardless, the fate of P_o in Northern Hardwoods needs further investigation. The degree to which P_o is hydrolyzed and made available, or lost from the system, may determine long-term changes in ecosystem P retention and therefore nutrient limitation (Perring et al. 2008). For microbial biomass P, the most comparable study to our own examined organic horizons in Northern Hardwoods of New Hampshire (Fiorentino et al. 2003). Our measures of microbial biomass P are slightly higher (mean 138 $\mu g\ g^{-1}$) compared to 81 $\mu g\ g^{-1}$ in New Hampshire. In agreement with other studies we found that microbial biomass P is large in comparison to extractable P (Walbridge et al. 1991).

Sherman et al. (2006) hypothesized that soil acidity arising from atmospheric deposition could increase mobilization of Al and Fe along with any bound P, and that changes in P would depend upon forest type. In hardwood forests, biotic uptake of mobilized P prevents P loss. In softwood forests where uptake is lower or less efficient than hardwoods, P losses are predicted. We found few changes in mineral soil P fractions due to fertilization. While we found that fertilized hemlock soils did have slightly (though not significantly) decreased P concentrations compared to controls, we also found this trend for fertilized beech soils (Table 4). Further, there were no consistent increases in mineral soil P concentrations due to fertilization of other hardwoods. We suggest that changes in soil P, Al and Fe caused by N deposition may vary at the species level,

and not simply be a dichotomy between hardwood- and softwood-type responses.

Hemlock, P limitation and pests

Our study showed few signs that increased N leads to P limitation. However, hemlock, the only softwood in our study, showed the greatest indications that additional N additions may cause P deficiency. While P concentrations and N:P ratios in hemlock foliage were not unusual compared to hemlocks in the FCD-NERC data set, the response of phosphatase in fertilized hemlock plots suggests that (if phosphatase is an important mechanism for P acquisition), hemlocks receiving additional N inputs could experience P limitation in the future. Unlike hardwoods, fertilization tended to decrease phosphatase activity (and other enzymes—data not shown) in hemlock soils. Soils under softwoods may be poorly buffered compared to hardwoods (Boggs et al. 2007) and therefore decreases in phosphatase may have been the result of increased acidity (Carreira et al. 2000) in the fertilized plots. However, hemlock soils were not the most acidic in our study (beech soil and maple soils tended to be more acid), so it seems unlikely that acidification alone was responsible for the decline. We speculate that microbial communities beneath hemlocks differ from those of hardwood soils, and their response to N additions and/or soil acidification may also differ. Future investigations comparing these microbial communities, e.g., focusing on the mycorrhizal communities, and their abilities to access P, could elucidate these responses.

With ongoing atmospheric N deposition, P limitation may add yet another stress to hemlock trees whose survival is already threatened by forest pests. The hemlock woolly adelgid, an introduced forest pest, is currently spreading throughout the eastern deciduous forest and is present in many areas of the Catskill Mountains (Lovett et al. 2006). Recent research suggests that foliar P concentrations may determine resistance to the pest (Pontius et al. 2006). Future studies examining the P status of hemlocks in relation to disease intensity or tree mortality are needed. These studies could be used to determine whether hemlocks growing in P rich soils may be the best targets for protection or conservation efforts.

Conclusions

Our study suggests that, as with N cycling, P cycling in Northern Hardwoods varies by tree species. Comparisons of N and P characteristics suggest that some species may influence N and P similarly (e.g., hemlock soils appear to be relatively poor in both N and P), while other species affect each nutrient differently (oak soils appear to be abundant in available P, but have little available NO_3). Future studies are needed to understand the interspecific differences in P cycling suggested here. For example, within Northern Hardwoods we know little regarding interspecific differences in organic forms of P, controls on P mineralization from litter, mycorrhizal associations important for P acquisition, or mechanisms of P uptake. Interspecific differences in the microbial controls on N cycling (Templer et al. 2003) and in preferred N nutrition (Templer and Dawson 2004) have been observed, and parallel studies could be done for P. Unlike other studies, we found little evidence that increased N inputs altered the P status of the trees or soil. It is possible that historical N deposition affecting all the plots caused P limited conditions prior to the experiment, leading to only weak biotic responses to the N fertilizer. Alternatively, it is possible that the amount of N fertilizer we added was insufficient to see a response in P indicators because N limited conditions still exist in the fertilized plots (this is supported by the increased foliar N:P in fertilized plots). Factors such as nutrient input and retention, site productivity, and substrate age may all determine the strength of biogeochemical linkages between N and P cycling and the occurrence of N-induced P limitation. Species with P demands in excess of supply will of course be more susceptible to P limitation. Our study suggests that hemlock has P demands in excess of P supply, though N additions did not affect most of these indicators. Detecting the actual P status of plants and determining P limitation may be difficult. First, key aspects of the P cycle are difficult to accurately measure, e.g., P mineralization rates and actual biological P demand. Second, indicators of P limitation may vary within an ecosystem, and their occurrence may not be synchronous in time or space. For example, N additions resulted in few changes to the P chemistry of tree foliage, but herbaceous plants, with smaller biomass and no woody tissue, may be early detectors of

changes in the P status of the ecosystem (Tessier and Raynal 2003). The difficulties in detecting P limitation do not preclude its occurrence or the importance of its study.

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