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A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest

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Abstract. Experimental evidence for limitation of net primary productivity (NPP) by nitrogen (N) or phosphorus (P) in lowland tropical forests is rare, and the results from the few existing studies have been inconclusive. To directly test if N or P limit NPP in a lowland tropical wet forest in Costa Rica, we conducted a full factorial fertilization experiment (4 treatments \times 6 replicates in 30 \times 30 m plots). We focused on the influence of tree size and taxa on nutrient limitation, because in these forests a wide variety of tree functional traits related to nutrient acquisition and use are likely to regulate biogeochemical processes. After 2.7 years, a higher percentage of trees per plot increased basal area (BA) with P additions ($66.45\% \pm 3.28\%$ without P vs. $76.88\% \pm 3.28\%$ with P), but there were no other community-level responses to N or P additions on BA increase, litterfall productivity, or root growth. Phosphorus additions resulted in doubled stem growth rates in small trees (5–10 cm diameter at breast height (dbh); [$P \leq 0.01$]) but had no effect on intermediate (10–30 cm dbh) or large trees (>30 cm dbh). Phosphorus additions also increased the percentage of seedling survival from 59% to 78% ($P < 0.01$), as well as the percentage of seedlings that grew ($P = 0.03$), and increased leaf number ($P = 0.02$). Trees from *Pentaclethra macroloba*, the most abundant species, did not increase growth rates with fertilization ($P = 0.40$). In contrast, the most abundant palms (*Socratea exorrhiza*) had more than two times higher stem growth rates with P additions ($P = 0.01$). Our experiment reiterates that P availability is a significant driver of plant processes in these systems, but highlights the importance of considering different aspects of the plant community when making predictions concerning nutrient limitation. We postulate that in diverse, lowland tropical forests “heterogeneous nutrient limitation” occurs, not only driven by variability in nutrient responses among taxa, but also among size classes and potential functional groups. Heterogeneous responses to nutrient additions could lead to changes in forest structure or even diversity in the long term, affecting rates of NPP and thus carbon cycling.

Key words: basal area; Costa Rica; EARTH; fertilization; litterfall; net primary production (NPP); nitrogen; nutrient limitation; phosphorus; tropical forest.

INTRODUCTION

Nutrient availability controls key processes in all ecosystems on earth. Net primary productivity (NPP), nutrient use efficiency (NUE) by plants, and nutrient turnover through decomposition, are all processes affected by nutrient availability. Nitrogen (N) and phosphorus (P), either individually or in combination, limit primary productivity in most terrestrial ecosystems (Vitousek and Howarth 1991, Elser et al. 2007, Vitousek et al. 2010). In turn, plant adaptations to limitation by these nutrients feed back strongly to control ecosystem rates of nutrient cycling (Chapin 1980, Hobbie 1992, Vitousek 2004). Most lowland tropical forests occur on highly weathered soils, where much of the original P-rich

parent material has been lost, and most of the remaining P is occluded on iron and aluminum oxides (Sanchez 1976, Miller et al. 2001). Nitrogen, by contrast, accumulates over time through biological fixation, and is therefore expected to be relatively more available than P in old soils. Thus, it is generally believed that NPP is limited by P in lowland tropical forests (Walker and Syers 1976, Vitousek 1984, Vitousek and Farrington 1997).

Historically, our conceptual understanding of nutrient limitation was one derived from Liebig’s law of the minimum, where the single scarcest nutrient in relation to plant demand, usually N or P, was the most limiting (Liebig 1842). However, recent investigations showing synergistic interactions between limited supplies of N and P are widespread across aquatic and terrestrial systems (Elser et al. 2007, Harpole et al. 2011), and there is emerging evidence of limitation by other nutrients in lowland tropical forests (e.g., Kaspari et al. 2008, Wright et al. 2011). Consistent with these findings, nutrient limitation in tropical forests is probably not a

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question of N vs. P, but instead one that includes complex interactions among multiple nutrient cycles, and their linkages with biological processes (Townsend et al. 2011). For example, N inputs have been shown to accelerate phosphorus cycling rates through enhancement of soil and root phosphatase activity (Marklein and Houlton 2011), and tree species composition is known to influence rates of nutrient turnover through decomposition (Wieder et al. 2009).

The influence of biological processes on nutrient cycling is expected to be of particular importance in lowland tropical forests, where there is a high diversity of flora (Losos and Leigh 2004). Here, a wide variety of tree functional traits related to nutrient acquisition and use are likely to influence biogeochemical processes, creating complex linkages with nutrient cycles (Townsend et al. 2008). For example, large interspecific differences in foliar nutrient concentrations and resulting litter quality can influence plant–soil microbial feedbacks related to nutrient use (Vitousek 2004, Wood et al. 2011). In a lowland tropical forest in the south of Costa Rica, tree species variation in foliar P and carbon (C) chemistry were associated with tree-specific differences in both free-living N fixation and soil respiration (Reed et al. 2007, 2008, Wieder et al. 2008). In addition, differences among species in the functional use of resources (e.g., nitrogen fixers vs. nonfixers, shade-tolerant vs. light-demanding, species with superficial roots vs. species with deep roots) can enable them to mitigate nutrient limitation by accessing different nutrient pools (Vance et al. 2003). Therefore, to understand nutrient limitation in lowland tropical forests it is imperative to consider the influence of species-specific effects on nutrient cycling.

Another understudied aspect of nutrient limitation in tropical forests is the relationship between different demographic groups and nutrient cycling. In monodominant forests (e.g., Hawaii, summarized by Vitousek 2004), population growth rates (λ) will only be determined by the vital rates of a single tree species, and thus changes in nutrient availability will not have consequences for community composition and biodiversity. By contrast, in diverse tropical forests, alterations in vital rates of different species due to changes in nutrient availability can lead to shifts in community composition (Ceccon et al. 2004), which in turn affect plant–soil–microbial feedbacks. Additionally, there may be resource partitioning in space and time among age groups, and different age groups may be limited by different nutrients. For example, adult trees, which intercept the majority of light reaching the canopy and possess greater root area, may assimilate more nutrients than light-limited saplings and seedlings (Lambers et al. 1998). Seedlings in the understory may access nutrients added in fertilizer, but may be unable to incorporate them in their tissues due to light co-limitation (Burslem et al. 1995).

To our knowledge, only two fertilization experiments have been conducted in lowland tropical forests to directly test nutrient limitation of NPP. One found evidence for N and P co-limitation (Mirmanto et al. 1999), and one found evidence of limitation by N, P, and K (Wright et al. 2011). These results differ from the traditional view that lowland tropical forests are P limited. Moreover, the study by Wright et al. (2011) reported that in the lowland tropical forest in Panama where their study was conducted, different tree age groups and different fractions of NPP were limited by different nutrients. None of these studies explored the effects of tree species composition and identity on the observed responses to nutrient additions. The scarcity of fertilization experiments in lowland diverse tropical forests, the conflicting results obtained from these few existing studies, and the critical role that these forests have on the global C cycle motivated our research.

Our first objective was to test if N and P limited NPP. Because theory (Walker and Syers 1976) and indirect evidence from stoichiometry (Hedin 2004, McGroddy et al. 2004, Reich and Oleksyn 2004) and soil microbial dynamics (Cleveland and Townsend 2006) suggest that lowland tropical forests in highly weathered, clayey soils are P limited, we hypothesized that P fertilization would increase productivity by enhancing stem diameter growth and fine litterfall production.

Our second objective was to investigate the effect of fertilization on stem diameter increase for trees from different size classes. Consistent with Wright et al. 2011, we predicted that different age groups would respond differently to nutrient additions. Large trees (>30 cm diameter at breast height [dbh]) usually have slow growth rates (Lieberman and Lieberman 1994) or may utilize extra nutrients for reproduction instead of growth, and small trees (5–10 cm dbh) are usually light limited (Lambers et al. 1998). Thus, we predicted that intermediate-sized trees (10–30 cm dbh) would have the largest response to fertilization (mainly with P) because these trees have moderate access to light but must reach the canopy to obtain full sun. Small trees and seedlings in the understory may access nutrients added in fertilizer, but may be unable to incorporate them in their tissues as biomass due to light co-limitation (Givnish 1988, Burslem et al. 1995, Gilbert et al. 2006). Therefore, we predicted no difference in stem diameter increase for small trees and no difference in stem length or number of leaves in seedlings with increased nutrient availability.

Our third objective was to explore the effect of fertilization on stem diameter increase for trees from different taxa. Because fast-growing, light-demanding species acclimate faster to increases in light or nutrient regimes (Lambers et al. 1998), we predicted that fast-growing canopy species (e.g., *Pentaclethra macroloba* and *Goethalsia meiantha*) would demonstrate greater diameter increases after fertilization (especially with P) than slow-growing, shade-tolerant trees. In addition, we

TABLE 1. Means (and standard errors [SE]) for various soil parameters measured at three depths (0–10 cm, 10–30 cm, 30–50 cm) on each plot ($N = 24$) at the beginning of the experiment.

Soil parameters	0–10 cm		10–30 cm		30–50 cm	
	Mean	SE	Mean	SE	Mean	SE
Bulk density (mg/cm ³)	0.71	0.03	0.78	0.02	0.83	0.04
pH H ₂ O	4.04	0.04	4.20	0.03	4.32	0.02
Extractable P (μg/g)	2.14	0.36	1.40	0.25	1.02	0.25
Total P (μg/g)	1601.25	104.47				
Total C (%)	4.83	0.14	2.51	0.07	1.71	0.06
Total N (%)	0.49	0.01	0.28	0.01	0.18	0.01
Dissolved inorganic nitrogen (μg N/g)	19.67	1.47	10.86	1.23	6.89	0.56
Net N mineralization (μg N·g ⁻¹ ·d ⁻¹)	1.68	0.28	0.50	0.17	0.36	0.09
Net nitrification (μg N/g)	1.74	0.35	0.54	0.10	0.35	0.09

Notes: Methods used for soil analyses are described in Appendix B. The empty cells indicate that total P was not analyzed at those depths.

predicted that legumes (*Pentaclethra macroleoba* and *Inga*) would not respond to N additions, because of their ability to acquire N through N₂ fixation (McKey 1994).

METHODS

Site description

The study was conducted at the EARTH University Forest Reserve (Escuela de Agricultura de la Región del Trópico Húmedo), in Guácimo, Limón, Costa Rica (10°11' N and 84°40' W). This 900-ha private reserve is located at an altitude of 30 m, has a mean annual temperature of 25.1°C, and mean annual precipitation (MAP) of 3464 mm, distributed in a bimodal pattern with no months receiving <100 mm of precipitation (Alvarez-Clare 2012; Appendix A: Fig. A1). Parent material is of volcanic origin (Sancho et al. 1990), and soils are clayey, with ~50% clay, 20% silt, and 30% sand (Alvarez-Clare 2012). Overall, total N and P and macronutrient concentrations are relatively high for a lowland tropical forest (Table 1; Appendix A: Table A1). However, resin-extractable P (not shown but below 1 μg/g) and Melich-extractable P (2.14 ± 0.36 μg/g; mean ± SE) are low, suggesting that although total P pools are large, P bioavailability is low. Indicators of N bioavailability (Table 1) were high, consistent with other forests in Costa Rica (e.g., Robertson 1984, Cleveland et al. 2003, Baribault et al. 2012).

The experiment was conducted on mature forest, which has not been altered in the past 25 years, but where some selective, low-density logging is presumed to have occurred in the past. Within our study plots (total of 0.96 ha), which did not include swampy or riparian areas, we identified 104 tree species comprising 82 genera and 46 families (Appendix A: Table A2). Mean tree density was 542 ± 143 trees/ha and mean basal area was 34.43 ± 13.19 m²/ha. Although tree density and basal area varied among plots in our study site, there were no differences among blocks or among assigned treatments in these parameters when the experiment was established (Appendix A: Fig. A2). The leguminous canopy tree *Pentaclethra macroleoba* was the dominant species, contributing almost 30% of the total basal area within

our plots. The canopy or subcanopy palm *Socratea exorrhiza* had the highest stem density (~19% of stems >10 cm dbh). The forest at EARTH resembles that found at La Selva Biological Station in various aspects, including tree density (446 trees/ha; Lieberman and Lieberman 1994) and species composition (Hartshorn and Hammel 1994).

Experimental design

In May 2007, we established 24 30 × 30 m plots and randomly assigned them to three fertilizer treatments or a control in a complete block design ($n = 6$). Though fertilization treatments are ongoing, data described here were collected from August 2007 to March 2010. In addition to the six control plots, the three treatments included +P (47 kg P·ha⁻¹·yr⁻¹ as triple superphosphate), +N (100 kg N·ha⁻¹·yr⁻¹ applied as ammonium nitrate and urea), and +NP (N and P added together in quantities as in +N and +P plots). Fertilizer is broadcast by hand twice per year on the surface of the 900-m² plots. All measurements are restricted to the central 400 m² of each plot (20 × 20 m) to reduce edge effects, and all plots are at least 50 m apart to avoid between-plot contamination. Plot size, replication, and the amount of fertilizer added are consistent with similar studies conducted in montane (Tanner and Kapos 1992, Harrington et al. 2001), secondary (Davidson et al. 2004), and lowland seasonal (Wright et al. 2011) tropical forests. Each plot includes at least one *Pentaclethra* tree and one *Socratea* palm, two of the most common species in this forest. At the onset of the experiment, and one year, and then two years after fertilization, we collected soils and conducted various chemical analyses to evaluate the fate of the fertilizer. Methodological details for soil analyses can be found in Appendix B.

Tree growth measurements

In May 2007, all trees larger than 9 cm dbh in each 20 × 20 m subplot were identified to species or genus and labeled with a metal tag nailed to the bole. For larger trees with buttresses, or trees with irregular, difficult-to-

measure boles (~10 trees per plot), we installed dendrometer bands constructed with aluminum packing tape and springs as described by Keeland and Joy Young (2004). We removed lianas and loose bark at the point of attachment and installed dendrometers ≥ 10 cm above identification tags or higher when buttresses were present. Dendrometers were allowed to settle at least one month prior to initial marking. In addition in each plot, we labeled 10 trees of common species with a dbh of 4–9 cm with plastic tags attached to a plastic string. We measured diameter increase with either calipers (for trees with dendrometers) or with a dbh tape (for remaining trees) every six months until ~2.7 years after initial fertilization, for a total of six censuses.

We used two metrics to test the tree community response to fertilization at the plot level ($n = 24$). First, we calculated total basal area increase for a plot (in square meters per hectare per year) from the total increase in tree diameter for a plot during a time interval. This metric was calculated as the plot sum of all the differences in basal area [BA] between two consecutive measurements divided by the time interval, and was dependent on the number and size of the trees in a plot. This metric only considers trees that increased in diameter. Trees that died, broke, shrank or did not change in BA were excluded from the calculations. Second, we evaluated community response to fertilization at the plot level by calculating the percentage of trees that increased in BA relative to the total number of trees in a plot for a given time interval (the percentage of trees that grew per year per plot). In this case we considered all trees in a plot.

To evaluate the effect of fertilization on the tree community at a finer scale, we used individual trees as the experimental unit and relative growth rate (RGR) as the metric of tree growth. In this case we considered all trees for analysis, including those that did not grow, although excluding “non-growers” did not change statistical results. To evaluate the effect of fertilization on trees from different sizes, we classified trees into three categories based on their dbh: 5–10 cm, 10–30 cm, and >30 cm. To evaluate treatment effects on different taxa, we selected four common species that had replicates in multiple plots (*Dendropanax arboreus*, *Goethalsia meiantha*, *Pentaclethra macroleoba*, and *Socratea exorrhiza*) and two common genera (*Inga* and *Protium*), and that differed in several ecological characteristics (Appendix A: Table A3).

In addition, to evaluate the effect of fertilization on the seedling community, in July 2007 we randomly selected, marked, and photographed 20 seedlings or saplings from the most common species in each plot that had expanded leaves and were <1 m high. In August 2008 and 2009, we conducted censuses of the marked seedlings and recorded survival, growth (measured as increase in stem length), number of leaves, and level of herbivory.

Litterfall productivity

We collected litterfall every two weeks for three years from the onset of the experiment, from two traps installed in each plot. Traps measuring 50×50 cm were constructed of mesh and a polyvinyl chloride frame mounted on 1 m high metal rods. Samples from both traps were combined to generate one sample per plot, dried at 60°C , separated into foliar, reproductive, and woody subsamples, and weighed; the woody fraction included all sticks smaller than 2 mm in diameter. Not all litterfall collection intervals were regular, because at times inclement weather prevented collection or traps were stolen. We therefore calculated monthly litterfall productivities by extrapolating daily litterfall rates (grams of biomass per day) calculated for each collection period. For months where there were no collections, we used the average from the previous and following month for that year. The sum of all monthly productions 12 months following initial fertilization was labeled as “year one” (which corresponded to August 2007–July 2008). Litterfall production for “year two” refers to August 2008–July 2009.

Root biomass and growth

To estimate the effect of fertilization on root biomass, we collected root cores using a pounding corer measuring 4.75 cm diameter \times 15 cm height. Prior to treatments, followed by one year and two years post treatments, we collected root cores at four points per plot adjacent to where soil samples were collected, and combined them into one composite sample for each plot. Intact root cores were refrigerated at 4°C for up to three months and then separated into large (>2 mm diameter), small (<2 mm diameter), live, and dead roots. For samples collected in 2007 and 2008, we separated roots using variable-speed electric drills affixed on a wooden structure, where paint-stirring attachments extended down into 7.6-L (two-gallon) pails filled with water, as described by Espeleta and Clark (2007). For samples collected in 2009, we dissolved the samples in the same pails filled with water but stirred them manually. Each year, immediately after cleaning and separating the roots, we dried them at 60°C for at least one week and measured dry mass. To obtain an index of root growth, we randomly installed two cylindrical, closed-bottom root ingrowth cores (2-mm mesh, 10 cm deep and 7.7 cm diameter) per plot. To install the cores we collected a soil sample, removed all visible roots, placed the mesh ingrowth cores in the hole from which the core was removed, and then filled the cores with the root-free soil, following Cuevas and Medina (1988). Cores were carefully retrieved with a small shovel after two years, and roots analyzed as described previously.

Statistical analysis

To test the effect of fertilization on community-level parameters (total basal area increase, proportion of tree

growth, litterfall productivity, and root biomass), we used plot-averaged values ($n = 24$) in repeated-measures MANOVAs with measurements at different times as dependent variables and treatment and block as independent variables. We selected this approach over univariate repeated-measures ANOVA because in some cases the sphericity assumption was not met (Field 2009). In August 2008, a strong storm with severe winds destroyed plots 17 and 22, (both +N treatments). Therefore, to preserve a balanced design, in the MANOVA analyses we replaced the values for these plots with averages from other plots of the same treatment, at the same time point.

To test the effect of fertilization on trees from specific size classes or taxa, we used RGR from individual trees and conducted one-way ANOVAs with RGR (ln-transformed; originally measured in $\text{mm}\cdot\text{mm}^{-1}\cdot\text{yr}^{-1}$) as the dependent variable and treatment as the independent variable. We calculated RGR for each tree as the slope of a line from the log-transformed dbh at each census ($n = 6$). Because RGR is calculated as a slope, it incorporates the differences between measurements and provides an integrated index of growth throughout the time-span of the experiment. We used Dunnett's method to test if treatment means were different from the control.

It was difficult to detect treatment effects on the seedling community using plot means. For example, random events such as trampling by vertebrates, damage due to falling litter, or changes in light availability (S. Alvarez-Clare, unpublished data) influenced the magnitude of the measured response to fertilization. Therefore, we used logistic regressions to evaluate the effect of fertilization in the proportion of seedlings surviving, growing, or increasing the number of leaves in each plot one and two years after initial fertilization. For all models, N addition, P addition, and the interaction of the two were included as fixed effects. We included random effects of species and block to quantify variation and account for any lack of independence in observations. We used Wald Z tests to test for significant effects.

All analyses were conducted in JMP 8.0 (SAS Institute 2009) except logistic regressions, which were done in R version 2.15.0 using the function `glmer` in the package `lme4` version 0.999375-42.F (R Development Core Team 2012).

RESULTS

Effect of fertilization on soil parameters

After two years, there was a mean increase in Melich extractable P of surface soils (0–10 cm) of $59\% \pm 9.8\%$ (mean \pm SE) and $87\% \pm 25\%$ for the +P and +NP treatments, respectively. By contrast, there was a $9.3\% \pm 23\%$ and $20\% \pm 5.6\%$ decrease in Melich P in the control and +N treatments respectively (Appendix B: Fig. B2). Total P increased $7.2\% \pm 2.2\%$ and $8.6\% \pm 2.5\%$ in the +P and +NP treatments, respectively, but decreased by

$1.8\% \pm 1.0\%$ and $4.6\% \pm 3.4\%$ in the control and +N treatments, respectively (Appendix B: Fig. B2). The observed total P increase represents a recovery of 97% and 93% of the P added as fertilizer in the +P and +NP treatments, respectively. There was not an observable increase in N availability in the soil after fertilization (measured as total %N, dissolved inorganic N, or net mineralization), probably because soil samples were collected six months after the last fertilizer addition had been conducted (Appendix B).

Community-level response to fertilization

Repeated-measures MANOVAs revealed a significant time effect in all analyses (Fig. 1; Appendix C: Table C1), highlighting the temporal heterogeneity of the measured parameters. Within the two initial years of the experiment, there was a trend toward larger biomass of fine roots and a smaller litterfall productivity, with no evident treatment differences in mean basal area increase, foliar or reproductive litterfall productivity, or fine- or coarse-root biomass (Fig. 1; Appendix C: Table C1). It was not until 2.7 years after initial fertilization that there was a detectable effect of fertilization in the mean proportion of trees that grew per plot. This was significantly higher in the +P and +NP treatments than in the control ($P < 0.01$; Fig. 1B). However, at this time point, the proportion of tree growth in the +N treatment was not significantly different from that in the control ($P = 0.84$), and the proportion of tree growth in the +NP treatment did not differ from that in the +P treatment ($P = 0.88$), suggesting that the response was driven by P and not N additions. Consistently, there was a positive correlation between the percentage of change in extractable P in surface soils (0–10 cm) after two years and the proportion of trees that grew between 2 and 2.7 years after initial fertilization ($r^2 = 0.33$, $df = 20$, $P = 0.01$). On the contrary, we could not detect a relationship between community-level tree growth and any indicator of N availability (but see *Influence of tree size and taxa on response to fertilization*).

Root growth measured with ingrowth cores in the 0–10 cm soil profile averaged $0.36 \pm 0.04 \text{ Mg C}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ and did not differ among treatments or blocks ($F_{\text{tr.}} [F \text{ for treatments}] = 0.52$, $P = 0.67$, $F_{\text{block}} = 1.57$, $P = 0.67$, $df = 3, 5$). There was no relationship between this root growth index and fine- or large-root biomass before fertilization or after fertilization (Alvarez-Clare 2012).

Influence of tree size and taxa on response to fertilization

In addition to the community-level response to fertilization, we explored how N and P additions influenced growth of trees of different size classes and taxa. There were important differences in the responses of trees from different size classes and ontogenetic stages. Small trees (5–10 cm dbh) had highest RGR when N and P were added simultaneously (Fig. 2A). Furthermore, small trees in the +P and +NP treatment

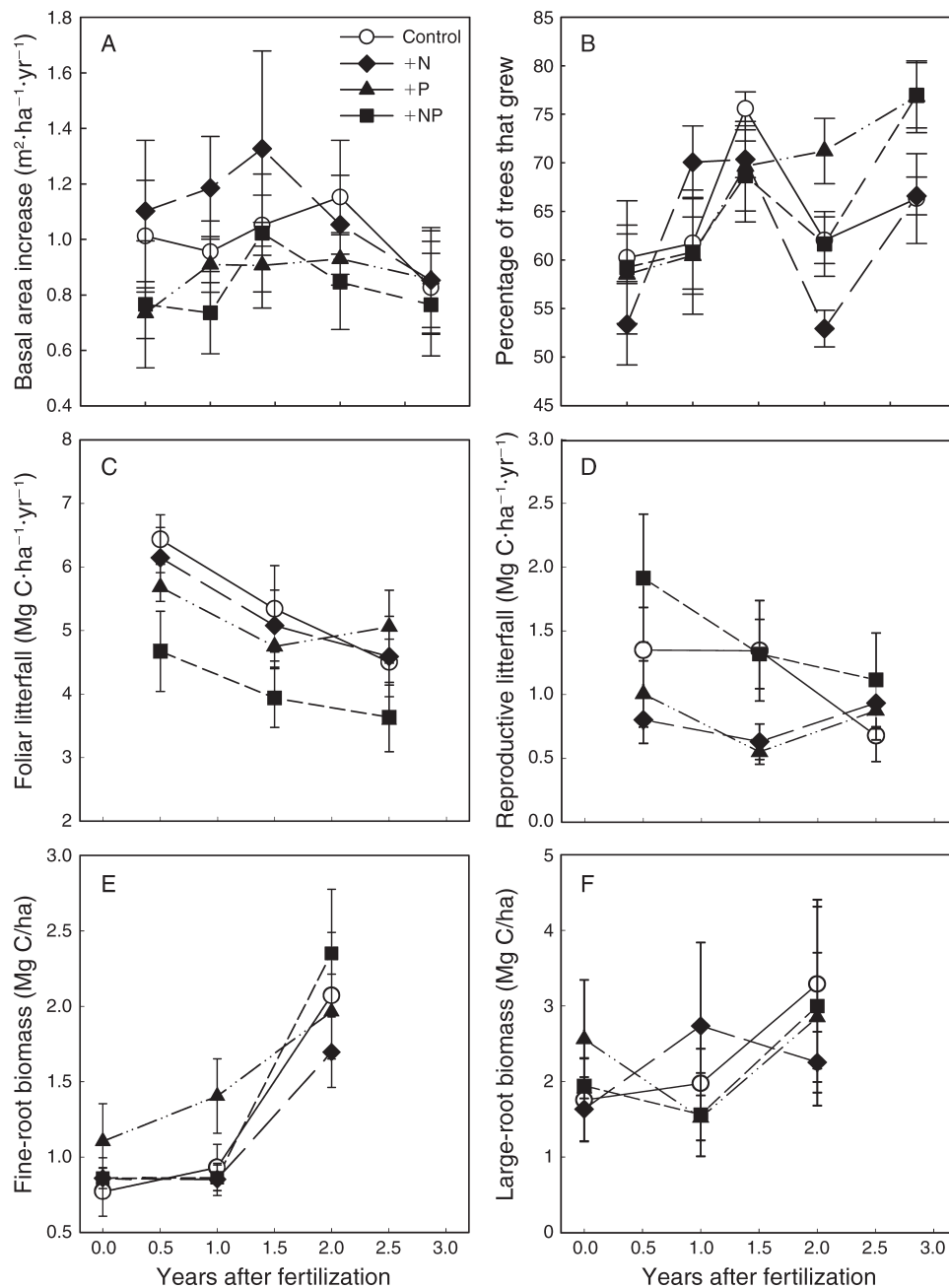


FIG. 1. Community-level responses (mean \pm SE) to fertilization: (A) basal area increase for trees larger than 5 cm dbh; (B) percentage of trees that grew, per plot, per year; (C) foliar litterfall productivity; (D) reproductive litterfall productivity; (E) fine-root biomass at 0–10 cm depth; and (F) large-root biomass at 0–10 cm depth.

were 2.16 and 2.68 times more likely to have positive RGRs than trees in the control plots (or in the +N plots), suggesting that the effect was primarily driven by P and not N additions. This is supported by higher mean RGRs of small trees between 2 and 2.7 years after initial fertilization in plots where there was a higher percentage change in extractable P in surface soils (0–10 cm; Appendix D: Fig. D1). In contrast, we could not detect an effect of fertilization on large trees (>30 cm dbh) at

this timescale, even though an average of $97\% \pm 1.6\%$ of trees in this size class showed some growth during the study.

Consistent with responses of small trees, seedlings showed significant treatment differences two years after initial fertilization (Fig. 3). Logistic regressions revealed that P additions increased seedling survival ($P < 0.01$) and the percentage of seedlings that exhibited some growth both in the +P and +NP treatments ($P = 0.04$).

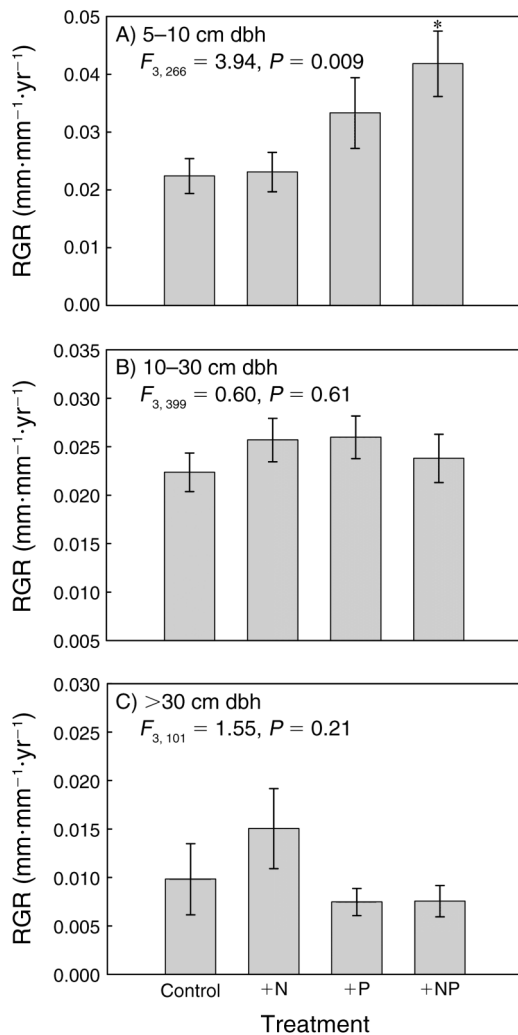


FIG. 2. Relative growth rates (RGR; mean \pm SE) of (A) small trees, (B) medium trees, and (C) large trees measured during 2.7 years after initial fertilization, in the four nutrient addition treatments. Also shown are results from one-way ANOVAs with treatment as the dependent variable. An asterisk indicates that for small trees, the +NP treatment is different ($P < 0.05$) from the control (Dunnett's test).

However, the percentage of seedlings that increased leaf number was significant in the +P ($P = 0.03$) but not +NP treatment ($P = 0.26$; Fig. 3C). The importance of P additions for seedling performance is highlighted by a positive correlation between percentage change in extractable P in surface soils (0–10 cm) after two years and the number of seedlings that grew between 2 and 2.5 years after fertilization (Appendix D: Fig. D1).

From the four species and two genera where we analyzed RGR individually, *Socratea*, a fast-growing canopy palm, showed higher RGR with both N and P fertilization (Fig. 4F). In addition, there was a positive correlation between the percentage change in extractable P in surface soils (0–10 cm) after two years and RGR of *Socratea* between 2 and 2.7 years after fertilization. In

contrast, RGR of *Socratea* in this period was negatively correlated with net nitrification rates (Appendix D: Fig. D1). *Protium* had higher RGR when N and P were added together, although this difference was not statistically significant (Fig. 4E). *Goethalsia meiantha* had the highest RGR in the control treatment (Fig. 4B), although this pattern was highly influenced by a single tree with high RGR in the control treatment and should therefore be interpreted with caution.

DISCUSSION

Nutrient limitation to NPP

This study illustrates, with experimental evidence, the complexity of nutrient limitation in lowland diverse tropical forests (Vitousek et al. 2010, Cleveland et al.

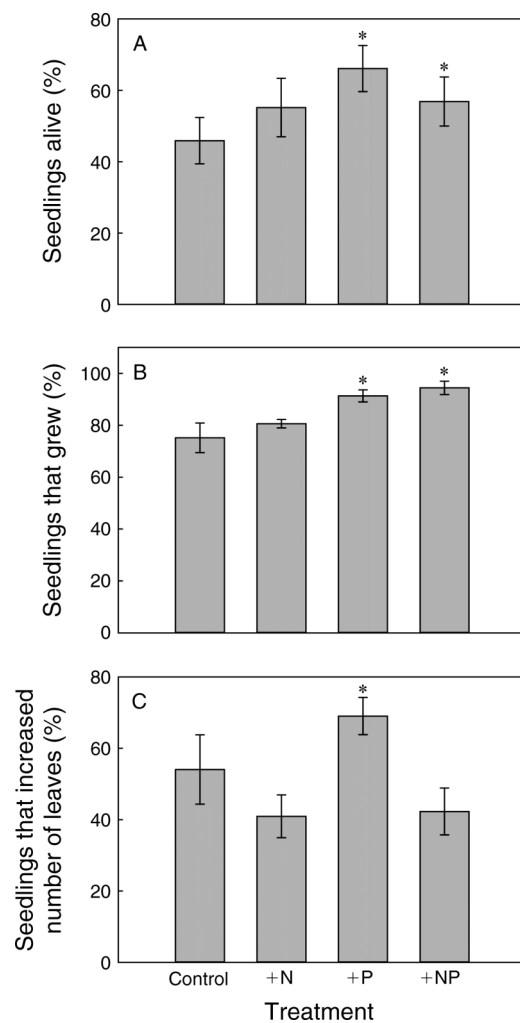


FIG. 3. Percentage (mean \pm SE) of seedlings (A) alive, (B) with increased stem length, and (C) with increased number of leaves, measured two years after initial fertilization, in the four nutrient addition treatments. An asterisk indicates that the treatment is different ($P < 0.05$) from the control (see Results: Influence of tree size and taxa on response to fertilization, for logistic regression results).

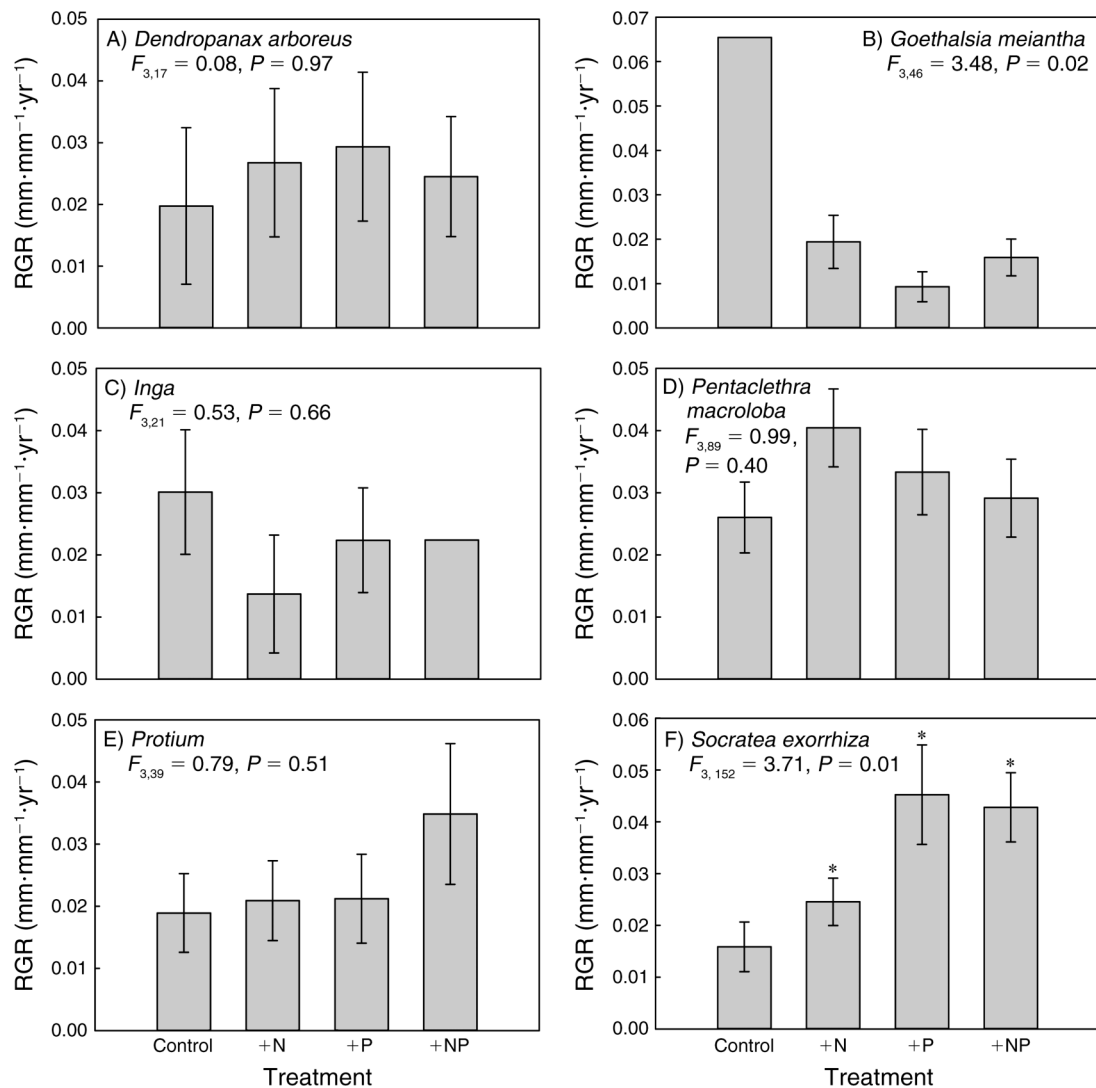


FIG. 4. Relative growth rates (RGR; mean \pm SE) for six common tree taxa, measured during 2.7 years after initial fertilization, in the four nutrient addition treatments. Also shown are results from one-way ANOVAs with treatment as dependent variable. An asterisk indicates that the mean for the treatment differs ($P < 0.05$) from the control (Dunnnett's test).

2011, Townsend et al. 2011), and complements data suggesting that these forests do not generally show a strong community-level response to either N or P fertilization in the short term (Mirmanto et al. 1999, Newbery et al. 2002, Wright et al. 2011). In our study, we could not detect treatment effects on tree BA increase, litterfall productivity, or root biomass or growth, two years after initial fertilization. There are various possible explanations for this lack of community-wide response to fertilization, some of which are evident only after exploring specific aspects of the tree community, such as differential responses among tree size classes and taxa.

One possibility for the lack of community response to fertilization is that the treatments were not effective. We observed, however, significant increases in soil available

and total P (Appendix B: Fig. B2), root P concentrations, litter N concentrations, and taxa-specific foliar N and P concentrations (S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*) with fertilization, demonstrating that the fertilizer was taken up by trees.

A second possibility is that a stronger NPP response at EARTH Forest was not observed because the plant community is co-limited by a nutrient other than N or P, potentially potassium or a combination of macronutrients (Herbert and Fownes 1995, Kaspari et al. 2008, Townsend et al. 2011, Wright et al. 2011, Baribault et al. 2012). A recent study conducted at a nearby and similar forest (La Selva Biological Station) demonstrated that availability of other macronutrients, such as K and Ca, were positively correlated with tree growth (Baribault et al. 2012). Therefore, we cannot rule out the potential

importance of other nutrients for NPP at EARTH Forest. However, treatment differences in size-specific and taxa-specific RGR, as well as in the proportion of trees and seedlings that grew in each treatment, highlight an undeniable importance of P as a driver of plant processes in this forest (Vitousek et al. 2010, Cleveland et al. 2011). This was substantiated by the positive relationship between significant parameters (small tree RGR, seedling growth, *Socratea* RGR) and the increase in extractable P, two years after initial fertilization. In addition, the negative relationship between each of these plant parameters and net nitrification (Appendix D: Fig. D1) suggests that N availability is also tightly coupled with plant growth (LeBauer and Treseder 2008), at least in some taxa and tree size classes.

A third possibility for the lack of community response to fertilization observed at EARTH Forest is the high spatial and temporal variability in individual tree growth (Clark and Clark 2011), litterfall (Wood et al. 2009), and root biomass (Espeleta and Clark 2007), which are characteristic of forests in this area and reduced statistical power to detect treatment differences. In the case of litterfall production, treatment differences have been observed in less than two years in other experiments (Herbert and Fownes 1995, Mirmanto et al. 1999, Wood et al. 2009). However, in a montane forest in Venezuela, there was an effect of both N and P additions on litterfall only after four years (Tanner and Kapos 1992). As recently highlighted by Clark and Clark (2011), temporal variability in NPP in lowland tropical forests makes long-term observations particularly important and necessary. In our experiment, it is possible that more time is required for the fertilizer additions to override the preexisting heterogeneity in soil nutrient availability. Nevertheless, a treatment effect on the proportion of trees that increased BA per plot could be a precursor of an effect on the actual magnitude of BA increase. We also observed a trend toward higher fine-root biomass in the NP treatment after two years, which may become stronger with continued fertilization.

The lack of strong and prompt community-level responses to fertilization observed in our study (and in an 11-year fertilization experiment in Panama [Wright et al. 2011]) make us question the effectiveness of conducting fertilization experiments in the tropics as a means to describe “community-wide” nutrient limitation in these diverse, heterogeneous, and extremely dynamic systems. Contrary to low-diversity island systems, where historic fertilization experiments were conducted (e.g., Vitousek 2004), continental forests present a wider array of tree functional traits related to nutrient acquisition and use, which are likely to influence biogeochemical processes, creating complex linkages with nutrient cycles (Townsend et al. 2008). For this reason, in the present study we complemented community-level parameters with observations conducted at a finer scale, such as individual tree size classes and taxa, including different

functional groups. These detailed observations provided, in the short term, evidence for P limitation by specific taxa and tree size classes. We also conducted foliar, litterfall, and root chemistry (N and P) analyses and explored how stoichiometric relationships in these tissues responded to nutrient additions. These measurements lead us to a slightly different set of conclusions, including evidence for both N and P limitation (S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*). In brief, this comprehensive approach was essential for discovering biological processes related to nutrient use that would have been dismissed if only community-level measurements of NPP had been conducted.

Nutrient limitation and tree size

Trees from different size classes showed contrasting and interesting responses to nutrient additions. For example, because of light co-limitation of small trees (Givnish 1988) and slow growth rates of large trees (Clark and Clark 1992), we had predicted that medium (10–30 cm dbh) trees would preferentially respond to nutrient additions. However, small trees were the only size class that differed among treatments, showing the highest RGRs with P additions. A combination of P limitation (*sensu* Chapin et al. 1986), overall higher growth rates of trees from this size class (Clark and Clark 1992), and relatively high understory light levels at EARTH forest (Alvarez-Clare 2012), likely caused the observed results. The lack of response of larger trees could be attributed to allocation of extra nutrients to other functions (e.g., photosynthetic capacity, reproduction, or branch growth). We found an increase in foliar nutrient concentrations with fertilization in “large trees” of several species (S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*).

We hypothesized that seedlings would not respond to nutrient additions because low light levels in the understory would prevent them from incorporating extra nutrients as biomass (Denslow et al. 1990, Burslem et al. 1995, Gilbert et al. 2006). However, although this was probably true in some cases, a higher proportion of P-fertilized seedlings survived, grew, and increased leaf number after two years than seedlings from other treatments. This suggests that (1) the canopy at EARTH Forest is relatively open, and enough light reaches the understory to allow seedling growth (Lawrence 2003, Gilbert et al. 2006, Alvarez-Clare 2012), and (2) the seedling community at EARTH may be limited by P (Lawrence 2001, Palow and Oberbauer 2009, Holste et al. 2011). Interestingly, for leaf number there seemed to be an effect on the +P but not the +NP treatment. Perhaps, +N cancels the +P effect by increasing herbivory if N is incorporated into tissues, increasing their palatability (Andersen et al. 2011, but see Campo and Dirzo 2003). However, we did not observe a significant difference in the percentage of seedlings exhibiting leaves with herbivory (data not shown). Further research is necessary to test this hypothesis.

Effect of taxa on nutrient limitation

From the studied taxa, only *Socratea* (the most abundant canopy palm) significantly responded to P additions, suggesting that this species is P limited (Baribault et al. 2012). However, consistent with multiple resource limitation theory (Chapin et al. 1987, Gleeson and Tilman 1992, Danger et al. 2008), individuals from this species showed the highest RGRs in several plots where there was a combination of P fertilization plus available light due to a tree or branch fall (S. Alvarez-Clare, *personal observation*). Moreover, because these palms usually reach a maximum stem diameter around 30 cm (Hartshorn and Hammel 1994), the response was mainly due to small individuals. Larger individuals responded by increasing foliar P instead of stem diameter with P additions (S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*). The species-specific and size-specific response of *Socratea* to fertilization illustrates the importance of considering “within-community” processes when interpreting nutrient limitation in this type of system.

We had predicted that *Pentaclethra maculosa*, the most abundant tree species in this forest and a fast-growing legume, would increase growth with P but not N fertilization. Interestingly, however, we could not detect a growth response to either N or P additions after 2.7 years. On the contrary, *Pentaclethra* trees increased foliar N concentrations in the +NP treatment (S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*). The lack of growth response of *Pentaclethra* is interesting because as the most important species in this forest (up to 30% of the basal area and 14% stems [Alvarez-Clare 2012]; Appendix A: Fig. A2) it has a strong influence over the community-averaged response to fertilization. Further investigation is needed to decipher if this species is allocating the P added in fertilizer to other functions, such as branch growth, or fruit production.

Our experimental data validate recent observations in a natural fertility gradient at La Selva Biological Station showing a strong correlation of palm growth (including *Socratea*) with P and base cations (but not N), but no relationships between legume growth (dominated by *Pentaclethra*) and N, P, or base cation availability (Baribault et al. 2012). Yet further fertilization experiments that include a treatment with base cations are needed to corroborate the role that these have on nutrient limitation of lowland diverse tropical forests (Wright et al. 2011, Baribault et al. 2012).

Conclusions

This study adds to the body of experimental evidence showing that lowland tropical forests are not exclusively limited by P (Mirmanto et al. 1999, Newbery et al. 2002, Wright et al. 2011), or at least do not respond to either N or P fertilization in the short term, as is the case in montane forests (Tanner et al. 1998, Vitousek 2004). Although the plant community as a whole at EARTH

Forest did not respond to N or P additions in the short term (<3 years), our data suggest that seedlings, small trees (5–10 cm dbh) and a palm species (*Socratea*) were limited by P. This substantiates the traditional view that P availability is an important driver of plant processes in lowland tropical forests (e.g., Walker and Syers 1976, Reich and Oleksyn 2004, Vitousek 2004). On the other hand, the contrasting responses of *Socratea* and *Pentaclethra* (the dominant species at EARTH Forest) illustrate how plant communities are not black boxes of trees reacting in concert to nutrient availability. On the contrary, “community nutrient limitation” (Vitousek et al. 2010, Harpole et al. 2011) is a heterogeneous phenomenon, resulting from the competing and conflicting responses of different biological and biochemical processes.

Recent attention has been given to the complex nature of “community nutrient limitation” (Townsend et al. 2008, Vitousek et al. 2010, Cleveland et al. 2011, Harpole et al. 2011) and mechanisms for multiple nutrient co-limitation have been proposed as an alternative to the historical Liebig’s Law of the Minimum (e.g., Chapin et al. 1987, Gleeson and Tilman 1992, Danger et al. 2008). Based on experimental data from this study, we expand this idea and propose that in diverse lowland tropical forests “heterogeneous nutrient limitation” occurs, not only driven by variability in nutrient responses among taxa but also among size classes. This heterogeneous response to nutrient additions could lead to changes in forest structure, or even diversity in the long term, and could have important implications for plant–soil–microbial feedbacks concerning nutrient limitation (Vitousek 2004; S. Alvarez-Clare and M. C. Mack, *unpublished manuscript*). Finally, this heterogeneity highlights the importance of considering different aspects of the plant community, such as forest structure and species composition, when making predictions concerning nutrient limitation in these forests.

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SUPPLEMENTAL MATERIAL

Appendix A

Supplementary site description data, including climate, soil, and vegetation characteristics ([Ecological Archives E094-139-A1](#)).

Appendix B

Methods and results for soil analyses conducted at the onset of the experiment, one year, and two years after fertilization ([Ecological Archives E094-139-A2](#)).

Appendix C

Results from repeated-measures MANOVA analyses for tree community responses to fertilization ([Ecological Archives E094-139-A3](#)).

Appendix D

Correlations between the two most significant soil parameters vs. within-community indicators of plant growth ([Ecological Archives E094-139-A4](#)).