THE ROLE OF PHOSPHORUS AVAILABILITY IN THE RESPONSE OF SOIL NITROGEN CYCLING, UNDERSTORY VEGETATION AND ARBUSCULAR MYCORRHIZAL INOCULUM POTENTIAL TO ELEVATED NITROGEN INPUTS

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Abstract. The impacts of increased nitrogen (N) inputs into temperate ecosystems via atmospheric nitrogen deposition on nitrogen cycling and nitrogen retention have been described in a variety of ecosystem types. The role of secondary nutrients such as phosphorus (P) in ecosystem responses to increased N inputs is less well-understood. N and P availability are likely to interact to influence ecosystem productivity and N cycling rates, and this interaction would be expected to vary as N inputs increase. Furthermore, N and P inputs may affect plant-mycorrhizal associations and the ability of arbuscular mycorrhizae (AM) to colonize roots. We added nitrogen (97 kg ha^{-1} yr^{-1}) and phosphorus (30 kg ha^{-1} yr^{-1}) to an oak-maple forest in southwestern Virginia (U.S.A.) from 1994 through 1996. Inorganic nitrogen concentrations, net nitrogen mineralization, net nitrification rates and arbuscular mycorrhizal inoculum potential (MIP) were assessed during the growing season in 1996. Responses of the understory vegetation and soil N cycling to N addition suggested that the ecosystem was strongly N-limited. Nitrogen cycling rates were not affected by P inputs, though P addition increased P availability and decreased MIP. It was hypothesized that P availability may have more significant influences on N cycling and the plant-mycorrhizal association in ecosystems showing stronger symptoms of nitrogen saturation. Results suggest that the use of P fertilization would be effective in alleviating P-deficiency in vegetation receiving elevated atmospheric N deposition, but perhaps at the cost of benefits that associations with arbuscular mycorrhizae provide.

Keywords: arbuscular mycorrhizal inoculum potential, atmospheric nitrogen deposition, effect of phosphorus on soil nitrogen cycling, nitrogen, nutrient addition, phosphorus

1. Introduction

Nitrogen (N) inputs via atmospheric deposition have begun to alter the nutrient status of ecosystems over much of eastern North America and northern Europe (Aber *et al*., 1989, 1998; Fenn *et al*., 1998). Increases in N cycling (McNulty *et al*., 1990), streamwater nitrate concentrations (Stoddard, 1994; Dise and Wright, 1995; Gunderson, 1995), and soil acidification (Shultze, 1989; Fenn *et al*., 1998) have been reported in a number of forest systems. Ecosystems impacted most severely

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by elevated N inputs may lose the ability to retain N inputs and, consequently, export large amounts of inorganic N via leaching losses (Aber *et al*., 1989; Stoddard, 1994), a profound change from the highly conservative N cycling in N-limited ecosystems (Hedin *et al*., 1995; Schlesinger, 1997).

Increased N inputs can also affect the cycles of other essential nutrients in an ecosystem. For example, export of nitrate into groundwater contributes to leaching losses of cations such as Ca^{2+} and Mg^{2+} (Stoddard, 1994), soil acidification increases Al mobility and reduces the availability of P to biota (Schlesinger, 1997), and N-stimulated growth leads to biotic uptake of a variety of essential nutrients. For example, Ca^{2+} and Mg^{2+} deficiency have been implicated in the decline of red spruce and Norway spruce forests in N-saturated ecosystems in the Great Smoky Mountains of North Carolina (Johnson *et al*., 1991; Van Miegroet and Johnson, 1993) and Bavaria (Schultze, 1989), respectively. Furthermore, decreased leaf P and K concentrations in European forests receiving elevated inputs of N have suggested P or K deficiency (Mohren *et al*., 1986; Houdijk and Roelofs, 1993; Flückiger and Braun, 1998).

It is less well-understood how the availability of other essential nutrients besides N affect N cycling as an ecosystem receives increasing N inputs. Aber and colleagues described that chronic N addition saturates an ecosystem's ability to retain N inputs, at which point biotic activity is no longer limited by N availability (Aber *et al*., 1989; Fenn *et al*., 1998). The cycling rates and availability of other nutrients in the ecosystem besides N play a key role in the relaxation of N limitation since growth limitation is defined by the relative abundances of essential nutrients.

Though phosphorus (P) availability less frequently limits biotic activity in the temperate region than N availability, P is an element frequently in short supply (DiTommaso and Aarssen, 1989) and may be expected to interact with N availability to influence ecosystem productivity and N cycling rates. The interaction between N and P availability would likely vary with the N-status of the ecosystem (*sensu* Aber *et al*., 1989). For example, in strongly N-limited ecosystems, where N limits biotic activity, N addition, but not P addition, would likely result in increased net primary productivity (NPP) and increased biotic demand for essential nutrients including P. However, it has been suggested that P or P-K fertilization of N-saturated ecosystems may be effective in increasing biotic uptake of N and reducing symptoms of N-saturation such as nitrate leaching losses below the rooting zone (Stevens *et al*., 1993; Fenn *et al*., 1998).

We established a nutrient addition experiment in a deciduous forest in southwestern Virginia to examine the interaction between N and P addition on soil N cycling and P availability. We hypothesized that N and P availability would interact to influence nutrient cycling rates and vegetation productivity, and that the interaction would vary with the N status of the ecosystem. Specifically, we predicted that, in a non-N-saturated ecosystem, N addition would decrease P availability in the soil while P addition would have little effect on either vegetation productivity or N cycling rates. On the other hand, N addition to a N-saturated ecosystem would

contribute to such symptoms of N saturation as increased net nitrification rates, the relaxation of N-limitation of NPP, and nutrient deficiencies in vegetation. P addition to an N-saturated ecosystem would be predicted to slow the development of such symptoms.

Our experimental approach examined the vegetation and soil responses in a series of 100 m^2 plots within a single forest watershed. The establishment of the experimental units within a single area eliminated any concerns about pseduoreplication (*sensu* Hurlbert, 1984) that watershed-scale experiments encounter, but limited our ability to assess the response of trees in the ecosystem. Instead, we focused on the understory layer of the forest, a component that has been ignored in previous examinations of the impact of nutrient addition on forest ecosystems. We also examined the symbiosis between plants and mycorrhizal fungi, which is an important factor in plant P nutrition (Smith and Read, 1997) by testing whether nutrient addition reduces the number of infective propagules of arbuscular mycorrhizae (AM).

2. Methods

2.1. Study site

The study was conducted in a *Quercus rubra – Acer rubrum* forest adjacent to the Mountain Lake Biological Station (MLBS) in southwestern Virginia, at 37°22′37.5″N and 80°31′35.7″W at an elevation of 1061 m. Logging in the 1920's gave rise to a relatively even-aged overstory including such overstory dominants as *Q. rubra* (L.), *A. rubrum* (L.), *Q. velutina* (Lam.) and *Quercus alba* (L.). The understory community is dominated by woody shrubs such as *Vaccinium* spp. and *Menziesia pilosa* (Michaux) and perennial forbs such as *Aster acuminatus* (Michaux), *Amianthium muscaetoxicum* (Gray), and *Viola* spp. (Corbin, 1997).

The mean annual temperature was 8 °C, ranging from –3 °C in January to +19 °C in July. The mean annual precipitation was 130.1 cm. Monthly precipitation was highest from May–July (ca. 12 cm $month^{-1}$) and lowest from December–February (ca. 8.5 cm $month^{-1}$). The soils were acidic (pH 3.5–4.1) and highly weathered. Soil texture was approximately 15% clay, 60% silt, and 25% sand. A recording station for the National Atmospheric Deposition Program (NADP) located <10 km from the study site reported wet deposition of nitrate + ammonium of 4.50 kg N ha^{-1} in 1994, 3.95 kg N ha^{-1} in 1995, and 5.24 kg N ha^{-1} in 1996 (National Atmospheric Deposition Program, 1998). N inputs have increased from <3.0 kg N ha^{-1} since data recording began in 1979.

2.2. EXPERIMENTAL DESIGN

In June 1994 a series of eight replicate blocks were established in a 0.25 km^2 area of relatively homogenous understory vegetation and level topography. Each of the eight blocks was divided into four 10 × 10 m plots in a randomized complete block design for a total of 32 plots (4 treatments × 8 blocks). The plots in each block were randomly assigned to one of four treatments: +N, +P, +N+P, and control (which received no experimental N or P addition). A 1 m wide buffer was included within each plot to minimize edge effects, and all measurements were confined to the central 64 m^2. Each plot was separated from adjacent plots by at least 1 m.

N (NPK 34-0-0 using commercial NH_4NO_3) and P (NPK 0-22-0 using reagent grade Na_2HPO_4) fertilizer pellets were cast by hand throughout the entire 100 m^2 of a plot. The experimental addition amounted to a yearly input of 97 kg N ha^{-1} yr^{-1} and 30 kg P ha^{-1} yr^{-1}. Additions were made each month from April through October to simulate the periodic nutrient addition characteristic of atmospheric deposition. This level of N addition was approximately three times the inputs to highly polluted regions (Gunderson, 1995; Fenn *et al*., 1998). The level of yearly P addition was approximately half of the P contained in above-ground vegetation and the forest floor found in a survey of temperate deciduous forests (Cole and Rapp, 1981). Treatments began in July 1994 and continued through October 1996.

2.3. FIELD SAMPLING

Soil samples were collected 14 days after the monthly nutrient addition in July 1995 and each month in May–August 1996. The sampling date was chosen halfway between any two nutrient additions so that transient responses to the nutrient pulse were not measured. At each sample period, three samples of mineral soil (after removal of forest floor and organic horizons, where present) were collected from each plot to a depth of 10 cm, bulked, and sieved (<2 mm). Four of the eight blocks were sampled in 1995 while all eight blocks were sampled in 1996.

Above-ground biomass production of the understory was sampled at peak biomass in 1996 by harvesting all individuals <50 cm tall in three 4 m^2 subplots. Regrowth following harvest was minimal. During the harvesting, species were separated and dried at 60 °C for 24 hr the day of harvest, then again for 48 hr immediately prior to weighing. For the purpose of analysis, measurements of biomass for the three subplots in each plot were pooled, resulting in one estimate of above-ground biomass for each of the 32 plots. Because of the small number of trees present in any given plot and the likelihood that tree roots extended well beyond plot borders, responses of canopy vegetation to treatments were not sampled.

2.4. LABORATORY METHODS

2.4.1. *Soil Analyses*

Three 5 g subsamples from each plot's pooled soil sample were immediately extracted with 50 mL 2.0 M KCl for determination of extractable ammonium and nitrate. Three other subsamples were incubated in the laboratory for 14 days at 20 °C to determine potential net N mineralization and nitrification. Each subsample was incubated in capped polyethylene sample bottles at field moisture. The incubated subsamples were extracted with KCl as above. The filtered extracts were frozen until further analysis, no more than three months after extraction. An additional subsample was weighed and dried overnight at 100 °C for gravimetric water content (GWC) correction factors. Ammonium and nitrate concentrations in the KCl extracts were measured using a Bran & Luebbe (Technicon) Traacs 800 Autoanalyzer. Potential net mineralization of nitrogen was calculated as extractable nitrate + ammonium in the incubated sample minus extractable nitrate + ammonium in the initial extracts. Potential net nitrification was calculated as incubated minus initial extractable nitrate.

One subsample of soil (100–120 g) from each plot's pooled soil sample was dried and sent to Brookside Laboratories (New Knoxville, Ohio) for analysis of extractable P using standard Mehlich III methods (Mehlich, 1984).

2.4.2. *Foliar Chemistry*

Foliar chemistry was analyzed for three species collected during above-ground biomass harvesting. Following weighing, leaves from *Acer rubrum*, *Amianthium muscaetoxicum*, and *Aster acuminatus* were ground in a Wiley Mill (20-mesh). Each sample (0.04 g) was digested in sulfuric and salicylic acid, using the standard Kjeldahl method (Haynes, 1980). N and P concentration of the digested samples and standards (National Bureau of Standards Pine) were analyzed for N and P concentration on a Bran & Luebbe (Technicon) Traacs 800 Autoanalyzer.

2.4.3. *Arbuscular mycorrhizal inoculum potential*

Two to four conetainers (Stuewe and Sons, Inc., Corvallis, OR) were filled with approximately 135 g of soil collected from each plot in October 1996 as above. One pre-germinated seed of sorghum-sudan grass (*Sorghum bicolor* (L.) Moench) was planted per conetainer as a mycorrhizal trap plant and grown under greenhouse conditions. Roots were harvested at regular intervals in adjacent conetainers to determine the optimal time to assay mycorrhizal inoculum potential (MIP). Colonization was poor in all conetainers until week 6. All plants were harvested after seven weeks and soil was gently removed from roots as completely as possible. The roots were stored in 50% ethanol until further analysis.

Roots of each plant were cleared of cytoplasm by autoclaving at 121 °C at 20 psi for 15 min in 10% KOH. After rinsing with deionized water, roots were acidified with 1% HCl for 5 min. Roots were stained for 24 hr at 20 °C in 0.1% Chlorozol

Black E in a 1:1:1 solution of lactic acid, glycerol, and deionized water (Brundrett *et al*., 1994). To destain, the roots were transferred to a 50% glycerol solution for 24 hr at room temperature.

MIP was determined for each sorghum-sudan plant by counting the number of colonization units formed from single entry points per length of root observed (modified after Franson and Bethlenfalvay, 1989). After staining, roots were cut into ∼3 cm or less lengths and arranged in 50% glycerol on a glass slide with a cover slip. Each slide had approximately 15 cm of root, and between 1 and 7 slides were made per plant depending on the amount of root production. The slides were observed microscopically at 10 and 40×. Infection units were counted as the number of identifiable and discrete clusters of appressoria, hyphae, arbuscules, and/or vesicles developed during primary colonization of the root and prior to secondary spread along a root. Since harvest coincided with the first sign of extensive colonization, which should have minimized secondary colonization events, these clusters were believed to have developed during primary colonization rather than any secondary spread. Inoculum propagules could have come from spores, hyphal fragments, or infective mycorrhizal roots, all of which are taken into account with this method. Furthermore, this method provides the advantage of directly relating the number of infection units to the number of inoculum propagules in a soil while other infectivity measures do not (INVAM, 2001), allowing a robust measure of the infective propagules and their response to fertilization.

2.5. Statistical analysis

Data from each subplot or conetainer were pooled by plot to provide a single estimate in each plot for each variable. The effects of N and P addition on extractable P, foliar N and P concentrations, understory biomass, and MIP were tested using ANOVA (PROC GLM in SAS version 6.10). Each ANOVA model included the main effects of block, N, and P, and the N × P interaction. Because extractable ammonium and nitrate, and potential net N mineralization and nitrification were measured multiple times in 1996, repeated measures analysis of variance (ANOVAR) was used for analysis of these variables in 1996.

3. Results

3.1. Extractable N and P

Extractable ammonium and nitrate levels were significantly higher in plots that received supplemental N (+N and +N+P plots) than in plots that did not receive N (control and +P plots) (Table IA). The influence of N input changed over the course of the season, however. Ammonium in N-treated plots decreased from May–August 1996 relative to ammonium in plots that did not receive N (Month × N, Table IA; Figure 1A). Extracted nitrate in N-treated plots was highly variable

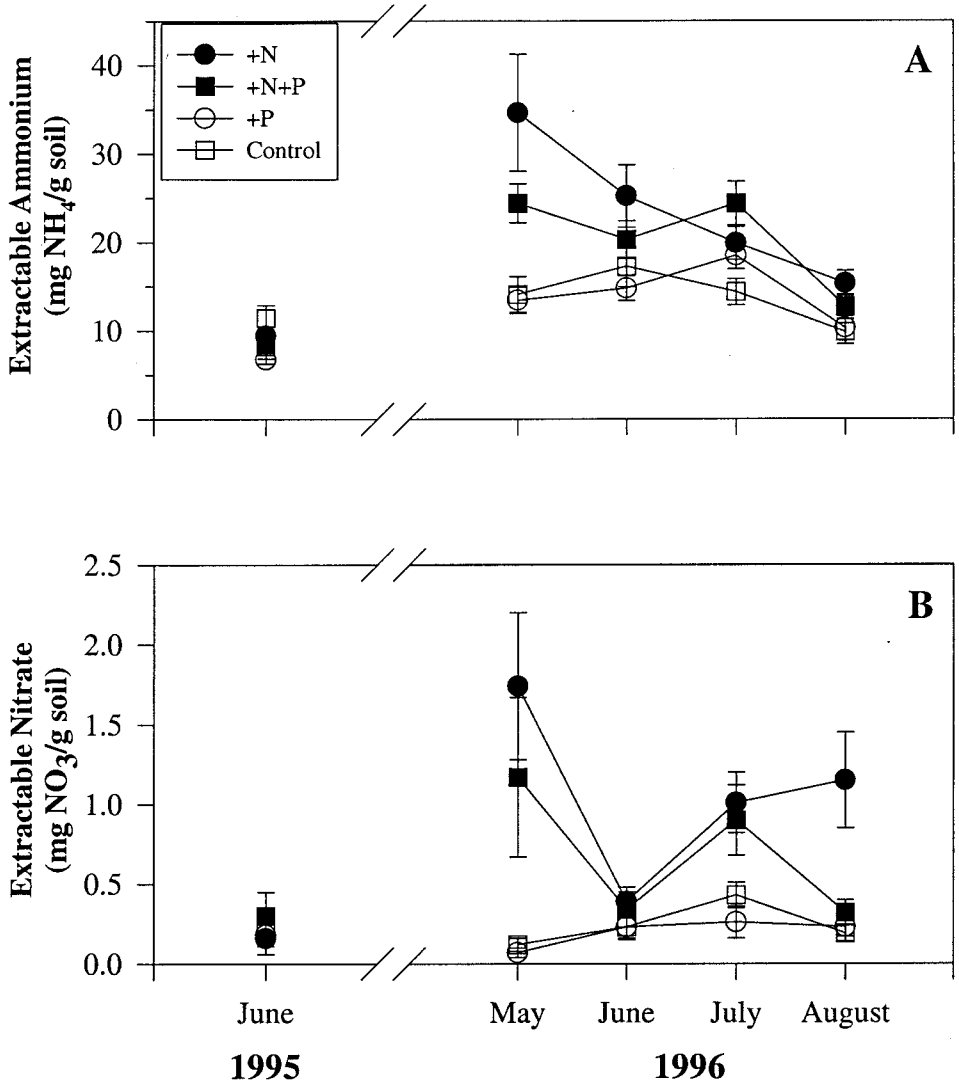

Figure 1. Extractable (A) ammonium and (B) nitrate in soil samples. Data points represents the mean of four (1995) or eight (1996) plots. Error bars represent one S.E.

through the season. Nitrate in +N plots was at least twice as abundant as in +P and control plots in every sample period except June 1995 and June 1996 (Figure 1B). Nitrate in +N+P plots showed a large amount of variation from sample period to sample period (Figure 1B).

Nitrate in control and +P plots was at or near detection limits ($\sim$0.25 μg g^{-1} soil) for each sample period and did not change between May and August 1996 (Figure 1B).

TABLE I

Repeated measures analysis of variance for (A) extracted ammonium and nitrate and (B) potential net N mineralization and nitrification in 1996, after three years of fertilization

(A)		Extracted ammonium		Extracted nitrate	
Source	d.f.	F	$p < F$	F	$p < F$
Block	7	1.44	0.24	0.54	0.79
N	1	58.52	**0.0001**	21.49	**0.0001**
P	1	2.00	0.17	3.66	0.07
N × P	1	3.05	0.10	0.98	0.33
Error (plot)	21	–	–	–	–
Month	3	10.22	**0.0001**	5.38	**0.009**
Month × Block	21	0.96	0.53	1.52	0.15
Month × N	3	4.06	**0.01**	7.34	**0.002**
Month × P	3	2.66	0.06	0.67	0.51
Month × N × P	3	0.64	0.59	1.97	0.15
Error (Month)	63	–	–	–	–
Total	127	–	–	–	–

(B)		Net N mineralization		Net nitrification	
Source	d.f.	F	$p < F$	F	$p < F$
Block	7	1.69	0.17	1.15	0.37
N	1	4.65	**0.04**	3.72	0.07
P	1	1.20	0.29	0.02	0.89
N × P	1	0.67	0.42	0.63	0.44
Error (plot)	21	–	–	–	–
Month	3	14.76	**0.0001**	106.6	**0.0001**
Month × Block	21	1.30	0.21	2.49	**0.003**
Month × N	3	0.27	0.84	3.10	**0.03**
Month × P	3	0.24	0.87	0.37	0.77
Month × N × P	3	1.86	0.14	1.07	0.37
Error (Month)	63	–	–	–	–
Total	127	–	–	–	–

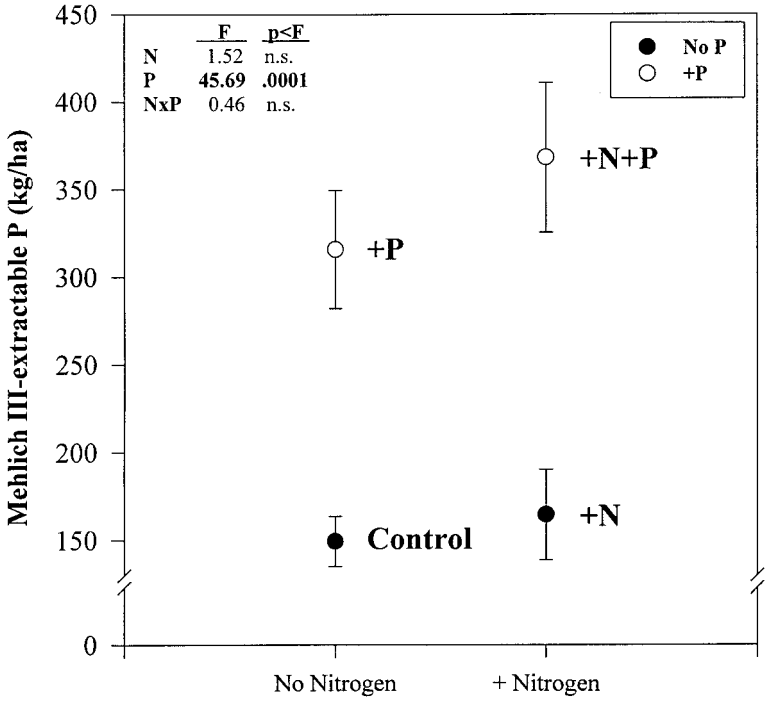

Figure 2. Mehlich III–extractable soil P in each treatment. F-statistics and p-values reported are from ANOVA of the effect of N addition, P addition and the N × P interaction on soil P levels. Labels identify treatment. Error bars represent one S.E.

P addition had no significant effect on either extracted ammonium or nitrate (Table IA). There was no interaction between N and P for either ammonium or nitrate (Table IA), indicating that the response of extractable inorganic N to addition of N or P did not depend on the addition of the other nutrient.

Mehlich III–extractable P was twice as high in plots that received P addition as compared to plots that did not receive P (Figure 2). There was no effect of N addition or an interaction between N and P.

3.2. Potential net mineralization and nitrification

Potential net mineralization of N in 1996 was greater in plots that received N addition than in plots that did not receive additional N (Table IB; Figure 3A).

N addition only affected potential net nitrification in July and August of the third year of N additions, when nitrification in N-fertilized plots was significantly lower than nitrification in plots that did not receive N (July: $F = 10.57$, $p < 0.004$; August: $F = 6.29$, $p < 0.02$). Net nitrification showed a strong seasonal trend through the course of 1996, as the nitrification decreased through the growing season until August, when it increased in all treatments (Month, Table IB; Figure 3B). In June

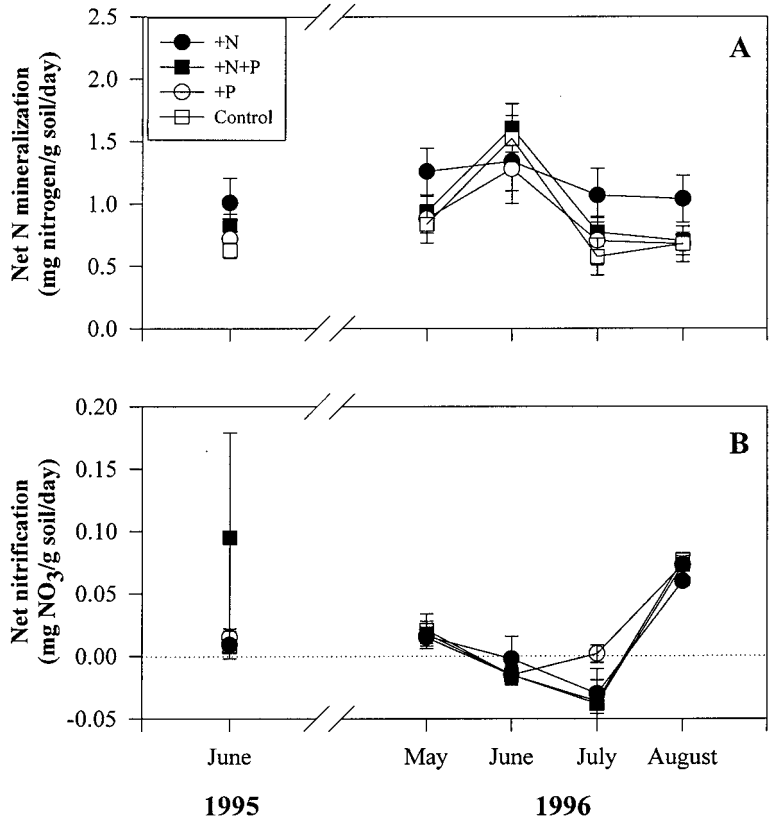

Figure 3. (A) Net N mineralization and (B) net nitrification in soil samples. Data points represent means of four (1995) or eight (1996) plots. Error bars represent one S.E.

and July 1996, net nitrification was negative in all treatments except +P plots in July 1996.

Net nitrification was 2–5% of the total net N mineralization in all treatments, and neither the main effects of N or P nor the interaction between N and P were significant for the proportion of mineralized N that was nitrified.

3.3. Foliar N and P concentrations

Foliar N concentrations of *Acer rubrum, Amianthium muscaetoxicum*, and *Aster acuminatus* leaves were significantly higher in +N and +N+P plots as compared to control and +P plots (Table II; Figure 4). P fertilization resulted in a significant decrease in the foliar N concentration of *Aster* (Table II), particularly in the +N+P plots as compared to +N plots (Figure 4). Foliar P concentration increased in all three species following P addition (Table II; Figure 5). In *Aster* and *Acer*, N addition significantly decreased the foliar P concentration (Table II; Figure 5). The negative effect of N on foliar P concentration was more pronounced in plots

TABLE II

ANOVA tables of tests for effect of N and P addition on the concentration of N and P in leaf tissue for *Acer rubrum, Amianthium muscaetoxicum*, and *Aster acuminatus*

Species	Source	d.f.	Leaf N		Leaf P	
			F-ratio	p-value	F-ratio	p-value
Acer rubrum	Block	7	0.77	0.62	0.29	0.95
	N	1	66.44	**0.0001**	9.98	**0.005**
	P	1	0.39	0.54	155.05	**0.0001**
	N × P	1	1.00	0.33	7.13	**0.01**
	Error	21	–	–	–	–
Amianthium muscaetoxicum	Block	7	0.82	0.58	2.60	**0.04**
	N	1	24.77	**0.0001**	0.43	0.52
	P	1	1.12	0.30	132.56	**0.0001**
	N × P	1	1.90	0.18	0.48	0.50
	Error	21	–	–	–	–
Aster acuminatus	Block	7	0.56	0.78	1.14	0.38
	N	1	49.53	**0.0001**	46.87	**0.0001**
	P	1	5.34	**0.03**	294.71	**0.0001**
	N × P	1	0.83	0.37	21.75	**0.0001**
	Error	21	–	–	–	–

that received experimental P addition than in plots that did not (N × P interaction, Table II)

3.4. Understory vegetation biomass

Understory vegetation biomass (herbaceous + woody species) was not significantly higher in +N and +N+P plots than in +P and control plots ($F = 3.34$, $p < 0.082$; Figure 6). Understory above-ground biomass was significantly lower in plots that received P addition ($F = 13.85$, $p < 0.01$; Figure 6). There was no significant interaction between N and P ($F = 1.17$; $p < 0.29$).

3.5. Total nutrient content of understory biomass

By taking the above-ground biomass of the three common understory species, *Acer rubrum, Amianthium muscaetoxicum* and *Aster acuminatus* into account, we estimated the N stored in the above-ground biomass of the dominant species in the understory (Table IIIA). N storage was greater in N-fertilized plots ($F = 5.29$, $p < 0.032$), primarily due to the response of *Aster acuminatus*, which contributed 70–90% of the N stored by the three species. The increase in N storage was primar-

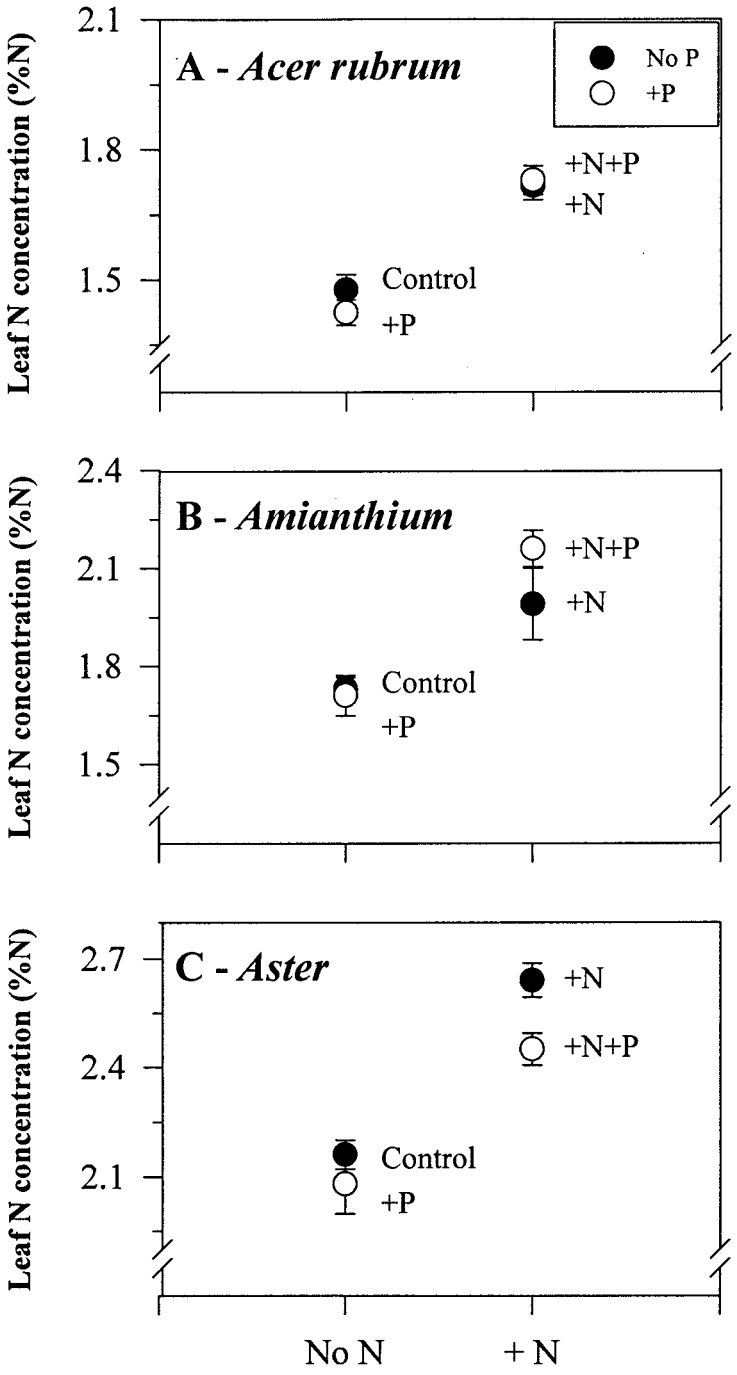

Figure 4. Foliar nitrogen concentration in (A) *Acer rubrum*, (B) *Amianthium muscaetoxicum*, and (C) *Aster acuminatus*. Labels identify treatment. Error bars represent one S.E.

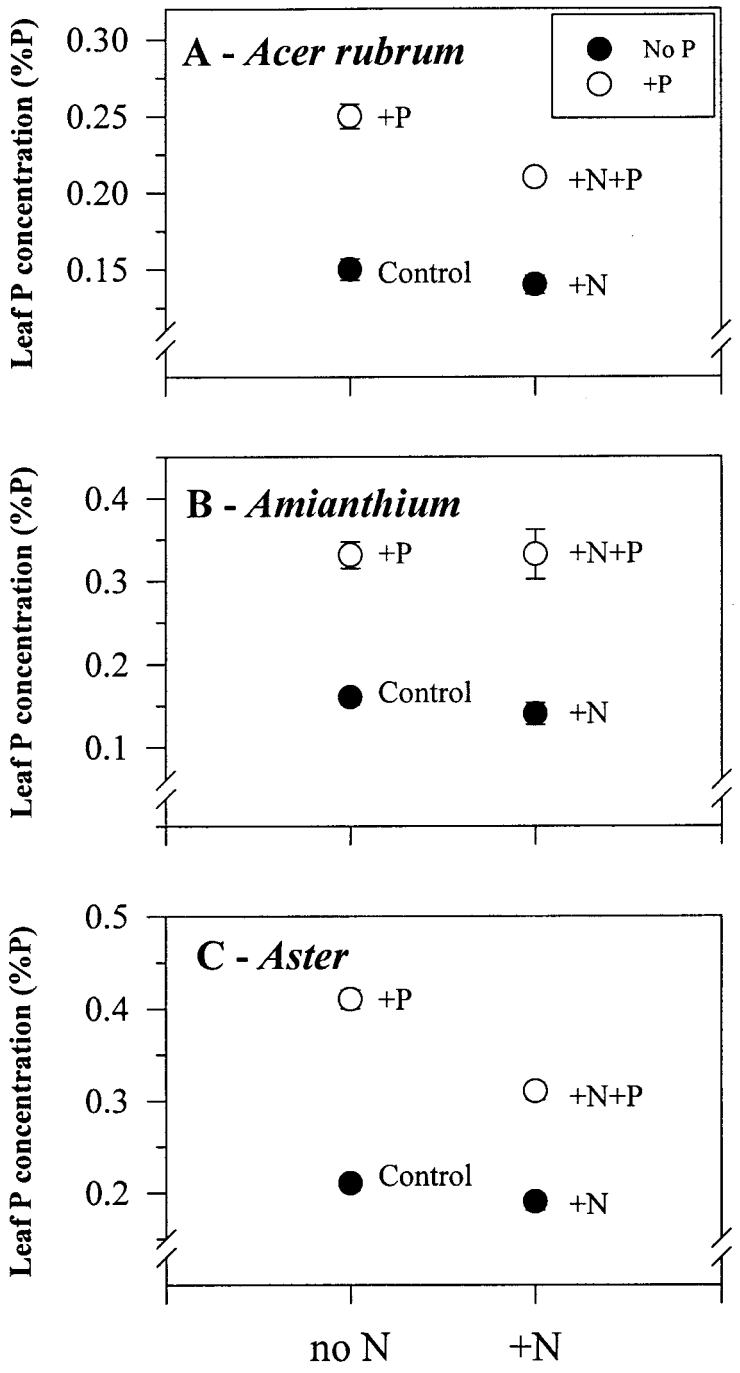

Figure 5. Foliar phosphorus concentration in (A) *Acer rubrum*, (B) *Amianthium muscaetoxicum*, and (C) *Aster acuminatus*. Labels identify treatment. Error bars represent one S.E.

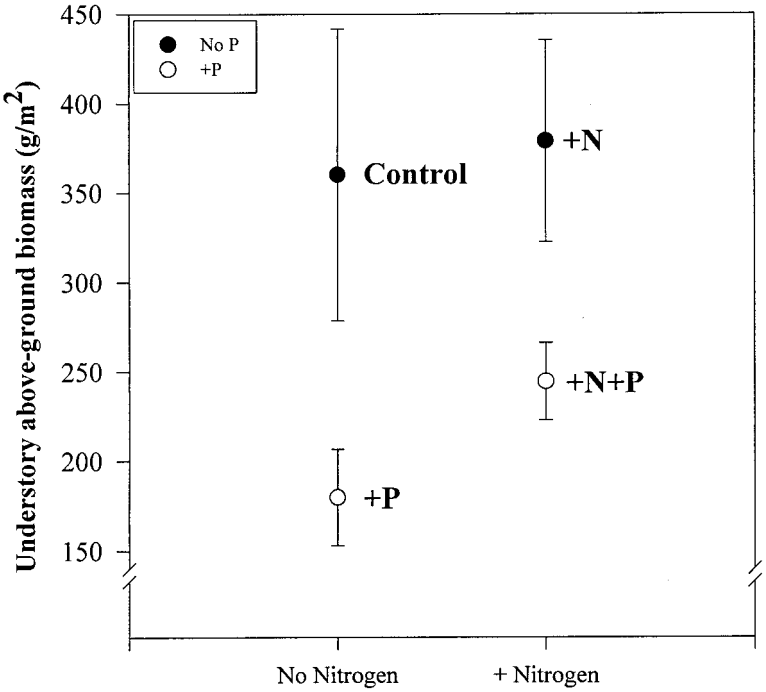

Figure 6. Above-ground biomass of all understory species (<50 cm height) in 1996. Error bars represent one S.E.

ily via increased incorporation of N into tissue rather than increased above-ground biomass, though a modest increase in above-ground biomass of *A. acuminatus* was observed. Extrapolated to a per hectare basis, N content of these three species in +N and +N+P plots was 4.6 kg N ha^{-1}, as compared to 3.0 kg N ha^{-1} in control and +P plots. N storage in the three understory species was significantly lower in plots receiving P addition ($F = 7.38$, $p < 0.013$; Table IIIA). There was no interaction between N and P on N storage ($F = 0.7$, $p < 0.4$).

Neither N or P addition had a significant effect on the total P contained in above-ground tissue of *Acer rubrum* or *Aster acuminatus* (Table IIIB; $p > 0.05$), in spite of the fact that both these species' tissue P concentrations responded to nutrient addition. Only *Amianthium muscaetoxicum* experienced decreased P content in N-amended plots ($F = 6.16$, $p < 0.02$).

3.6. Arbuscular mycorrhizal inoculum potential

MIP was significantly lower in plots that received P addition as compared to those that did not (Figure 7). MIP was slightly lower in +N and +N+P plots as compared to control and +P plots, respectively, though this difference was not significant.

TABLE III

(A) N content and (B) P content in each treatment of three common understory species, and the sum of all three species' N and P content. N and P content was calculated by multiplying above-ground biomass by tissue nutrient content. Values are means (in kg ha^{-1}) of eight replicates with one S.E. in parentheses

Treatment	*Acer rubrum*	*Amianthium muscaetoxicum*	*Aster acuminatus*	Sum
(A)				
Control	0.33 (0.25)	0.84 (0.22)	3.05 (0.86)	4.22 (1.03)
+N	0.23 (0.12)	0.53 (0.13)	4.47 (0.95)	5.24 (0.94)
+P	0.03 (0.00)	0.24 (0.07)	1.55 (0.41)	1.82 (0.41)
+N+P	0.10 (0.03)	0.19 (0.06)	3.64 (3.80)	3.93 (0.40)
(B)				
Control	0.003 (0.002)	0.008 (0.002)	0.031 (0.009)	0.042 (0.011)
+N	0.001 (0.001)	0.004 (0.001)	0.029 (0.007)	0.034 (0.007)
+P	0.002 (0.001)	0.004 (0.001)	0.033 (0.007)	0.038 (0.007)
+N+P	0.001 (0.001)	0.003 (0.001)	0.046 (0.005)	0.049 (0.005)

4. Discussion

4.1. Effect of N and P addition on soil nutrient cycling

Nitrogen addition increased the availability of N in the soil, as indicated by the increased concentration of soil inorganic N and increased rates of net N mineralization. Total understory biomass did not increase in N-amended plots, though biomass of *Aster acuminatus*, the understory dominant, and the total amount of N in understory vegetation was significantly higher in N-treated plots (Table III; Corbin, 1997). Canopy vegetation was not quantified in this study, but it likely played a significant role in the ecosystem responses to N and P addition. Watershed-level studies of N addition have reported N uptake by canopy individuals in the range of 15–30 kg N ha^{-1} (Aber *et al.*, 1993; Magill *et al.*, 1997), far higher than the 1.6 kg N ha^{-1} incorporated into understory biomass in our study. Changes in the tissue chemistry and litter decomposition rates of trees, too, would influence nutrient cycling rates in N- or P-treated ecosystems. Future studies designed to assess canopy-level responses to nutrient addition should consider the role of trees in the interaction between N and P availability.

The response of the ecosystem to three years of experimental N addition resembles results from strongly N-limited forests in the northeastern United States

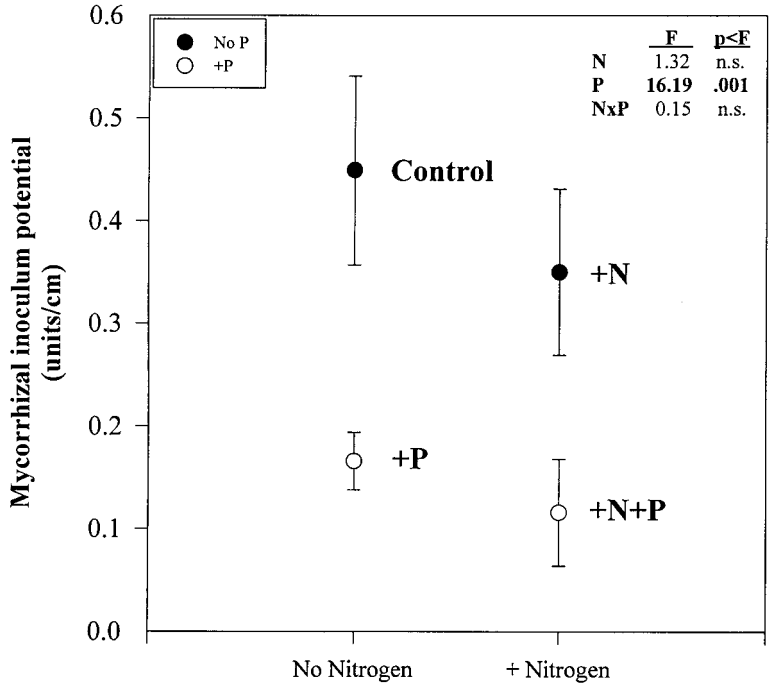

Figure 7. Arbuscular mycorrhizal inoculum potential in 1996, as assayed by counting colonization on roots of conetainer-grown sorgham-sudan grass. See methods for description of quantification of root colonization. F-statistics and p-values reported are from ANOVA of the effect of N addition, P addition and the N × P interaction on MIP. Labels identify treatment. Error bars represent one S.E.

(e.g. McNulty and Aber, 1993; Magill *et al.*, 2000). Net immobilization of nitrate was high, particularly in plots that received additional N. Net nitrification was negative for all treatments in two out of four sample periods, and addition of N resulted in even greater sequestration of nitrate. The low proportion of mineralized N that was nitrified (2–5% in all treatments) is characteristic of ecosystems that are strongly N-limited (McNulty *et al.*, 1990; Magill *et al.*, 2000).

P addition increased P availability, but had little effect on the cycling of N within the ecosystem. Neither the quantity of extractable ammonium and nitrate nor the rates of net N mineralization and net nitrification were significantly affected by P addition. P addition also had little effect on the ecosystem's response to N addition, as measured by the NxP interaction in the ANOVA models. This is consistent with our prediction that P addition would have little effect on N cycling rates in an N-limited ecosystem.

Our study leaves unresolved the nature of the interaction between N and P availability in a N-saturated ecosystem. We hypothesized that P addition would reduce such symptoms of N saturation as high rates of net nitrification, the relaxation of N-limitation of NPP, and N leaching losses. However, experimental N additions to our oak-maple ecosystem did not relax N-limitation. Further research should

examine the influence of P availability as N saturation develops in an ecosystem. We expect that N cycling and N retention in ecosystems receiving N additions alone will exhibit symptoms of N saturation sooner than comparable ecosystems receiving N and P additions. The addition of P to N-saturated ecosystems may also be capable of reversing symptoms of excess N. If primary productivity of a N-saturated ecosystem is controlled by P availability, then addition of P could enhance N uptake capacity of microbial populations and vegetation (Stevens *et al*., 1993; Fenn *et al*., 1998).

4.2. Effect of nutrient addition on understory N and P content

N addition decreased the concentration of P in leaf tissue in two of the three species, *Acer rubrum* and *Aster acuminatus*. This is consistent with observations of P-deficiency of coniferous trees in Norway (Flückiger and Braun, 1998), the Netherlands (Mohren *et al*., 1986), and Switzerland (Houdijk and Roelofs, 1993) experiencing elevated atmospheric N deposition.

The decrease in foliar P in N-fertilized plots was observed even though the N addition did not lead to N saturation, so that P-deficiency may have begun developing in the vegetation before other symptoms of N saturation became evident.

However, N addition in our study had no significant impact on the total P contained in the understory biomass, or on Mehlich-III-extractable P in the soil. A number of possibilities might explain the apparent contradiction between the lack of effect of N on extractable soil P and total understory P content while leaf P concentrations decreased. For example, N-stimulated growth of *Aster* (Corbin, 1997) may have diluted the concentration of P in plant tissue per unit plant mass without influencing P uptake. This mechanism, however, is unlikely for *Acer rubrum*, as this species did not experience a similar growth response to N addition. Alternatively, the understory species may have reduced their root: shoot ratios in response to elevated N inputs, thereby reducing P uptake. Such a response has been observed in forests receiving elevated N inputs (Wallenda *et al*., 1996; Boxman *et al*., 1995) and has been used to explain reductions in foliar P concentrations in trees (Flückiger and Braun, 1998) as well as reductions in species diversity in grasslands following elevated N inputs (Bobbink, 1991). While no difference in below-ground biomass was found between control and +N plots (Levy and Corbin, unpublished data), we did not attempt to sample responses to N addition of root biomass or root:shoot ratios for individual plants.

P fertilization increased leaf tissue P concentrations in all three species, and was able to counteract the negative effect of N addition on leaf P concentrations in the *Aster* and *Acer*. Thus, we conclude that P fertilization is able to alleviate P-deficiency in vegetation where elevated atmospheric N deposition has reduced leaf P concentration (Mohren *et al*., 1986; Houdijk and Roelofs, 1993; Stevens *et al*., 1993; Flückiger and Braun, 1998). Leaf foliar N concentrations in *Aster*, however, were significantly lower following P fertilization. While P fertilization

may be effective in increasing biotic uptake of N in N saturated systems (Stevens *et al*., 1993; Fenn *et al*., 1998), the effect of P inputs on leaf N concentrations and plant-mycorrhizal associations should be considered, as well, in determining the impact of P fertilization on uptake of N by biota.

4.3. Effect of nutrient addition on arbuscular mycorrhizal inoculum potential

The decrease in MIP with the addition of P is consistent with expectations that AM fungi trade P to vascular plants for fixed carbohydrate (C). The symbiosis is generally considered to be controlled by plants (Koide and Li, 1990) since AM fungi depend entirely on plants for C while plants can acquire P by direct root uptake. Mutualistic theory predicts that if P availability is high, plants will decrease allocation to the symbiosis since the mycorrhizal fungi do not provide any relative advantage under these conditions (Schwartz and Hoeksma, 1998). Decreased C allocation to mycorrhizal fungi would likely result in less root colonization and fewer fungal extramatrical hyphae and spores, each of which are components of MIP. Laboratory and grassland studies showing the decrease in mycorrhizal activity with the addition of P have consistently supported this view (Mosse, 1973; Menge *et al*., 1978; Bentivenga and Hetrick, 1992; Martensson and Carlgren, 1994; White and Charvat, 2000).

While we found that P addition reduces the number of AM inoculum propagules, N addition had no significant effect on MIP. It is unclear, however, how the plant-mycorrhizal symbiosis would be expected to respond as N addition continues and the ecosystem becomes N saturated. On the one hand, if P availability becomes limiting for plant growth and plants are capable of increasing belowground allocation to AM when P is scarce (Daft and Nicholson, 1969; Hayman and Mosse, 1971), then MIP would be expected to increase. On the other hand, AM may facilitate the uptake of N by plants (Subramanian and Charest, 1999; Mader *et al*., 2000; Hodge *et al*., 2001), in which case MIP could decline as N inputs continue. Observation of plant-mycorrhizal symbioses as ecosystems transition from the condition of N limitation to N saturation would help reconcile these competing predictions of how MIP would respond to N inputs.

5. Conclusions

Our study confirmed hypothesized interactions between N and P availability in a non-N saturated forest ecosystem, but left unresolved how N and P availability would interact in N-saturated ecosystems. P addition had no effect on either soil N cycling rates or N content of understory vegetation. While N addition did decrease tissue P concentration in two abundant understory species, we did not find evidence that increased biotic demand for P led to lower availability of P in the soil. P addition did decrease MIP, indicating that P availability to vegetation plays an important

role in the relationship between plants and arbuscular mycorrhizae. P addition may be effective in alleviating P-deficiency in vegetation receiving elevated N inputs, but perhaps at the cost of the benefits that associations with AM provide.

We have hypothesized that the importance of P availability to soil N cycling, N retention and plant-mycorrhizal symbioses is likely to increase as N availability increases and an ecosystem becomes N saturated. Further research should examine the impact of P inputs as symptoms of N saturation develop, or test whether addition of secondary nutrients such as P are capable of increasing N retention in N-saturated ecosystems.

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