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EFFECTS OF SOIL NUTRIENT AVAILABILITY ON INVESTMENT IN ACQUISITION OF N AND P IN HAWAIIAN RAIN FORESTS

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Abstract. We determined the influence of nutrient availability on the mechanisms used by plants to acquire nitrogen and phosphorus from the soil. Extracellular acid phosphatase production, mycorrhizal colonization, and N and P uptake capacities were measured in control, N-, and P-fertilized forests in three sites that varied in nutrient status from N limited to relatively fertile to P limited. Nitrogen fertilization increased extracellular phosphatase activity in all sites. Phosphorus additions consistently reduced phosphatase activity, mycorrhizal colonization, and P uptake capacity across sites. Our results indicate that these plants efficiently allocate resources to nutrient acquisition as suggested by an economic model. Investment in acquisition of a nutrient was greatest when that nutrient was limiting to growth, and plants appeared to allocate excess N to construction of extracellular phosphatases to acquire P. This increase in phosphatase production with N fertilization implies that even P-limited systems might respond to N deposition with greater productivity.

Key words: *extracellular acid phosphatases; fertilization; Hawaii; Metrosideros polymorpha; mycorrhizal colonization; nitrogen uptake capacity; nutrient availability; nutrient limitation; phosphorus uptake capacity; plant economics; resource allocation; root physiology.*

INTRODUCTION

Nitrogen and phosphorus, either individually or in combination, limit primary productivity in most terrestrial ecosystems, and investment by plants in acquisition of these soil nutrients can have important implications for plant growth and nutrient dynamics. Economic-based theories suggest that plants optimize growth by allocating more reserves to the acquisition of nutrients that most limit growth (Bloom et al. 1985, Read 1991). It follows that plants may use resources that are available in excess to capture those that are not. For example, carbon, if abundant, may be used to acquire P through allocation to mycorrhizal fungi. In addition, an increase in availability of one nutrient may increase the demand for, and investment in procurement of, another. For instance, a greater P availability may elicit greater allotment to N acquisition. Because nutrients interact in this manner, it is important to consider the availability of several essential resources (e.g., C, N, and P) simultaneously to adequately assess regulation of N and P acquisition by plants.

It is equally important to concurrently examine each of several acquisition methods, because N or P can be procured through various mechanisms. Plants can obtain P through the manufacture of enzymes that mineralize organic P in the soil, through the cultivation of associations with mycorrhizal fungi, or through the production of P-carrier enzymes in roots. Likewise,

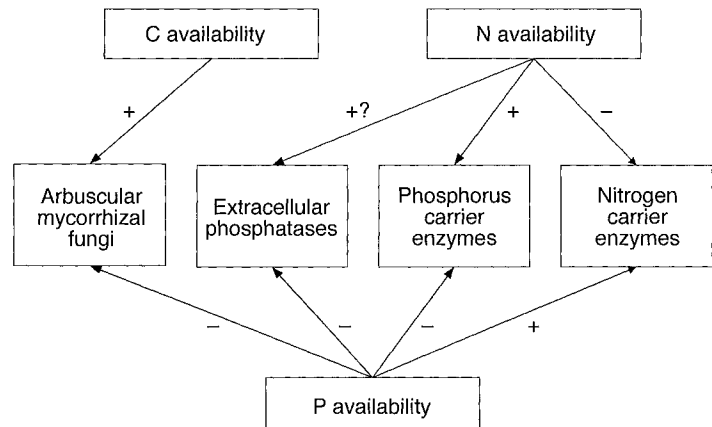
nitrogen can be acquired through N-carrier enzymes. In addition, root production increases the foraging capacity for several nutrients, including N and P. Each option demands distinct levels of investment of carbon, nitrogen, or phosphorus, and each has its own potential consequences for nutrient cycling. Environmental characteristics such as nutrient availability may determine the optimal combination of methods in each system, yet few studies have assessed investment comprehensively. Fig. 1 synthesizes potential and documented effects of C, N, and P availability on investment in these mechanisms, and each will be addressed individually.

One widespread mechanism, through associations with arbuscular mycorrhizal (AM) fungi, expands the volume of soil from which plants may draw phosphorus. Because P is relatively immobile in the soil, contributions by mycorrhizal fungi are particularly important for its procurement (Allen 1991, Smith and Read 1997 and references therein). AM fungi require primarily carbon from the plant for fungal growth, and are more efficient foragers than are plant roots, as their hyphae have smaller diameters (Smith and Read 1997). Therefore, AM fungi may be the most efficient option for plants that are P limited and have carbon available in excess of that needed for growth (Fig. 1). Several recent studies have indicated that elevated carbon dioxide levels augment investment in AM fungi, either by increasing the carbon available for investment, or by increasing demand for soil nutrients (Diaz 1996, Hodge 1996, Staddon and Fitter 1998). Likewise, many studies have also noted that colonization levels of AM fungi decrease upon an increase in P availability (Fig. 1; e.g., Mosse 1973, Baath and Spokes 1989, Furlan

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FIG. 1. Potential and documented effects of carbon, nitrogen, and phosphorus availability on investment in methods of nutrient acquisition. Arbuscular mycorrhizal fungi, extracellular phosphatases, and phosphorus carrier enzymes are used to obtain P from soil. Nitrogen carrier enzymes assist in N acquisition. Literature documenting individual nutrient effects is cited in the *Introduction* and *Discussion* sections.



and Bernier-Cardou 1989, Raju et al. 1990). In contrast, effects of increased N on the growth of AM fungi in field systems are inconsistent, and may be positive (Lussenhop et al. 1998, Eom et al. 1999) or negative (Klironomos et al. 1997).

Another mechanism, extracellular enzymes, enables plants to exploit pools of organic nutrients in the soil. Acid phosphatase activity, in particular, may provide a significant portion of phosphorus for plants (Harrison and Pearce 1979, Kroehler and Linkins 1988, Moorhead et al. 1993), although its role in phosphorus acquisition has not been directly demonstrated (Colpaert et al. 1997). These extracellular enzymes are present on root surfaces and are also secreted by roots into the rhizosphere. AM hyphae produce extracellular phosphatases as well (e.g., Gianinazzi et al. 1979, Dodd et al. 1987). Phosphatases hydrolyze the ester-phosphate bonds in soil organic P, which releases the phosphate into soil solution for uptake by nearby roots or microbes (Tarafdar and Claassen 1988, Duff et al. 1994, Pant and Warman 2000). Extracellular phosphatases anchored on the root or hyphal surface ensure that mineralization occurs in close proximity to P uptake, although other plants may receive some fraction of the mineralized P.

As proteins, phosphatases have relatively high N concentrations (between 8% and 32%), and may represent a significant investment of N. Therefore, increased nitrogen availability may raise the extracellular phosphatase activity of plants (Fig. 1), although few studies have examined that possibility. Clarholm and Rosengren-Brinck (1995) found that N fertilization increased phosphatase activity in soils under a Norway spruce forest, although free-living soil microbes could have contributed to phosphatase production. Numerous studies have reported reductions in extracellular acid phosphatase activity per unit root in response to increased P availability (Fig. 1; see McLachlan 1980, McLachlan and DeMarco 1982, Caradus and Snaydon 1987).

Plants may also invest in P- or N-carrier enzymes in roots to improve their ability to compete for P or N,

respectively. Nye and Tinker (1977) showed that the rate of nutrient uptake by a single, isolated root is limited primarily by the speed of diffusion of nutrients through the soil, but they also noted that under competitive conditions, "absorbing powers" of roots from different individuals may also affect the nutrient uptake rates of each. Reductions in P uptake capacity in the field upon P fertilization (Fig. 1; see Harrison and Helliwell 1979, Harrison et al. 1991, Jones and Dighton 1991, McDonald and Malcolm 1991) and in N uptake capacity upon N fertilization (Fig. 1; see Jones and Dighton 1991, Jones et al. 1991) have been observed. Moreover, previous studies have indicated that an increase in nitrogen status can raise uptake capacity for phosphorus (Fig. 1; see Cole et al. 1963, Thien and McFee 1972, White 1972), and vice versa (Williams 1948, Hills et al. 1970, Jones and Dighton 1991). As with phosphatases, these enzymes have a relatively high N content and may also represent a notable N investment.

Overall, because these different methods of nutrient acquisition vary in their resource demands, plants may shift among them depending on which nutrients are available in excess. For example, in a P-limited system, plants may increase investment in extracellular phosphatases, but not in AM fungi, in response to N deposition. This change may essentially produce a shift toward phosphatases (vs. AM fungi) as the dominant mechanism. In this case, measurements of AM fungi only would limit the interpretation of possible consequences of N deposition on P uptake and plant growth. In this study, we examined the potential of ecosystems to modify allocation of resources among several nutrient acquisition mechanisms (including extracellular phosphatases, mycorrhizal fungi, and nutrient carrier enzymes) in response to N and P availability. Few studies have measured these variables concurrently, even though such a synthesis could provide insight into ecosystem-level responses to changes in nutrient accessibility.

We tested the relationship between nutrient availability and N and P acquisition in N- and P-fertilized

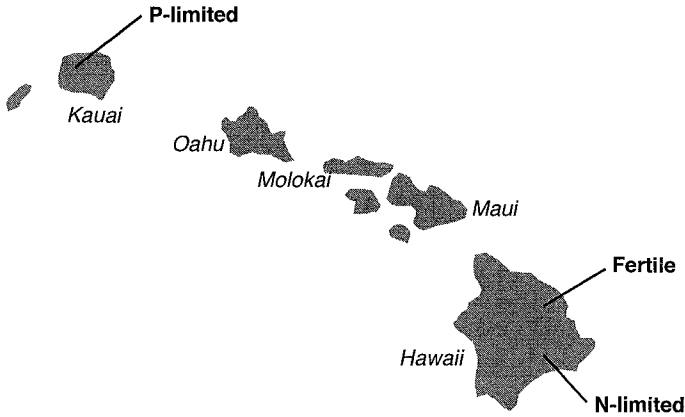


FIG. 2. Location of study sites across the Hawaiian Island chain. The N-limited, fertile, and P-limited sites are 300, 20 000, and 4 100 000 yr old, respectively.

plots in three rain forests in Hawaii (Fig. 2). These sites are at different stages of soil development (300, 20 000, and 4 100 000 yr old) and therefore vary in soil nutrient availability. Phosphorus availability is low in the youngest and oldest sites and greatest at the 20 000-yr-old site. Nitrogen availability is lowest at the youngest site, moderately high in the intermediate-aged site, and highest at the oldest site (Table 1; see Crews et al. 1995). Moreover, nutrient availability exerts a strong influence on plant growth across the sites. Long-term fertilization experiments have indicated that above-ground productivity is limited primarily by N in the youngest site (Vitousek et al. 1993), P in the oldest site (Herbert and Fownes 1995), and by neither N nor P independently in the relatively fertile 20 000-yr-old site (Vitousek and Farrington 1997). Because these forests vary in nutrient status, we were able to compare both baseline levels and responses to fertilization of N or P-limited vs. relatively fertile ecosystems.

SITES

The sites are described in detail by Crews et al. (1995). Briefly, all sites are near 1200 m elevation and have a mean annual temperature of $\sim 16^{\circ}\text{C}$. All receive ~ 2500 mm of rainfall annually, which is relatively evenly distributed throughout the year. At each site, volcanic tephra is the parent material, with slope angles $< 2^{\circ}$. The vegetation at each site consists of native forest dominated by the evergreen tree *Metrosideros polymorpha* (Kitayama and Mueller-Dombois 1995). *Cheirodendron trigynum* and *Ilex anomala* are also pre-

sent in the upper canopy. Subcanopy plants at all sites include *Coprosma* spp., *Myrsine* spp., *Vaccinium calycinum*, and tree ferns (*Cibotium* spp). An exotic ginger (*Hedychium gardenianum*) is abundant at the youngest and oldest sites.

Fertilized plots receive $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ of either N (half as ammonium nitrate and half as urea) or P (as triple superphosphate) in two applications per year (Vitousek and Farrington 1997). Fertilization has been ongoing for at least four years in each site. Plots are 15×15 m in the N- and P-limited sites. At the fertile site, plots are centered on a single adult *Metrosideros* individual and are 10 m in diameter. There are 4–6 replicate plots per treatment per site.

METHODS

Sample collection

All measurements involved sampling roots from control, N-fertilized, and P-fertilized plots in the three sites. Because the majority of root biomass occupies the top 10 cm of soil (Ostertag 2001), we used 5 cm diameter soil corers to excise roots growing to 10 cm depth. One core was collected at a random location in each of 3–4 plots per treatment per site. Roots were not sorted by species, and thus represent the community of plants at each site.

Roots were collected in October 1997 for analyses of extracellular phosphatase; in January and March 1996 and July and October 1997 for mycorrhizal colonization; in June 1998 for P-uptake capacity; and in May 1998 for N-uptake capacity. For mycorrhizal colonization, samples were only taken from plots in the N- and P-limited sites in January and March 1996, and from the fertile site in July 1997.

Assays were performed within 12 h of root collection. Soil cores were stored at 4°C prior to starting lab analyses. Live roots < 2 mm in diameter were selected and were gently washed four times in deionized water.

Extracellular phosphatase activity

Following McLachlan (1980), with modifications by Z. Cardon (*personal communication*), we placed roots

TABLE 1. In situ phosphorus and nitrogen availability (mean ± 1 SE), by site nutrient status and age.

Site status	Site age (yr)	Resin P ($\mu\text{g}\cdot\text{bag}^{-1}\cdot\text{d}^{-1}$)	Resin N ($\mu\text{g}\cdot\text{bag}^{-1}\cdot\text{d}^{-1}$)
N limited	300	0.20 ± 0.08	3.31 ± 1.56
Fertile	20 000	1.21 ± 0.28	12.37 ± 3.32
P limited	4 100 000	0.41 ± 0.17	14.41 ± 7.20

Notes: Resin N is the sum of resin ammonium-N and resin nitrate-N. Data are from Crews et al. (1995).

in a 25-mL solution of 3.5 mmol/L para-nitro phenyl phosphate (PNPP) in 50 mmol/L sodium citrate buffer (pH 5.5) within 12 h of collection. Roots were incubated in the dark at 20°C for 1 h, after which 1 mol/L sodium hydroxide was added to stop the reaction. Roots were removed from the solution by filtration through Whatman Number 1 filter paper (Whatman, Maidstone, UK). The solution was frozen until it was transported back to Stanford University, where absorbance at 410 nm was measured on a spectrophotometer (U-2000, Hitachi, Tokyo, Japan) and was compared to a standard curve of para-nitrophenol. Roots were dried for 3 d at 70°C and weighed. Results are expressed as micromoles PNPP cleaved per gram of root per hour.

Arbuscular mycorrhizal colonization

We used ~10 mg of roots that had been dried at 70°C for 3 d. Roots were rehydrated and stained with Trypan blue as reported in Koske and Gemma (1989). Colonization of arbuscular mycorrhizal fungi was quantified using the magnified intersections method (McGonigle et al. 1990). Results are reported as percentage of root length with mycorrhizal structures (vesicles, arbuscules, or hyphae). We found no evidence of ectomycorrhizal fungi in our samples.

Phosphorus uptake capacity

Roots were first cut into 1 cm long pieces; from each sample, a subsample was weighed, dried for 3 d at 70°C, and was then reweighed to calculate the percentage dry mass. The remainder of each sample (4–30 mg) was weighed for fresh mass and was placed into a small scintillation tube. Following McDonald and Malcolm (1991), we incubated roots for 30 min in enough 0.5-mmol/L calcium sulphate to cover the roots (usually 1–2 mL). We then incubated the roots for 30 min in a solution containing 0.5-mmol/L calcium sulphate, 5- μ mol/L potassium dihydrogen orthophosphate, and 1 MBq 32 P/L as orthophosphate. Roots were washed by soaking in four changes of 0.5 mmol/L calcium sulphate plus 5- μ mol/L potassium dihydrogen orthophosphate for 5 min each. Enough scintillation fluid was added to cover the roots, and radioactivity was measured in a liquid scintillation counter (LS-9600, Packard Instruments, Meriden, Connecticut, USA). Data were corrected for radioactive decay, instrument efficiency, and dry mass, and results are reported as micromoles P per gram dry root per hour.

Nitrogen uptake capacity

Following Jackson and Reynolds (1996), ~25 mg of roots were placed in cheesecloth bags and incubated in a 0.5 mmol/L calcium chloride solution for 20 min. Roots were then incubated for 30 min in solutions containing 0.5 mmol/L calcium chloride and 0.01 mol/L sucrose plus 100 μ mol/L of either 15 NH₄Cl or K 15 NO₃. Roots were washed by soaking in four changes of 1 mmol/L potassium chloride for 5 min each. Roots were

dried for 3 d at 70°C and ground to 40 mesh, and 3–5 mg of sample were weighed and rolled in tin cups. Atomic percentage of 15 N and percentage of total N were measured in a mass spectrometer combined with an elemental analyzer (Tracermass, Europa Scientific, Waynesville, Ohio, USA) and compared to a peach standard (National Institute of Standards and Technology, Washington, D.C., USA). Results are reported as micromoles N per gram dry root per hour. Because preliminary results indicated that roots were taking up 1/10 as much nitrate as ammonium (~0.1 μ mol nitrate-[g dry root]⁻¹·h⁻¹), in later measurements we incubated roots in 15 NH₄Cl only. Because root excision can reduce absorption of ammonium, our measurements may underestimate actual uptake (Bloom and Caldwell 1988).

Statistics

Statistical analyses were performed with SYSTAT version 8.0 for Windows (SPSS 1998). Data were log-, square-root-, or arcsine-transformed. ANOVAs and Tukey post hoc tests were conducted with site and treatment as grouping variables. We tested for normal distribution of data by calculating standard deviates separately for each sample, pooling all deviates, then applying a Kolmogorov-Smirnov test for goodness of fit. We used an F_{\max} test to confirm homogeneity of variances (Sokal and Rohlf 1995). The latter two tests indicated that normality and homogeneity of variances were achieved for each ANOVA.

Calculation of "total" investment

All of the measurements described thus far were made per unit root mass or length. The product of these results and the standing stock of live roots per square meter of soil provides an approximation of total ecosystem-level investment. To estimate total investment, we used data reported by Ostertag (2001) for standing biomass and length of live, fine roots from control plots in the N-limited, P-limited, and fertile sites, and from N- and P-fertilized plots in the N- and P-limited sites. We used standing live biomass instead of annual root production for our calculations because each of the acquisition methods, especially secreted phosphatases and mycorrhizal fungi, requires investment and maintenance while roots are active. Root lifetimes are longest in the P-limited site (Ostertag 2001), so standing biomass is greatest there despite low root production. Table 2 summarizes these data and the estimated investment per unit soil area for each method.

RESULTS

Extracellular phosphatase activity

Fertilization significantly altered investment in phosphatases (Fig. 3; $F_{2,22} = 23.327$, $P < 0.001$). Specifically, phosphorus additions significantly and consistently reduced extracellular phosphatase activity per

TABLE 2. The length and biomass of live, fine roots (from Ostertag 2001) and calculated "total" investment in each acquisition method.

Site status	Fertilization treatment	Root mass (g/m) ² †	Root length (km/m) ² †	Extracellular phosphatase activity (mmol PNPP cleaved·m ⁻² ·h ⁻¹)
				Oct 1997
N-limited	control	257 ± 45	2.40 ± 0.35	7.40 ± 2.25
	N	331 ± 34	2.76 ± 0.32	16.15 ± 1.31
	P	292 ± 20	3.54 ± 0.23	4.73 ± 0.77
Fertile	control	354 ± 119	3.22 ± 0.43	12.22 ± 1.67
P-limited	control	457 ± 81	3.40 ± 0.38	14.67 ± 0.58
	N	467 ± 103	3.60 ± 0.52	35.27 ± 4.65
	P	544 ± 147	4.54 ± 0.67	9.17 ± 3.26

Notes: Total investment is the product of activity (or colonization) and standing root mass (or length) in each plot. Data are means (± 1 SE) of 2–4 replicates.

† Data are from Ostertag (2001).

gram root ($P < 0.023$). Nitrogen fertilization increased specific activity significantly across sites ($P < 0.002$) and more than doubled activity in the oldest site. There were no significant differences among sites.

Colonization of arbuscular mycorrhizal fungi

Nutrient additions significantly affected mycorrhizal colonization in October 1997 (Fig. 4; $F_{2,23} = 7.560$, $P < 0.004$). Fertilization with P consistently reduced the percentage of colonization across the sites and at each sampling date. Phosphorus fertilization effects were significant in October 1997 ($P < 0.004$) and marginally significant in March 1996 ($P < 0.068$). A significant site by fertilization interaction in March 1996 ($F_{2,18} = 4.843$, $P < 0.022$) was driven by response to N; i.e., N additions reduced colonization in the P-limited ($P < 0.057$) but not in the N-limited site. Otherwise, sites did not differ significantly from one another.

Phosphorus uptake capacity

Phosphorus uptake capacities were significantly altered by fertilization (Fig. 5; $F_{2,23} = 8.202$, $P < 0.003$). In particular, phosphorus fertilization markedly reduced specific phosphorus uptake capacity across sites ($P < 0.012$). Nitrogen fertilization had no significant effect on activity, and there were no significant differences among sites.

Nitrogen uptake capacity

Nutrient additions significantly affected N uptake capacity (Fig. 6; $F_{2,22} = 5.070$, $P < 0.016$). In particular, fertilization with N significantly reduced specific N uptake capacity ($P < 0.031$). There was a significant site by fertilization effect ($F_{4,22} = 3.118$, $P < 0.037$). Specifically, a trend toward reduction in uptake capacity was most apparent in the N-limited site, and did not occur in the N-rich, P-limited site where N uptake capacity was already low. Likewise, a trend toward an increase in N uptake capacity with P fertilization occurred only in the P-limited site.

Specific root vs. total investment

The effects of fertilization on ecosystem-level patterns of nutrient acquisition consistently mirrored those in investment per unit root (Table 2, Figs. 3–6). Because of the pattern of root biomass, the rank of each site was similar at both scales, although differences among sites were greater at the ecosystem level in mycorrhizal colonization and P uptake capacity (where the P-limited site had much higher levels of activity than did the N-limited site). Differences among sites were less pronounced in extracellular phosphatase activity (levels at the P-limited site were only slightly higher than those at the fertile site).

DISCUSSION

Phosphorus acquisition

One of the interesting findings of this study is that nitrogen availability markedly increased investment in extracellular phosphatases (Table 2, Fig. 3). Nitrogen-fertilized trees at these sites may be using the most efficient approach to obtain P by allocating N, which is relatively abundant following fertilization, to construction of phosphatases. This interaction has received little attention in past studies and may be widespread.

How much N is invested in exuded phosphatases? We estimated this amount using published information on kinetic properties of isolated phosphatases. Duff et al. (1994) lists four acid phosphatases that are known to be secreted from plants, and an additional 16 that have an undetermined cellular location. The maximum rate of activity (V_{\max}) of these enzymes ranges from 2.6 to 1250 $\mu\text{mol PNPP cleaved} \cdot [\text{mg enzyme}]^{-1} \cdot \text{min}^{-1}$, with a median of 148 $\mu\text{mol PNPP cleaved} \cdot [\text{mg enzyme}]^{-1} \cdot \text{min}^{-1}$. We used a V_{\max} of 1250 $\mu\text{mol PNPP cleaved} \cdot [\text{mg enzyme}]^{-1} \cdot \text{min}^{-1}$, with its corresponding K_M of 1.1 mmol/L (Gellatly et al. 1993), as a high estimate of the activity (and low estimate of enzyme mass) of the extracellular phosphatases produced in the Hawaiian sites. A V_{\max} of 2.6 $\mu\text{mol PNPP cleaved} \cdot [\text{mg enzyme}]^{-1} \cdot \text{min}^{-1}$ (and corresponding K_M of 0.35-mmol/

TABLE 2. Extended.

Mycorrhizal colonization (km mycorrhizal roots/m ²)			Phosphorus uptake capacity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$)	Nitrogen uptake capacity ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$)
Jan 1996	Mar 1996	Oct 1997	June 1998	May 1998
0.42 ± 0.30	0.54 ± 0.09	1.04 ± 0.21	33.37 ± 13.38	0.383 ± 0.108
0.46 ± 0.09	0.62 ± 0.10	0.78 ± 0.24	75.54 ± 8.46	0.193 ± 0.031
0.14 ± 0.11	0.35 ± 0.19	0.26 ± 0.07	8.81 ± 2.72	0.293 ± 0.035
...	...	0.88 ± 0.01	43.36 ± 6.04	0.254 ± 0.060
0.63 ± 0.40	1.61 ± 0.22	2.10 ± 0.21	95.70 ± 12.51	0.312 ± 0.030
0.40 ± 0.09	0.23 ± 0.13	0.79 ± 0.20	89.72 ± 19.44	0.387 ± 0.079
0.27 ± 0.08	0.71 ± 0.34	0.67 ± 0.47	9.91 ± 4.93	0.692 ± 0.188

L; Miernyk 1992) was used as a low estimate. We also assumed that the enzymes were 16% N by mass, and that phosphatases were not secreted from roots. With these constraints, we estimated that ~ 0.041 – 16.6 mg N/m² were invested in extracellular phosphatases in the standing stock of live roots in control plots at the P-limited site in October 1997 (calculated from data in Table 2). These numbers are a lower bound for N investment, as some fraction of extracellular phosphatases are secreted from roots into the soil (e.g., Rubio et al. 1990). Annual investment in production of these phosphatases may be much higher than the standing stock in roots at any given time.

There may be a limit to the cost effectiveness of extracellular phosphatases, even in the N-rich, P-limited site. As more phosphatases are exuded, the probability that mineralized P will be tapped by other plants increases. In other words, plants will receive diminishing, marginal returns of P as invested N increases (sensu Bloom et al. 1985). At some point, the increase in P acquisition provided by an increase in investment will not offset the cost of that additional investment. However, an increase in availability of N through fertilization could reduce the relative cost of N involved

in phosphatase production, and could raise the level of phosphatase production. This may explain the response seen even in the P-limited, N-rich site, where investment in extracellular phosphatases more than doubled with N fertilization (Fig. 3).

Another possible explanation for the increase in extracellular phosphatase activity with N fertilization is that N additions stimulate plant growth, which in turn raises demand for P. Although this scenario may not be a factor in the P-limited site, it could be important in the youngest site, where aboveground productivity increases with N fertilization. Regardless of the underlying mechanism, it appears that an increase in N availability could result in an increase in P availability through the production of extracellular phosphatases. This mechanism could allow even P-limited systems to respond to N deposition and other anthropogenic N inputs with an increase in productivity.

Nitrogen fertilization had a less striking impact on P uptake capacity. An increase in growth and demand for P may be responsible for the nonsignificant increase in uptake capacity per gram root (Fig. 5), and the more than twofold increase in ecosystem-level investment (Table 2) with N fertilization in the N-limited plot. An increase in the amount of N available for construction of carrier enzymes may also be a factor. However, this latter factor seems to have a much more subtle influence than that of extracellular phosphatases, possibly because uptake enzymes require less N for construction. Secreted phosphatases are lost from the plant and therefore are likely to be much more costly than root-bound carrier enzymes. In addition, there may be a maximum concentration of carriers that can be contained on the root surface, so the production of additional carrier enzymes may not always be feasible.

Although phosphatases represent a substantial cost to plants in terms of N investment, mycorrhizal fungi can require a large fraction of the net C fixed by plants (up to 15–20%; Jakobsen and Rosendahl 1990, Allen 1991). It is not surprising that P fertilization consistently decreased investment in each of these methods of acquisition. These responses have also been observed in many previous studies (Mosse 1973, McLachlan 1980, McLachlan and DeMarco 1982, Caradus

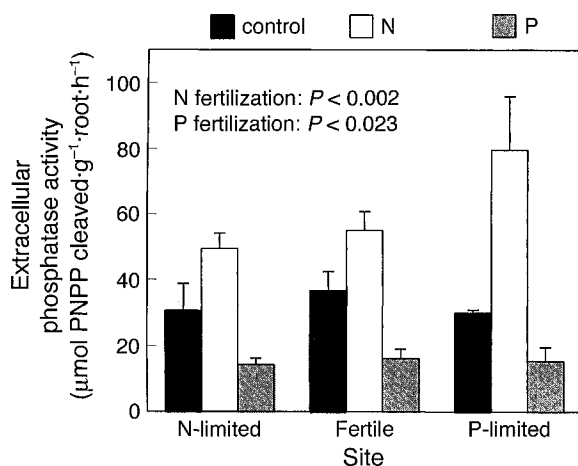


FIG. 3. Root-associated extracellular phosphatase activity in roots from control and fertilized plots. Error bars are ± 1 SE. Each bar represents an average of 3–4 plots per site.

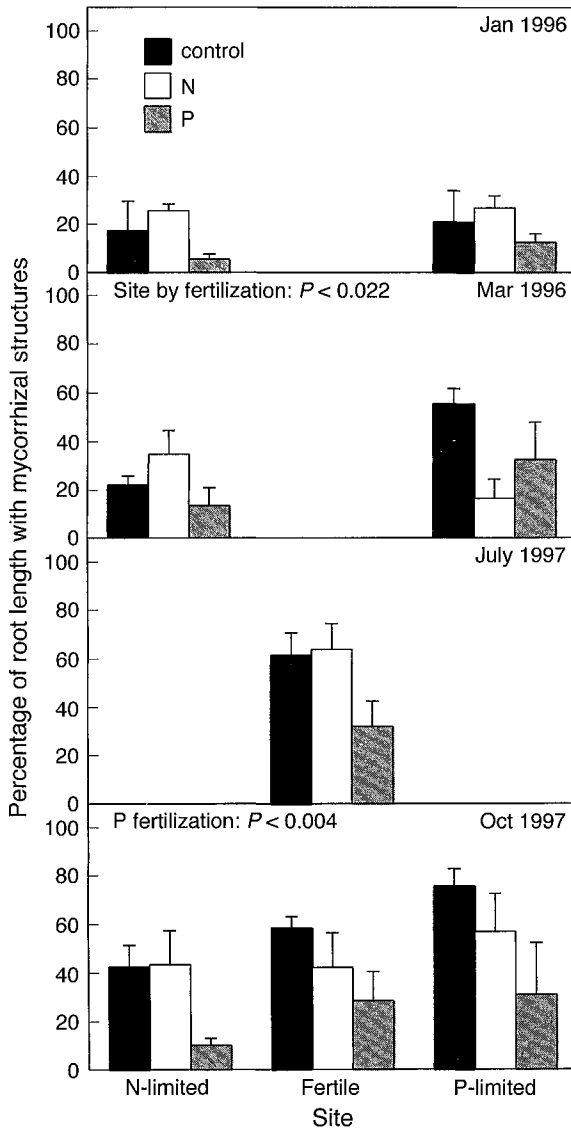


FIG. 4. Percentage of arbuscular mycorrhizal colonization of roots from control and fertilized plots. Error bars are ± 1 SE. Each bar represents an average of 3–4 plots per site. In January and March 1996, samples were not collected from the fertile site. In July 1997, samples were collected from the fertile site only.

and Snaydon 1987, Baath and Spokes 1989, Furlan and Bernier-Cardou 1989, Raju et al. 1990, McDonald and Malcolm 1991). Nitrogen fertilization either reduced (P-limited site, March 1996) or had no effect on AM fungi. Because extracellular phosphatase activity increased in the same conditions, N fertilization seems to induce a shift among P acquisition methods in this system.

Finally, root biomass and production in these sites have been characterized by Ostertag (2001), who found that the P-limited site has the lowest rates of root production. Moreover, although nitrogen fertilization had no effect on root production in the N-limited or P-

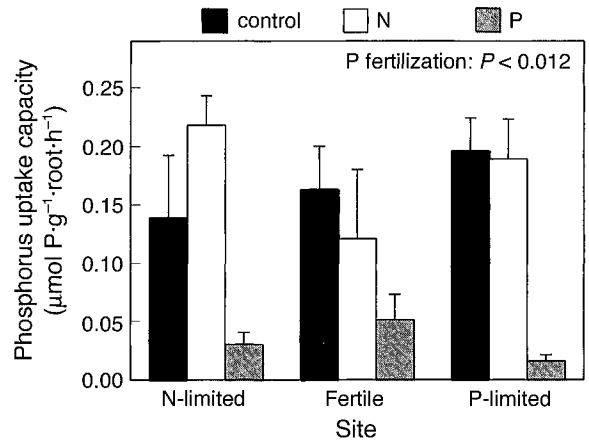


FIG. 5. Phosphorus uptake capacity of roots from fertilized plots. Error bars are ± 1 SE. Each bar represents an average of 3–4 plots per site.

limited sites, phosphorus fertilization significantly increased it in the P-limited site. This response suggests that plants in the P-limited site lack the necessary phosphorus reserves to maximize root growth (Ostertag 2001). We suggest that alternate methods of nutrient acquisition, such as arbuscular mycorrhizal fungi and extracellular phosphatases, may be more efficient in the P-limited site.

Nitrogen acquisition

Nitrogen uptake capacity responded to both N and P fertilization. Investment in carrier enzymes decreased with N fertilization in all but the P-limited, N-rich site (Fig. 6). Nitrogen previously allocated to N-carrier enzymes could have been invested instead in photosynthetic enzymes, reproductive biomass, or tissue growth. Finally, the observed increase of investment in N-carrier enzymes with P fertilization in the P-limited site

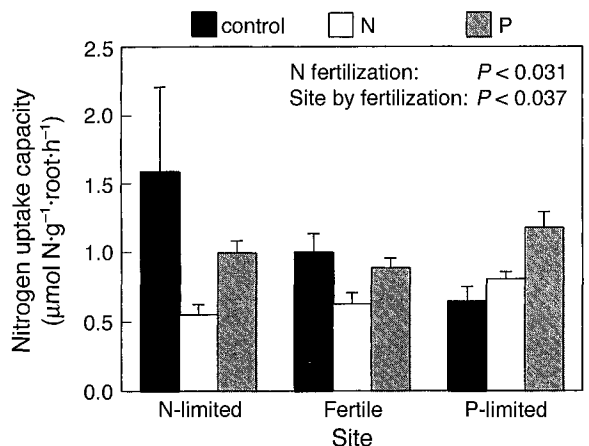


FIG. 6. Nitrogen uptake capacity of roots from fertilized plots. Error bars are ± 1 SE. Each bar represents an average of 3–4 plots per site.

(Fig. 6) may be attributable primarily to a higher growth demand for N.

Conclusion

As suggested by Bloom et al. (1985), plants appear to invest resources efficiently in nutrient acquisition. Extracellular phosphatase activity increases substantially with nitrogen fertilization, while investment in AM fungi decreases or has no response. Plants therefore seem to shift investment in P acquisition among different methods depending on the status of excess nutrients. Finally, our observation that N fertilization consistently increases extracellular phosphatase production in forests with a wide range in N availability has the implication that both N-limited and P-limited ecosystems could respond to inputs of anthropogenic N with increased productivity.

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