

Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests

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Received: 18 October 2009 / Accepted: 5 January 2010
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Abstract How plants respond to long-term nutrient enrichment can provide insights into physiological and evolutionary constraints in various ecosystems. The present study examined foliar concentrations after fertilization—to determine if nutrient accumulation responses of the most abundant species in a plant community reflect differences in N and P uptake and storage. Using a chronosequence in the Hawaiian Islands that differs in N and P availability, it was shown that after fertilization, plants increase foliar P to a much greater degree than foliar N, as indicated by response ratios. In addition, foliar P responses after fertilization were more variable and largely driving the observed changes in N:P values. Across species, both inorganic and organic P increased but neither form of N increased significantly. This pattern of P accumulation was consistent across 13 species of varying life forms and occurred at both the N-limited and P-limited site, although its magnitude was larger at the P-limited site. Foliar P accumulation after nutrient enrichment may indicate nutrient storage and may have evolved to be a general strategy to deal with uncertainties in P availability. Storage of P complicates interpretations of N:P values and the determination of nutrient limitation.

Keywords Leaf nutrients · Luxury consumption · N-to-P ratios · Nutrient cycling · Soil chronosequence · Stoichiometry

Introduction

Nutrients limit plant growth in many ecosystems, and many species have adaptations either to obtain more nutrients or to avoid losses (Chapin et al. 1990; Lambers et al. 2008), particularly for nitrogen and phosphorus. Nitrogen is abundant in amino acids, proteins, nucleic acids, ATP and importantly, RuBisCo, the enzyme that catalyzes photosynthesis. Phosphorus is the primary constituent in ATP, as well as an important element in nucleic acids, phospholipids and other cellular metabolites (Elser et al. 1996; Sinclair and Vadez 2002). Although low nitrogen (N) and phosphorus (P) soils are found worldwide, the ecological effects and consequences of limitation may vary by element due to fundamental differences in the soil mobility, availability, uptake, and cellular uses of N and P (Lambers et al. 2008). While past studies have often focused on plant adaptations under infertile site conditions versus fertile ones, there has been a recent emphasis on identifying the cause and nature of the infertility (Vitousek 1998), including new meta-analyses that have demonstrated that nitrogen (N) and phosphorus (P) limitation are widespread in terrestrial, freshwater, and marine ecosystems (Elser et al. 2007; LeBauer and Treseder 2008).

Responsible Editor: Michael Denis Cramer.

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Nutrient limitation of ecosystems is typically determined by fertilization experiments, with increased biomass or growth rates taken as evidence of limitation (Ulrich and Berry 1961; DiTommaso and Aarssen 1989; Tanner et al. 1998; Elser et al. 2007; LeBauer and Treseder 2008). A less direct index of nutrient limitation is foliar nutrient concentration, which is predicted to increase in response to addition of the limiting nutrient, although the positive relationship between biomass and foliar nutrients is not necessarily a linear one. This index is reasonable given that foliar nutrient concentrations (expressed either as N concentration, P concentration, or a ratio of N-to-P) reflect soil nutrient concentrations (Shaver and Melillo 1984; Valentine and Allen 1990; Vitousek 1998; Han et al. 2005; Townsend et al. 2007; Ordoñez et al. 2009). Foliar N and P concentrations also relate to the functioning of plants, as comparisons across biomes have shown that they are correlated with physiological traits such as photosynthesis and dark respiration, and leaf properties that affect resource capture such as specific leaf area and leaf lifespan (Wright et al. 2005). Finally, foliar nutrient concentrations may have bottom-up effects in communities, influencing herbivore abundance (Ritchie 2000).

The Hawaiian Islands have been used as a model to study nutrient limitation because the dominant tree species there exhibits examples of both N and P limitation. The mechanism of nutrient limitation across this chronosequence has been determined to be depletion-driven for P, in which weathering drives long-term losses (Vitousek et al. 2010), while N limitation is a function of low rates of fixation and deposition. A series of sites in Hawaii that vary in soil age and fertility but are similar in elevation (1200 m), mean annual precipitation (2500 mm), mean annual temperature (16°C), and species composition have demonstrated that geologically young Hawaiian wet forest sites have *Metrosideros polymorpha* trees that are N limited, while older, highly weathered sites contain trees of the same species that are P limited (Vitousek 1998). Having similar environmental conditions occurring under both nitrogen and phosphorus limitation is a situation unparalleled in continental gradients and is invaluable for testing hypotheses. Understanding how plants respond to long-term increases in nutrient availability can provide insights into species' physiological and evolutionary constraints in nutrient use.

The present study examines foliar concentrations after fertilization—and addresses whether plant responses to nutrient addition are related more to the background level of soil fertility or to the nutrient added. Although inorganic N and P are the two most limiting nutrients to plant growth, they differ profoundly in their bioavailability in the soil, with N relatively mobile, while P is relatively immobile and often very patchily distributed both spatially and temporally (Brady 1990). In plants, inorganic N (N_i) is generally not stored in cells but quickly incorporated into proteins or amino acids, which are the main N storage compounds in plants (Chapin et al. 1990). Organic N (N_o) is also the primary form of N transport within plants (Bloom et al. 1985). In contrast, inorganic P (P_i), stored in plant vacuoles, may be as much as 2/3rd of total plant P (Sinclair and Vadez 2002), and P_i is the form that regularly moves through xylem and phloem (Bloom et al. 1985). The differing patterns of soil availability, storage capacity, and cellular uses of inorganic N and P compounds suggest that the costs and consequences of nutrient uptake may vary considerably for N and P.

I hypothesized that foliar P accumulation after P fertilization would be greater than foliar N accumulation after N fertilization—regardless of site fertility or species—due to the ability of plants to store a greater percentage of P_i than N_i , relative to the total P or N amounts. The Hawaiian Islands provide both N-limited and P-limited soils dominated by the canopy same species, allowing for a test of the influence of soil fertility. Thirteen species were examined to determine if species are individualistic in their fertilization responses or if all species respond in a similar direction to a given nutrient addition. The question of whether fertilization leads to inorganic nutrient storage has been suggested in the literature for phosphorus (Attiwill and Adams 1993), but has only been examined in individual species, mainly agricultural crops. The community-level approach here allows for discussion of the significance of nutrient storage within natural ecosystems.

Methods

Site description and field sampling

This study used two sites along a soil age chronosequence: a young, N-limited site (300-y-old) and a

weathered P-limited site (4.1-my-old) (see Vitousek 2004 for full descriptions). The young site (19°25' W, 155°15' N) is located at 1176 m elevation; soils are Hydric Dystrandepths, consisting of coarse tephra deposits overlaying an older pahoehoe lava flow (Riley and Vitousek 1995). The old site (22°08' W, 159°37' N) is at 1134 m elevation and has a highly weathered substrate that is classified as a Plinthic Acrothox (Riley and Vitousek 1995). Previous determination of nutrient limitation was done in 15 × 15 m plots that have been fertilized with 100 kg/ha N and/or P twice annually since 1985 (N-limited site) or 1991 (P-limited site). Each site had fertilization addition treatments of N alone (+N), P alone (+P), or in combination (+NP), with four replicate plots per treatment. Nutrient limitation was declared when diameter growth and litterfall of the tree *Metrosideros polymorpha* increased with the treatment. It is worth noting that these designations of N or P limited are relative; they do not indicate the absolute abundance of soil nutrients but those data are available in Vitousek (2004).

Although biological demand for N is about an order of magnitude greater than for P (White and Hammond 2008), the large amount of P was added to compensate for the fact that: 1) in many tropical volcanic soils, a large proportion of P becomes fixed into biologically unavailable forms and only a small part of total P content in the soil is P_i (Sánchez 1976; Vitousek and Sanford 1986); 2) P fixation tends to increase in older soils that have higher amounts of aluminum oxides (Crews et al. 1995); and 3) soil colloids need to be saturated in order to adequately test responses to nutrient addition (Ingestad 1974). The effect of soil age on P availability is supported by P fractionation work that found that the occluded-P fraction was only 7% of total P at the N-limited site, but 48% at the P-limited site (Crews et al. 1995).

Young, fully expanded leaves were collected with a pruning pole, shotgun, or clippers in control and long-term +N, +P, +NP fertilization plots at both sites. A total of 13 species that were common enough to be found in replicate plots of each treatment were chosen. Species at the N-limited site were: ferns (*Cibotium glaucum*, *Sadleria cyatheoides*), shrub (*Vaccinium calycinum*), trees (*Coprosma* spp., *Ilex anomala*, *Metrosideros polymorpha*, *Myrsine lessertiana*) and a non-native ginger (*Hedychium gardnerianum*). Species at the P-limited site were: vine (*Alyxia oliviformis*),

fern (*Elaphoglossum* sp.), shrub (*Vaccinium calycinum*), trees (*Cheirodendron trigynum*, *Metrosideros polymorpha*, *Psychotria* sp., *Syzygium sandwicensis*) and ginger (*Hedychium gardnerianum*). In most cases, three individuals per species were sampled per plot. Leaf area was measured on a LI-COR 3000 (LI-COR Inc. Lincoln NE) and leaf material was dried at 70°C for at least 48 hours and specific leaf area (SLA, in cm²/g) was calculated. The three individuals per species were averaged and the four plots were considered replicates.

Chemical analyses

Leaf tissue was ground in a Wiley mill (40 mesh) and analyzed for total N and P concentration using Kjeldahl digestion followed by colorimetric assay on an Alpkem autoanalyzer (OI Analytical, Wilsonville, OR). Peach leaves (NIST SRM 1547) were used as an international standard. To evaluate fractions of P and N, at least two control and two fertilized samples per species from plots at both sites were randomly selected. ³¹P-NMR was used to determine P_i and P_o ; samples were analyzed on a Varian Inova 400 WB with 4 mm CMX Apex HX CPMAS probe (Chemagetics, Fort Collins CO). N_i was determined by extracting dried powder with 1 M KCl and the resulting solution was analyzed for N_i on a Pulse Autoanalyzer III with Autosampler IV (Saskatoon, SK, Canada). Total N was determined on a Costech ECS 4010 CN Elemental Analyzer (Valencia, CA) and N_o was calculated by subtraction.

³¹P-NMR was used to determine P_i and P_o at the Solid-state NMR facility at University of Hawaii at Manoa Chemistry Dept. Dry powder was inserted into a MAS rotor (4 mm O.D., Varian Inc., Palo Alto CA). The samples were recorded on a Varian Inova 400 WB operating at a H-1 frequency of 399.992 MHz, 161.92 MHz for ³¹P. Samples are recorded in a 4 mm CMX Apex HX CPMAS probe (Chemagetics, Fort Collins CO) and were typically between 30 to 40 mg dry mass. Samples were spun at 8 kHz rotation speed at the magic angle. Spectra were recorded using cross polarization (CP)^{S1} at a gamma*B1 field of 50 kHz resulting in a 5 microS 90 Deg (pi/2) for both ³¹P and ¹H. The initial ¹H pulse was followed by simultaneous spin locking fields on ³¹P and ¹H for 2 mS resulting in polarization transfer. After polarization

transfer the signals were recorded under the influence of high power ^1H decoupling using a gamma*B1 field of 75 kHz (Pines et al. 1973). The resulting spectra were analyzed as follows. An exponential weighting function of 80 Hz was applied to the raw data followed by Fourier transformation. The spectra were phase corrected and individual components from the different P-containing species in the sample were determined by deconvoluting the frequency domain spectrum. The MestReC Vr. 4.9.9.6 program was used for all data analysis (Mestrelab Research SL, Spain). Most samples had three distinct peaks, one inorganic and two organic, and the area under these peaks was used to determine the concentration of each P fraction. Standards run were pyrophosphate, sodium biphosphate, 5' ATP, 5' AMP, and Glucose-6-phosphate.

Statistical analyses

Following the meta-analysis of Elser et al. (2007), data are presented using a response ratio metric (\ln (fertilized response/control)). An advantage of this metric is that it controls for differences between nutrients and absolute nutrient concentrations. Differences in response ratios and N:P among fertilized treatments were determined for each site separately using one-way ANOVA or Welch ANOVA when variances were unequal ($\alpha=0.05$).

For the three species encountered at both sites, a two-tailed t-test was used to compare whether the response ratios of a nutrient differed between sites. In addition to analyzing means, I also examined the variation in foliar N and P to test if one nutrient may have more variation in its concentrations. Analysis of the coefficient of variation ((standard deviation/mean) * 100) for N and P concentrations among species in control plots was done with a Wilcoxon signed-rank test.

Differences between the concentrations of inorganic and organic forms of nutrients (\ln transformed) were analyzed with two-tailed t-tests; species were averaged and replicates were 29 (N-limited site) and 22 (P-limited site). Allocation to inorganic nutrient forms (calculated as percent inorganic relative to total concentration) were analyzed with two-tailed t-tests. All analyses were done with JMP 6.0.0 (SAS Institute 2005).

Results

Response ratios for N concentration were not significantly different among treatments at the P-limited site. At the N-limited site, P addition (+P) led to a significant decrease in N concentration. Foliar P concentrations at both sites were significantly greater after +P (or +NP) additions than after +N additions (Fig. 1). Thus, a dramatic pattern across species and sites is that foliar N concentrations were only altered slightly after N fertilization, while foliar P concentrations after P fertilization were often an order of magnitude larger. Strong foliar P and weak foliar N increases were also seen when nutrients were expressed on an area basis and when each species was analyzed separately, but fertilization did not increase individual species' leaf specific leaf area (Table 1).

For the species occurring at both sites, the non-native ginger *Hedychium gardnerianum* did not show differences in N or P response ratios between sites, but the native tree *Metrosideros polymorpha* ($t_6=16.9$, $P<0.0001$) and the native shrub *Vaccinium calycinum* ($t_5=3.7$, $P<0.014$) had greater P response ratios at the P-limited site than the N-limited site (Fig. 2). N response ratios were not significantly different except for *Metrosideros* (Fig. 2; $t_6=6.1$, $P<0.001$), where it appears that N fertilization may actually lead to a foliar dilution effect at the N-limited site. Thus, although the general patterns of N versus P responses are similar, site fertility does influence the magnitude of the P response ratio for the two native species (Fig. 1).

Not only were there differences in the magnitude of the N versus P response, but also in the variance of the responses. Overall, visual inspection showed that the P response ratio covered a larger range of values at both sites (Fig. 3a). Across species, the coefficient of variation was significantly greater for P than N under unfertilized conditions (Fig. 3b, c; $P<0.004$).

It has been suggested that N and P are in close stoichiometric balance in most ecosystems (Elser et al. 2007) and therefore not independent of each other (Shaver and Melillo 1984). The N-to-P ratios at the two sites follow remarkably similar patterns with fertilization (Fig. 4). In the unfertilized control plots, N:P across species averaged 13.0 ± 1.0 SE at the N-limited site, and 17.2 ± 1.1 SE at the P-limited site, following values predicted in the literature

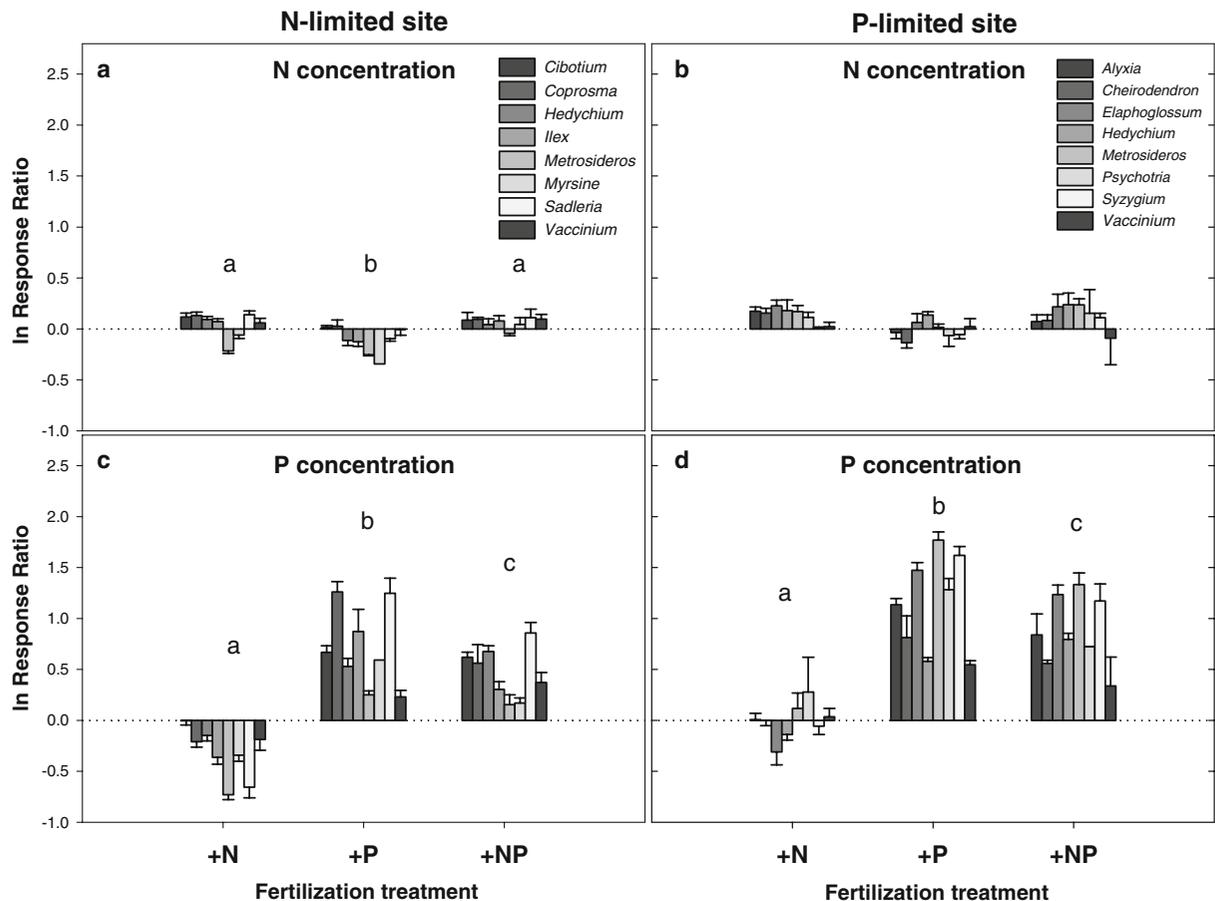


Fig. 1 Changes in foliar nutrient concentration (relative to the unfertilized controls) of Hawaiian forest plants after fertilization with N, P, or NP combined. Different lowercase letters represent significant treatment differences; statistics done combining all species within a site. Values are presented as ln response ratios (foliar nutrient concentration of treatment divided by control). **a** Fertilization treatment significantly changed the N concentration response ($F_{2,9}=8.2$, $P<0.010$; one-way ANOVA) but only due to a decrease under P-fertilization conditions; **b** No significant differences; **c** Fertil-

ization treatment significantly affected foliar P concentration response ($F_{2,9}=208.1$, $P<0.0001$; one-way ANOVA; **d** Fertilization treatment significantly affected foliar P concentration response ($F_{2,4,39}=949.5$, $P<0.0001$, Welch ANOVA). Means \pm SE of foliar ln N-response ratios across species in +N treatment: 0.04 ± 0.02 , $n=4$ (N-limited site) and 0.14 ± 0.04 , $n=4$, (P-limited site). Means of foliar ln P-response ratios across species in +P treatment: 0.72 ± 0.03 , $n=4$ (N-limited site) and 1.17 ± 0.22 , $n=4$ (P-limited site)

(Koerselman and Meuleman 1996). At both sites, the N-to-P ratio after fertilization with +NP together is more similar to the ratio after P fertilization alone, rather than after N fertilization alone—suggesting the larger influence of foliar P concentration increases in that ratio (Fig. 4). For example, the average decrease across species in the ratio after fertilization with both elements is 103% (N-limited site) and 142% (P-limited site), relative to N fertilization alone. Relative to P-fertilization alone, the average increase is 32% (N-limited site) and 41% (P-limited site). It is clear

from Fig. 1 that the ratios are being driven more by variation in foliar P than foliar N.

N fertilization at either site did not increase N_i or N_o concentrations or alter the allocation (percent inorganic) between the two forms (Fig. 5a–d; Table 2). In contrast, P fertilization led to increases in P_i and P_o at both sites (Fig. 5e–h; Table 3) but did not affect allocation. As hypothesized most of the N was in organic forms, while P was more variable; N_i concentrations of individual samples ranged from 0.1 to 6.5% of total foliar N, while P_i concentrations ranged

Table 1 Specific leaf area (SLA) measured in the same species as in Fig. 1. did not vary significantly among treatments; C control, N N fertilization, P P fertilization, NP N and P fertilization together

Site	Species	Treatment	N	SLA (cm ² /g) Average	SE
N-limited	<i>Cibotium glaucum</i>	C	4	114	8
N-limited	<i>Cibotium glaucum</i>	N	4	155	14
N-limited	<i>Cibotium glaucum</i>	P	4	104	8
N-limited	<i>Cibotium glaucum</i>	NP	4	118	14
N-limited	<i>Coprosma</i> spp.	C	4	101	23
N-limited	<i>Coprosma</i> spp.	N	4	113	22
N-limited	<i>Coprosma</i> spp.	P	4	101	12
N-limited	<i>Coprosma</i> spp.	NP	4	99	14
N-limited	<i>Hedychium gardnerianum</i>	C	4	191	12
N-limited	<i>Hedychium gardnerianum</i>	N	4	244	42
N-limited	<i>Hedychium gardnerianum</i>	P	4	165	15
N-limited	<i>Hedychium gardnerianum</i>	NP	4	188	11
N-limited	<i>Ilex anomala</i>	C	4	64	12
N-limited	<i>Ilex anomala</i>	N	4	65	6
N-limited	<i>Ilex anomala</i>	P	4	57	9
N-limited	<i>Ilex anomala</i>	NP	4	53	5
N-limited	<i>Metrosideros polymorpha</i>	C	4	49	13
N-limited	<i>Metrosideros polymorpha</i>	N	4	48	9
N-limited	<i>Metrosideros polymorpha</i>	P	4	39	1
N-limited	<i>Metrosideros polymorpha</i>	NP	4	36	2
N-limited	<i>Myrsine lessertiana</i>	C	2	211	85
N-limited	<i>Myrsine lessertiana</i>	N	3	150	91
N-limited	<i>Myrsine lessertiana</i>	P	1	177	–
N-limited	<i>Myrsine lessertiana</i>	NP	4	82	10
N-limited	<i>Saddleria cyatheoides</i>	C	4	188	68
N-limited	<i>Saddleria cyatheoides</i>	N	4	380	169
N-limited	<i>Saddleria cyatheoides</i>	P	4	83	19
N-limited	<i>Saddleria cyatheoides</i>	NP	4	126	3
N-limited	<i>Vaccinium calycinum</i>	C	4	273	26
N-limited	<i>Vaccinium calycinum</i>	N	4	277	59
N-limited	<i>Vaccinium calycinum</i>	P	4	191	26
N-limited	<i>Vaccinium calycinum</i>	NP	4	185	18
P-limited	<i>Alyxia oliviformis</i>	C	4	106	16
P-limited	<i>Alyxia oliviformis</i>	N	4	109	9
P-limited	<i>Alyxia oliviformis</i>	P	4	118	9
P-limited	<i>Alyxia oliviformis</i>	NP	4	118	22
P-limited	<i>Cheirodendron trigynum</i>	C	4	127	7
P-limited	<i>Cheirodendron trigynum</i>	N	4	122	6
P-limited	<i>Cheirodendron trigynum</i>	P	4	112	7
P-limited	<i>Cheirodendron trigynum</i>	NP	4	135	13
P-limited	<i>Elaphoglossum</i> sp.	C	4	108	7
P-limited	<i>Elaphoglossum</i> sp.	N	4	103	4
P-limited	<i>Elaphoglossum</i> sp.	P	4	125	9
P-limited	<i>Elaphoglossum</i> sp.	NP	4	129	19

Table 1 (continued)

Site	Species	Treatment	N	SLA (cm ² /g) Average	SE
P-limited	<i>Hedychium gardnerianum</i>	C	4	180	11
P-limited	<i>Hedychium gardnerianum</i>	N	4	158	8
P-limited	<i>Hedychium gardnerianum</i>	P	4	187	15
P-limited	<i>Hedychium gardnerianum</i>	NP	4	161	6
P-limited	<i>Metrosideros polymorpha</i>	C	4	58	2
P-limited	<i>Metrosideros polymorpha</i>	N	4	59	2
P-limited	<i>Metrosideros polymorpha</i>	P	4	76	15
P-limited	<i>Metrosideros polymorpha</i>	NP	4	63	2
P-limited	<i>Psychotria</i> sp.	C	4	89	9
P-limited	<i>Psychotria</i> sp.	N	4	117	35
P-limited	<i>Psychotria</i> sp.	P	2	142	53
P-limited	<i>Psychotria</i> sp.	NP	2	108	14
P-limited	<i>Syzygium sandwicensis</i>	C	2	66	6
P-limited	<i>Syzygium sandwicensis</i>	N	2	58	0
P-limited	<i>Syzygium sandwicensis</i>	P	4	73	7
P-limited	<i>Syzygium sandwicensis</i>	NP	4	61	2
P-limited	<i>Vaccinium calycinum</i>	C	4	296	40
P-limited	<i>Vaccinium calycinum</i>	N	4	293	24
P-limited	<i>Vaccinium calycinum</i>	P	2	237	1
P-limited	<i>Vaccinium calycinum</i>	NP	2	214	6

from 4.6 to 66.4%. When averaged across species and treatments, the percent of total foliar N as N_i was $0.50\% \pm 0.24$ SE, $n=29$ at the N-limited site and $0.16\% \pm 0.04$ SE, $n=22$ at the P-limited site, while the percent of total foliar P as P_i was $30.81\% \pm 3.21$ SE, $n=29$ at the N-limited site and $35.53\% \pm 3.40$ SE, $n=22$ at the P-limited site. Thus a much larger proportion of a species' total P than its total N, regardless of species or site fertility, is available in inorganic forms.

Discussion

Regardless of species or the soil fertility of the site, foliar P accumulation after P fertilization was relatively larger than foliar N accumulation after N fertilization. This larger response ratio for P than for N occurred across species of very different life forms as well as at the N-limited site where P has been shown to not increase tree growth (Vitousek 2004). This pattern has been reported in several independent studies (Vitousek 1998; Cordell et al. 2001; Harrington et al.

2001; Treseder and Vitousek 2001) and has also been noted for roots (Ostertag 2001). However, these studies reported on *Metrosideros polymorpha*, a species whose extremely broad ecological amplitude (Carlquist 1980) may not make it the most representative of the often specialized Hawaiian flora (Wagner et al. 1999). The present study clearly demonstrates that all species respond similarly in direction to P nutrient addition, but the pattern for responses after N addition was not uniform. The effect on nutrient addition on plant community structure was not investigated here because past research at these sites has shown that species diversity was not altered with fertilization, although the abundance of the non-native species increased (Ostertag and Verville 2002).

The foliar accumulation seen across species could represent substantial storage of P. When fertilized with P, species at the P-limited site had an average of 3.6 times more foliar P than unfertilized leaves (range 1.7–5.1), and even the N-limited site had an average of 2.2 times more foliar P (range 1.3–3.6) than unfertilized leaves. Given the low bioavailability of P in most soils (Lambers et al. 2008), the ability of

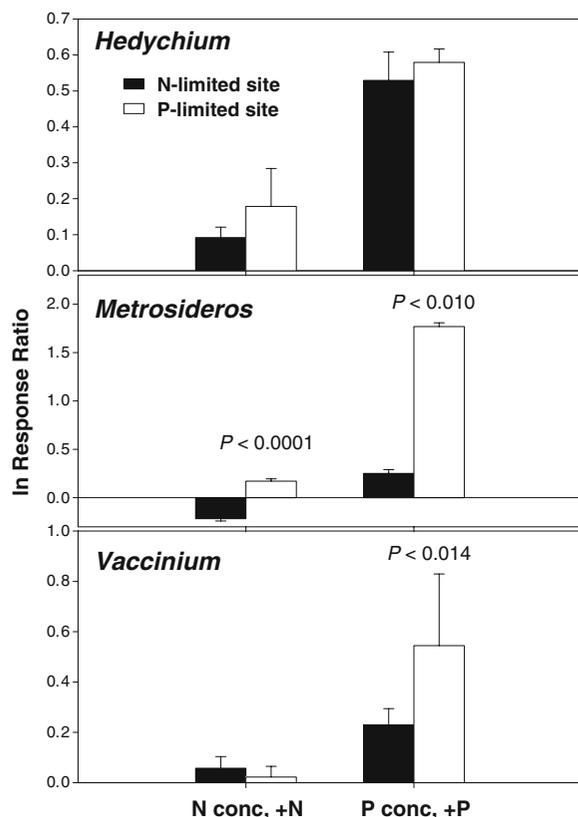


Fig. 2 Response ratios (treatment/control) on a ln scale for the three species common to both sites. The left column shows foliar N responses after fertilization with N and the right column shows foliar P responses after P fertilization. Values are means \pm 1 SE. Three of the t-tests were significant

plants to develop efficient mechanisms to take up and accumulate nutrients may be a strong selective pressure (Ingstad 1974; Mulligan and Sands 1988; Chapin et al. 1990). N may also be costly to store from an herbivory standpoint, given that many terrestrial herbivores have been shown to be N-limited (Huberty and Denno 2006). The fact that we see this foliar P response on both soil types suggests that a positive foliar accumulation response was triggered by the specific nutrient addition and not by necessarily by the background soil fertility, although the magnitude of the response appears to be related to soil fertility. The pattern of greater foliar P than foliar N accumulation after fertilization is not unique to Hawaiian tropical forests; it was also detected in many other ecosystems, including mangroves, wetlands, and pine forests (Table 4).

Due to the fact that more P fertilizer was added than N relative to biological demand, it is impossible

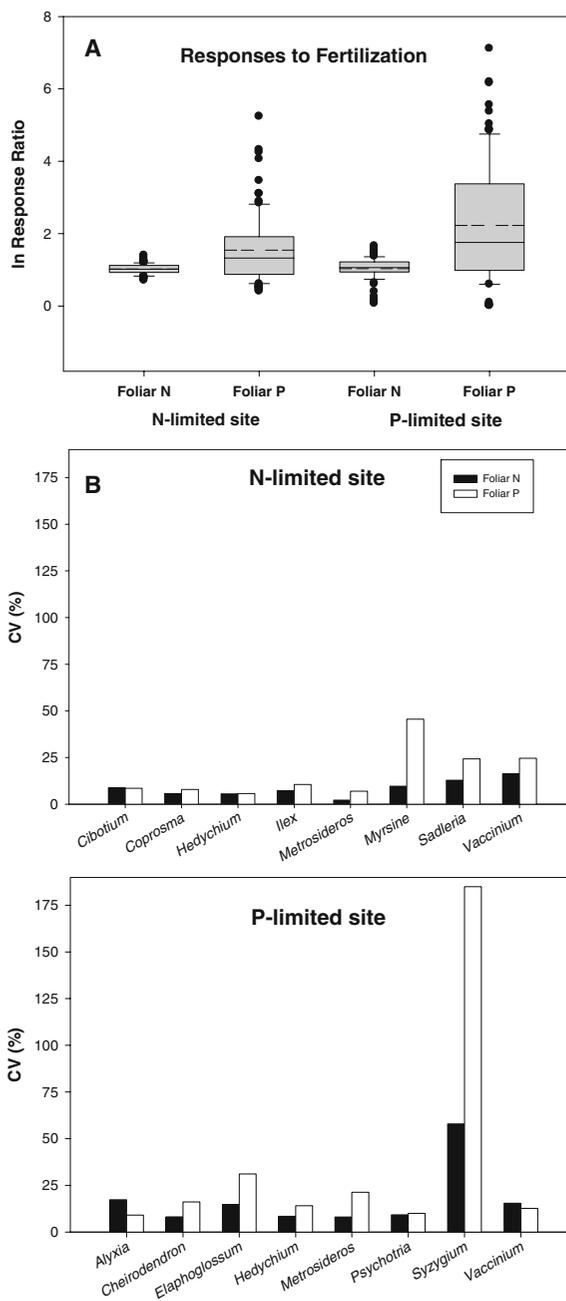


Fig. 3 a Box plots showing range of ln response ratio (treatment/control) for foliar N and P at both sites. Boxes represent 25–75th quartiles, error bars represent 10% and 90% of values, solid circles represent outliers, solid horizontal lines are medians and dashed lines are means. b Coefficient of variation in foliar N and P values in unfertilized control plots at the N-limited site and at the P-limited site

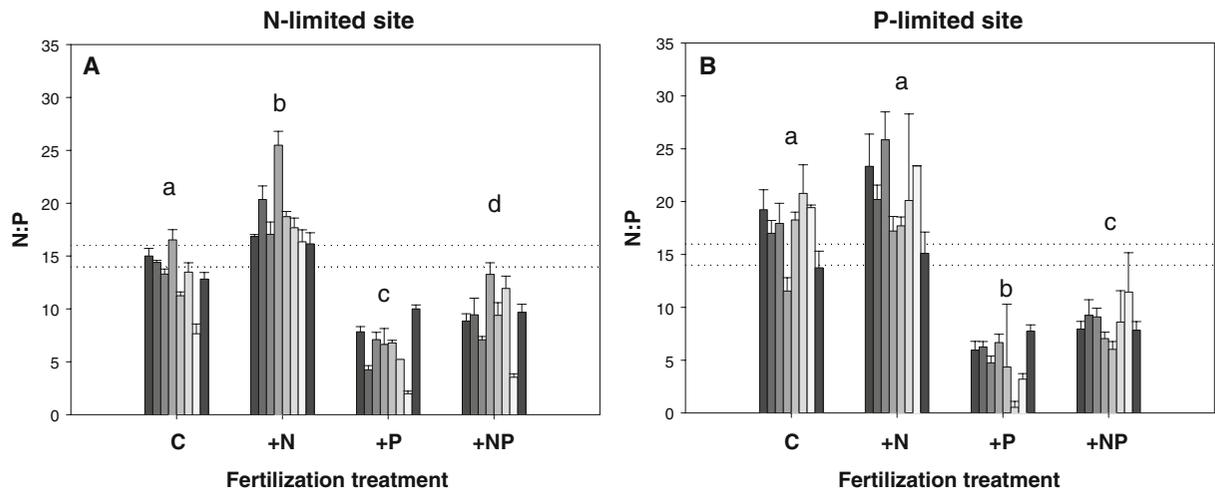


Fig. 4 Nitrogen-to-phosphorus ratios of Hawaiian forest plants in response to fertilization. Methods, species, and treatments as in Fig. 1 with C as unfertilized controls. Lines denote N limitation (<14) or P limitation (>16), as defined by (Koerselman and Meuleman 1996). **a** At the N-limited site, there is a significant difference among treatments ($F_{3,12}=349.9$, $P<0.0001$);

b At the P-limited site there is also a significant difference among treatments ($F_{3,12}=534.1$, $P<0.0001$). Means \pm SE across species for N fertilization: 18.8 ± 0.48 , $n=4$ (N-limited site) and 19.2 ± 0.86 , $n=4$ (P-limited site). Means \pm SE across species for P fertilization: 6.3 ± 0.33 , $n=4$ (N-limited site) and 6.0 ± 0.35 , $n=4$ (P-limited site)

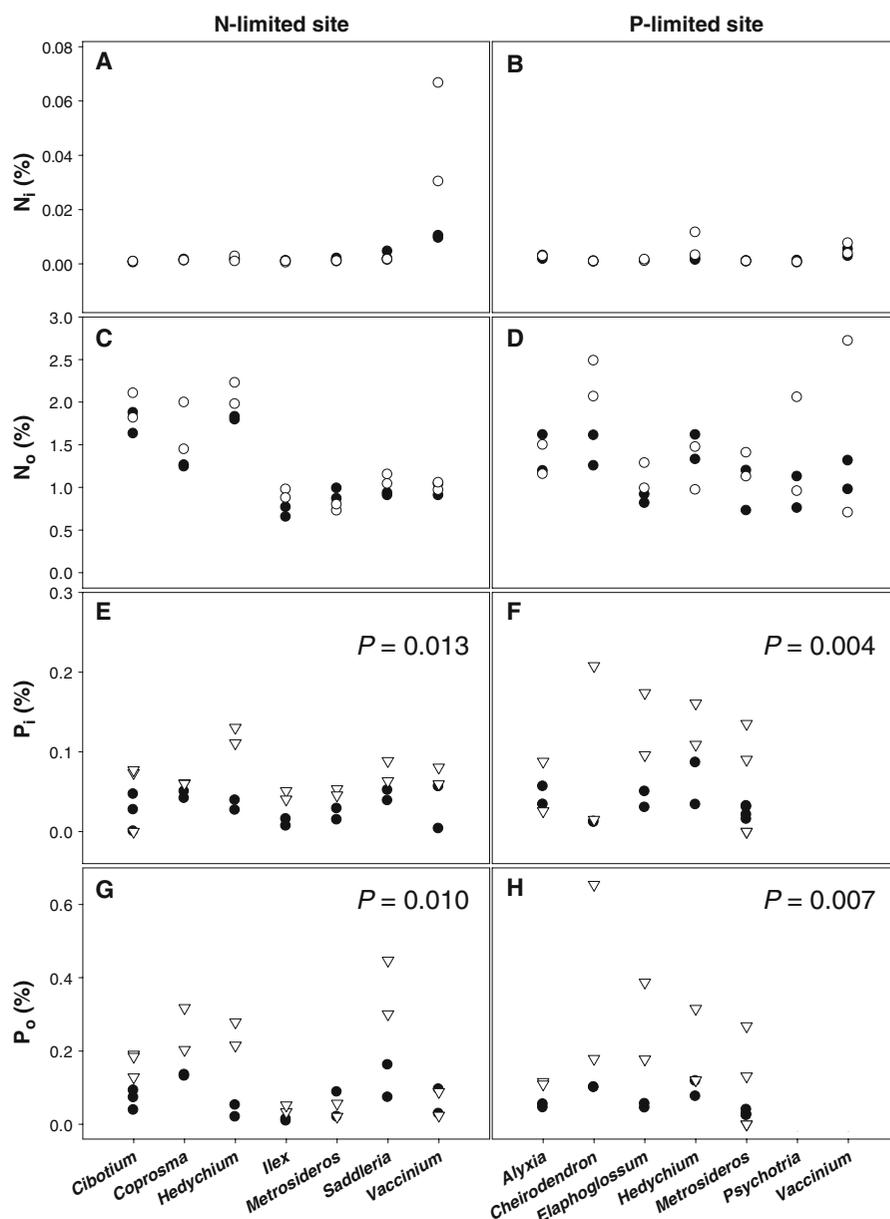
to determine conclusively whether this uptake represents an effort by the plant to store P. An alternative hypothesis to allocation to storage is that these species are unable to downregulate P uptake under high supply. Although much about P_i sensing and signaling is still unknown, a series of signaling regulators and hormones appear to be either stimulated or suppressed in response to P supply (Fang et al. 2009). Some species, particularly those adapted to P-poor environments may have an inability to downregulate P (Lambers and Shane 2007, Standish et al. 2007). For example, in greenhouse studies where P in solution was added in various concentrations, it was demonstrated that in both Proteaceae species that inhabit extremely P-poor Australian soils (Shane et al. 2004) and in creosote bush seedlings from southwestern US desert soils (Musick 1978), uptake of P in greenhouse-grown plants reached toxic levels. The high P concentrations given are not ones that these species are likely to ever experience in the wild. However, whether the mechanism is storage or lack of downregulation, the foliar accumulation of P seen across many species suggests the importance of acquiring P and hints at a potential evolutionary significance (Chapin et al. 1990; Lambers et al. 2008).

It could be argued that selection to take up P whenever available, even if not needed for growth at

the time, would lead to high variation among individuals in foliar P concentrations due to spatial and temporal variation of P in the soil environment. This greater variability in foliar P than foliar N was observed in two different ways: in response ratios (Fig. 3a) and in P concentrations under unfertilized conditions (Fig. 3b, c), suggesting a greater potential for luxury uptake. Exploring the prevalence of this variability in foliar N and P in other ecosystems is merited to determine if this is a general trend. On the one hand, there is evidence for relatively constant N:P values across taxa due to stoichiometric constraints and the need to maintain electrical neutrality in vacuoles (Broadley et al. 2004). However, in a literature survey of European wetlands, it was concluded that P concentrations were inherently more variable than N concentrations (Güsewell and Koerselman 2002). In a survey of mature tropical forests, N:P values among soil orders were mainly a function of soil P and not soil N (Townsend et al. 2007).

An additional result of P accumulation is that total P measurements or N:P ratios may not reflect immediate plant needs or the influence of background soil fertility levels. Storage of P complicates the interpretation of a N:P value. As an example, He et al. (2008) describe decoupling of N and P and hypothesize P storage as a mechanism to explain the low N:P ratios in Chinese desert grassland biomes.

Fig. 5 Inorganic and organic foliar N and P concentrations for species at both the low N and the low P sites. Each species had at least two samples from unfertilized control plots (filled circles) and two samples from fertilized plots (+N, open circles; +P, open triangles). *Psychotria* and *Vaccinium* were not analyzed for P forms at the P-limited site due to minimal leaf tissue. Statistics were done combining all species within a site. **a–d** N fertilization had no effect on N fractions; **e–h** P fertilization significantly increased both P fractions. Means in Tables 2 and 3



Another piece of evidence that suggests that storage can confound the interpretation of ratios is a modeling study that calculated N:P values separately for growth and storage organs using data from 365 measurements and 38 field experiments on crop species. The average N:P for growth tissues (mainly leaves) was 11.83, but it was 5.58 for storage-related tissues (Greenwood et al. 2008). If low N:P values are due to P storage, then some plants may be categorized as N-limited when they are perhaps have an adequate N supply.

While the limitation definitions of N:P values from 14–16 (mass based) shown of Fig. 1 do not apply in all systems (Drenovsky and Richards 2004, Soudzilovskaia et al. 2005), they have been widely applied in both aquatic and terrestrial systems (Güsewell 2004), and represent the concept of isolating the type of nutrient limitation. However, the hypothesis that foliar P may not correlate to present biochemical demand might help to explain physiological studies that have found that phosphate uptake was regulated not by the external supply but

Table 2 Mean concentrations and SE ($n=2$) of inorganic (NH_4^+ , NO_3^-) and organic N (N_o) fractions for species at both sites. When the species at a site were analyzed together, fertilization with N did not change the amounts of any fraction or allocation patterns

Site	Species	Control						N fertilizer					
		NH_4^+ ($\mu\text{g/g}$)		NO_3^- ($\mu\text{g/g}$)		N_o (%)		NH_4^+ ($\mu\text{g/g}$)		NO_3^- ($\mu\text{g/g}$)		N_o (%)	
		Average	SE	Average	SE	Average	SE	Average	SE	Average	SE	Average	SE
N-limited	<i>Cibotium</i>	0.83	0.22	3.17	0.48	1.75	0.12	1.91	0.02	4.80	0.22	1.96	0.15
N-limited	<i>Coprosma</i>	4.44	0.89	8.66	0.70	1.25	0.01	3.71	0.34	6.96	0.25	1.72	0.28
N-limited	<i>Hedychium</i>	8.39	0.38	6.48	0.89	1.81	0.02	5.53	1.87	11.17	7.36	2.10	0.12
N-limited	<i>Ilex</i>	4.11	0.09	5.04	0.48	0.71	0.06	2.41	1.06	2.60	1.06	0.92	0.05
N-limited	<i>Metrosideros</i>	10.83	2.47	4.79	0.58	0.92	0.06	4.13	0.65	3.47	0.42	0.76	0.04
N-limited	<i>Sadleria</i>	14.72	10.24	16.54	3.49	0.92	0.01	7.61	0.93	5.82	0.28	1.09	0.06
N-limited	<i>Vaccinium</i>	76.19	2.52	21.77	1.65	0.97	0.07	439.00	184.01	44.92	2.20	1.01	0.04
Site Average		17.07	10.01	9.49	2.64	1.19	0.16	66.33	62.12	11.39	5.69	1.36	0.52
P-limited	<i>Alyxia</i>	12.42	2.30	10.63	4.85	1.49	0.20	15.11	0.90	11.06	0.77	1.42	0.16
P-limited	<i>Cheirodendron</i>	4.55	0.76	3.00	0.40	1.52	0.17	4.11	0.66	2.60	0.06	2.31	0.20
P-limited	<i>Elaphoglossum</i>	6.06	1.25	4.40	0.72	0.98	0.05	6.37	2.00	5.06	0.82	1.24	0.14
P-limited	<i>Hedychium</i>	10.61	2.50	4.82	0.07	1.55	0.14	66.14	41.02	6.14	1.24	1.32	0.24
P-limited	<i>Metrosideros</i>	5.86	1.69	1.93	0.01	1.07	0.22	6.08	0.87	1.84	0.19	1.36	0.13
P-limited	<i>Psychotria</i>	4.91	1.53	3.59	0.61	1.05	0.17	1.52	0.39	2.17	0.81	1.59	0.52
P-limited	<i>Vaccinium</i>	30.46	10.75	9.04	2.19	1.25	0.16	36.33	15.16	19.10	4.32	1.78	0.95
Site Average		10.70	3.48	5.34	1.23	1.28	0.09	19.38	8.99	6.85	2.37	1.57	0.14

Table 3 Mean and SE of foliar inorganic and organic P fractions for species at both sites. When the species at a site were analyzed together, fertilization with P increased bothfractions but did not affect allocation patterns. For individual species, $n=2$ except where indicated by ^a($n=3$) or ^b($n=4$)

Site	Species	Control				P fertilized			
		P_i (%)		P_o (%)		P_i (%)		P_o (%)	
		Average	SE	Average	SE	Average	SE	Average	SE
N-limited	<i>Cibotium</i>	0.037	0.010	0.075	0.002	0.068	0.016 ^a	0.168	0.020 ^a
N-limited	<i>Coprosma</i>	0.046	0.004	0.060	0.0004	0.133	0.002	0.260	0.057
N-limited	<i>Hedychium</i>	0.033	0.006	0.121	0.010	0.035	0.016	0.247	0.032
N-limited	<i>Ilex</i>	0.011	0.004	0.046	0.005	0.011	0.003	0.043	0.009
N-limited	<i>Metrosideros</i>	0.021	0.007	0.050	0.004	0.054	0.034	0.039	0.018
N-limited	<i>Sadleria</i>	0.045	0.007	0.076	0.013	0.117	0.044	0.374	0.073
N-limited	<i>Vaccinium</i>	0.030	0.026	0.070	0.010	0.062	0.033	0.056	0.032
Site Average		0.032	0.005	0.071	0.009	0.068	0.016	0.170	0.049
P-limited	<i>Alyxia</i>	0.045	0.011	0.050	0.005	0.057	0.031	0.113	0.003
P-limited	<i>Cheirodendron</i>	0.012	0.001	0.100	0.001	0.111	0.096	0.417	0.238
P-limited	<i>Elaphoglossum</i>	0.040	0.010	0.050	0.006	0.135	0.039	0.282	0.105
P-limited	<i>Hedychium</i>	0.060	0.026	0.097	0.021	0.135	0.026	0.218	0.097
P-limited	<i>Metrosideros</i>	0.025	0.004 ^b	0.029	0.004 ^b	0.113	0.022	0.200	0.069
Site Average		0.036	0.008	0.065	0.014	0.110	0.014	0.246	0.051

Table 4 The effect of N and P fertilization on foliar N and P concentrations, respectively, across a variety of ecosystems and life forms, using the percent change in concentration relative to control as a common metric

Ecosystem/Location	Life form	% N change	% P change	Reference
Temperate estuary, US	seagrass	<10%	78–122	Murray et al. 1992
Arctic tundra, US	shrubs	14–25	118–201	Matthes-Sears et al. 1988
Alpine tundra, US	sedges/herbs	2–31	10–445	Bowman et al. 1993
Chalk grasslands, Holland	grasses	7–20	66–86	Bobbink 1991
Mangrove forests, Belize	trees	7–20	7–103	Feller et al. 2003
Loblolly pine, US	trees	–8–14	43–75	Sayer et al. 2004
European wetlands	angiosperms	20% avg	86% avg	Güsewell 2004

by some kind of internal control (Hommels et al. 1989; Veith and Komor 1993; Thomas et al. 2006), and plant level studies that show a poor relationship between biomass and foliar P concentrations for many species (McJannet et al. 1995; Xu et al. 1995).

Although the P_o increase was not expected based upon the agricultural literature (Sinclair and Vadez 2002), very few studies have documented forms of P in woody species growing in the wild (Chapin et al. 1986; Mulligan 1988; Hooda and Weston 1999). Because total P, rather than the forms of P, is usually measured, foliar P_i and P_o concentrations have received little attention. These species, regardless of site conditions, have much greater proportion of total P in inorganic form than total N in inorganic form. This inorganic pool may be highly advantageous given the difficulty in translocating P_o (Fang et al. 2009), and the ease of mobility of P_i in a plant's vascular system due to P_i transporters in cellular membranes (Bloom et al. 1985, Fang et al. 2009). P_i is also used in structural compounds to a larger extent than N (e.g., phospholipids, nucleic acids); more N_o is allocated to metabolism than to structural proteins and nucleic acids (de Groot et al. 2003). A consequence of an increase in foliar P_i may be faster rates of P cycling, due to lower resorption rates, and faster decomposition because P_i in leaf litter will not require mineralization and may be used more easily by microbes and plants (Hooda and Weston 1999; Turner et al. 2004).

In conclusion, using a model Hawaiian system that contains both N-limited and P-limited soils suggested that tropical plants from a variety of life forms and initial nutrient concentrations have foliar P values that are greater in magnitude and variability after P fertilization than are foliar N values after N fertilization. The foliar accumulation patterns reported here were more related to the nutrient added than to the

species or background site fertility. These Hawaiian forests provide a powerful test because of the similarity in climate and species between sites, but the patterns described also appear to be operating in a diversity of ecosystems. Based on these results a hypothesis to test further is that the differential soil availability and uses of N and P by plant cells may translate into different uptake strategies for each element. It is noteworthy that separate adaptations exist for maximizing N and P uptake (Wassen et al. 2005; Lambers et al. 2008) and thus it may be valuable to think about N- and P-limitation not just as infertile stressful conditions for plants, but as two fundamentally different phenomena. The ability to take advantage of increases in P availability by foliar accumulation of P may be an important bet-hedging strategy that could be convergent across many species.

Acknowledgements I thank D. Turner and S. Robinson (Stanford University) and R. Schneider (University of Hawaii at Hilo) for conducting elemental analyses, and W. Niemczura (University of Hawaii at Manoa) for NMR analyses and interpretation. H. Farrington, J. Schulten, and M. Yao assisted with field and sample preparation, and J. Schulten helped with data preparation. This work was supported by NSF CAREER 0546868 to R.O., but received facilitation through NSF EPSCoR 0237065 and 0554657 (J. Gaines PI, and D. Price and K. Kaneshiro co-PIs). I thank P. Vitousek, H. Lambers, P. Grierson, M. McGroddy, B. Turner, and G. Asner for useful discussions. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of the National Science Foundation.

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