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Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest

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Abstract. Questions remain as to which soil nutrients limit primary production in tropical forests. Phosphorus (P) has long been considered the primary limiting element in lowland forests, but recent evidence demonstrates substantial heterogeneity in response to nutrient addition, highlighting a need to understand and diagnose nutrient limitation across diverse forests. Fine-root characteristics including their abundance, functional traits, and mycorrhizal symbionts can be highly responsive to changes in soil nutrients and may help to diagnose nutrient limitation. Here, we document the response of fine roots to long-term nitrogen (N), P, and potassium (K) fertilization in a lowland forest in Panama. Because this experiment has demonstrated that N and K together limit tree growth and P limits fine litter production, we hypothesized that fine roots would also respond to nutrient addition. Specifically we hypothesized that N, P, and K addition would reduce the biomass, diameter, tissue density, and mycorrhizal colonization of fine roots, and increase nutrient concentration in root tissue. Most morphological root traits responded to the single addition of K and the paired addition of N and P, with the greatest response to all three nutrients combined. The addition of N, P, and K together reduced fine-root biomass, length, and tissue density, and increased specific root length, whereas root diameter remained unchanged. Nitrogen addition did not alter root N concentration, but P and K addition increased root P and K concentration, respectively. Mycorrhizal colonization of fine roots declined with N, increased with P, and was unresponsive to K addition. Although plant species composition remains unchanged after 14 years of fertilization, fine-root characteristics responded to N, P, and K addition, providing some of the strongest stand-level responses in this experiment. Multiple soil nutrients regulate fine-root abundance, morphological and chemical traits, and their association with mycorrhizal fungi in a species-rich lowland tropical forest.

Key words: Barro Colorado Nature Monument, Panama; fine roots; mycorrhizal fungi; nitrogen; phosphorus; potassium; root functional traits; specific root length; tissue density; tropical forest.

Introduction

Tropical forests account for a significant portion of global net primary productivity and contribute to the regulation of the global climate system (Field et al. 1998). How soil nutrients limit productivity across the tropical forest biome is poorly understood, creating uncertainty in projections of tropical forest response to CO_2 fertilization and changes in global climate (Gerber et al. 2010, Wang and Houlton 2010, Goll et al. 2012). Phosphorus (P) has long been considered the primary limiting element in lowland tropical forests because of leaching losses in highly weathered soils (Walker and Syers 1976, Vitousek and Sanford 1986, Vitousek et al. 2010). However, recent evidence indicates that substantial heterogeneity exists both among and within tropical forests in the way in which soil nutrients regulate primary productivity and other ecosystem processes. For example, nitrogen (N), P, potassium (K), calcium (Ca), and trace metals either singly or in combination constrain primary production, N_2 fixation, and decomposition in different forests (Mirmanto et al. 1999, Kaspari et al. 2008, Barron et al. 2009, Wright et al. 2011, Baribault et al. 2012, Wurzburger et al. 2012, Alvarez-Clare et al. 2013). The discrepancy between the long-standing focus on P limitation and the complex responses of recent studies raises new questions about how nutrient limitation arises and how it can be diagnosed among diverse tropical forests.

The means by which plants acquire soil nutrients are fundamental to the concept of nutrient limitation. Fine-root form and composition are evolved, adaptive traits that allow plants to acquire resources (e.g., water and nutrients) that limit their growth (Aerts and Chapin 2000). Root functional traits include a suite of morphological and chemical characteristics whose expression represents fundamental trade-offs between maximizing resource acquisition and minimizing costs associated with root tissue construction and maintenance. Thus, the concept of a root economic spectrum, similar to that

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documented for leaves (Westoby and Wright 2006), is gaining recognition, in which species associated with rapid resource acquisition tend to have fine roots with higher specific root length (SRL; cm/g), lower tissue density (g/cm^3), smaller diameters, higher N concentrations, and shorter life spans relative to species with a more conservative growth strategy (Eissenstat et al. 2000, Comas and Eissenstat 2004, McCormack et al. 2012). Indeed, along natural gradients of pedogenesis, community-level root functional traits assemble in predictable ways, such that nutrient-poor soils tend to be associated with plant species with resource-conservative root traits and vice versa (Holdaway et al. 2011).

A critical question remains as to whether fine roots can serve as diagnostic indicators of ecosystem nutrient status, such that root abundance and root functional traits respond in predictable ways to experimental nutrient addition. Fine-root biomass is the most commonly studied root response in the context of ecosystem fertilization experiments, and a reduction in fine-root biomass is typically interpreted as evidence for alleviation of nutrient limitation (reviewed in Ostertag 2001). However, fine-root length per unit soil volume more accurately depicts nutrient acquisition potential at the ecosystem scale (Aerts and Chapin 2000), because biomass can manifest as varying amounts of root length, depending on root diameter and root tissue density. Experimental manipulations of nutrient or water availability can induce intraspecific variability in root functional traits (i.e., SRL, tissue density, root diameter, and nutrient content) among woody plants (Eissenstat et al. 2000, Hendricks et al. 2000, Ostonen et al. 2007, Freschet et al. 2013); however, the nature and magnitude of these responses vary both among species and by functional trait (Einsmann et al. 1999, Freschet et al. 2013, Tobner et al. 2013).

Plant allocation to root symbionts can also serve as an indicator of ecosystem nutrient status. Arbuscular mycorrhizal (AM) fungi are a common symbiont among land plants and facilitate nutrient acquisition and assimilation in exchange for carbon (C) resources from the plant. The abundance of mycorrhizal fungi in fine-root systems varies widely, and tends to decline with increased nutrient availability (Smith and Read 2008). In the context of root functional traits, AM fungi serve as extensions of the plant root system, and therefore, add an additional layer of complexity to the expression of root traits in response to soil resources (Muthukumar et al. 2003, Heinemeyer and Fitter 2004). Therefore, quantifying root biomass responses to experimental fertilization and concomitant responses in the expression of functional traits and the abundance of root symbionts may improve our understanding of ecosystem nutrient limitation.

In a lowland tropical forest in Panama, we documented fine-root characteristics, including root abundance, root functional traits, and mycorrhizal abundance after 14 years of stand-level fertilization. This long-term experiment has demonstrated that additions of N and K together stimulate stem growth, and additions of P stimulate fine litter production (Wright et al. 2011). Because the addition of macronutrients has altered patterns in growth above ground, we anticipated that all three nutrients would trigger a response below ground. Indeed, our previous measures of standing fine-root biomass have shown that K addition has led to a decline of fine-root biomass (alone or in combination with N; Wright et al. 2011), increases in root turnover rates (Yavitt et al. 2011), and declines in seedling root : shoot ratios (Santiago et al. 2012).

We anticipated that long-term fertilization with N, P, and K would shift allocation away from fine-root biomass and AM fungi and change the expression of fine-root traits. Specifically, we hypothesized that nutrient addition would lead to reductions in fine-root biomass, diameter, tissue density, and the abundance of AM fungal structures. We also predicted that the N, P, and K concentration of root tissue would increase with the addition of each respective nutrient, indicating the limitation of forest growth by all three elements. We also evaluated responses of fine-root length and SRL, but made no a priori predictions due to mathematical relationships among SRL, root biomass, root length, and root diameter (see *Discussion*), and the potential for AM hyphae to augment root length.

Methods

Study site

The 38.4-ha study plot (9°06′31″ N, 79°50′37″ W) supports a highly diverse (~300 tree species) mature (>200 years old) forest and is located on the Gigante peninsula in the Barro Colorado Nature Monument in the Republic of Panama. The temperature averages 26°C and annual precipitation averages 2600 mm (Leigh 1999), with a distinct dry season between January and April. The soils are derived from a basaltic parent material and have been characterized as Endogleyic Cambisols and Acric Nitisols (Koehler et al. 2009).

We replicated the eight treatments of a $2 \times 2 \times 2$ factorial NPK experiment four times. We placed the four replicates perpendicular to a 36-m topographic gradient because soil properties (Yavitt et al. 2009) and tree distributions (S. J. Wright, *unpublished data*) parallel the gradient. Within each replicate, we blocked the N, P, K, and NPK treatments vs. the NP, NK, PK, and control treatments (see Wright et al. (2011) Appendix A). This balanced, incomplete-block design minimizes uncontrolled error associated with spatial variation, enables evaluation of main effects and two-way interactions, but limits power to evaluate the three-way interaction (Winer 1971). The 32 experimental plots each measured 40×40 m. The minimum distance between plots was 40 m, except for two plots separated by 20 m and a 3 m deep streambed (see Wright et al.

(2011) Appendix A). All measurements for this study took place within the central 20 × 20 m of each plot, with a 5 m wide treated buffer area on all sides. Fertilizer treatments have been applied by hand since 1998 in four equal doses each wet season, with 6–8 weeks between applications. Annual doses are 125 kg $N{\cdot}ha^{-1}{\cdot}yr^{-1}$ as urea, 50 kg $P{\cdot}ha^{-1}{\cdot}yr^{-1}$ as triple superphosphate, and 50 kg $K{\cdot}ha^{-1}{\cdot}yr^{-1}$ as potassium chloride. Fertilization has altered chemical properties of the soils. N fertilization reduced soil pH and extractable base cations and increased extractable nitrate and aluminum, P fertilization increased extractable P, and K fertilization increased extractable K (Yavitt et al. 2011, Turner et al. 2013).

Root sampling and analysis

In July of 2011, during the 14th year of nutrient addition, we sampled five soil cores (4 cm in diameter to a 10 cm depth) from each of the 32 plots. Cores were sampled from the center and each corner of the inner 20 × 20 m of each plot. Soil samples were refrigerated (4°C) and processed within five days of collection. Roots were carefully separated from soils under a gentle shower of tap water over 0.5-mm sieves. A test of our root-washing procedure showed negligible root tissue loss through the sieve. Root collection was conducted during the wet season, when we have documented greater availability of nutrients and microbial activity in soils (Turner et al. 2013, Turner and Wright 2014).

We sorted fine roots into two size classes (0–1 mm and 1–2 mm diameter), soaked them in distilled water, and gently brushed them to remove adhering soil and discarded dead roots. We sorted roots based on size class rather than root order because of the difficulty in accurately assigning root order to species-rich root samples. The 0–1 mm size class generally represented first- to third-order roots and the 1–2 mm size class represented third- or fourth-order roots. We acquired an image (300 DPI, CanoScan LiDE210, Canon, Melville, New York, USA) of roots in the 0–1 mm size class and then separated them into two subsamples: one subsample was scanned a second time, oven-dried at 60°C for a minimum of 72 h and then weighed; a second smaller subsample was preserved in 95% ethanol and refrigerated at 4°C for subsequent mycorrhizal analysis. To ensure equal representation, root fragments of each morphological group (potential species) were distributed into each of the subsamples. We then acquired an image of the entire root sample in the 1–2 mm size class and the tissues were oven-dried. Root images were analyzed with WinRhizo (Regent Instruments, Quebec, Canada). Images were analyzed for length (L) and average diameter ($\bar{D}$). We also measured the mass of oven-dried roots and used L and core-specific values of SRL to estimate dry mass for the subsample of 0–1 mm roots preserved in alcohol. We calculated specific root length (SRL) exactly as L/M. We estimated tissue density (TD) approximately as mass per volume or:

$$\mathrm{TD} = \frac{M}{\pi(\bar{D}/2)^2 L}. \quad (1)$$

Our calculation of TD is an approximation because total root length (L) should be multiplied by the average of the squared diameter and not by the average diameter squared. The average diameter squared will approximate the average of the squared diameter poorly if the distribution of root diameters is skewed.

The number of potential independent responses to nutrient addition is limited for two reasons. First, AM fungal hyphae extend the reach of roots, which complicates responses concerning L. Second, substituting the definition of SRL into Eq. 1 yields the following relationship among SRL, TD, and average diameter ($\bar{D}$) (Ostonen et al. 2007):

$$\mathrm{SRL} = \frac{4}{\pi \bar{D}^2 \mathrm{TD}}. \quad (2)$$

For these reasons, although we present the responses of L and SRL to nutrient addition, we do not make additional predictions concerning L and SRL.

Elemental analysis of root tissue

To determine the C, N, P, and K concentrations of root tissues, all oven-dried root samples were homogenized by plot and size class then ground into a fine powder. Total C and N were determined by Micro-Dumas combustion (Carlo Erba Stumentazione). Total P and K were determined by double acid extraction of ashed plant material and analyzed via colorimetry (Alpkem autoanalyzer, OI Analytical, Clackamas, Oregon, USA) and atomic absorption spectrophotometry (Shimadzu 6800, Shimadzu, Columbia, Maryland, USA), respectively. All analyses were conducted in the Analytical Chemistry Lab of the Odum School of Ecology, University of Georgia, Athens, Georgia, USA.

Mycorrhizal colonization

Preserved root samples were soaked in deionized water overnight and rinsed three times to remove ethanol. Roots were cut into 1-cm sections, cleared in 10% KOH at 70°C for 5–7 h, acidified briefly in 1% HCl, and stained with 0.05% trypan blue (in a 1:1:1 mixture of lactic acid, glycerol, and deionized water) for 15 min at 70°C. Roots were destained in a lactic acid glycerol solution for at least 8 h prior to observation. We studied roots under a compound microscope and quantified the number of mycorrhizal structures (arbuscules, vesicles, and hyphae) using a random-intercept method (McGonigle et al. 1990). Mycorrhizal colonization was calculated as the percentage of fine-root length and mycorrhizal density as the length of fine root colonized for arbuscules, vesicles, and hyphae.

Data analysis

We performed incomplete-block, factorial analyses of variance (ANOVA) for each response variable. The ANOVA models included main effects for N, P, and K; their two-way interactions; and spatial terms for replicate and blocks nested within replicates (Winer 1971). We used Bartlett's test to evaluate the homogeneity of variance of residuals over the eight factorial treatments for each ANOVA. Data transformation was unnecessary; however, one outlier was identified (for plot 28, root tissue density = 0.361 and 0.372 g/cm^3 for 0–1 mm and 0–2 mm roots, respectively). Results were qualitatively similar for analyses performed with and without this outlier, and results including all data are presented. We performed all analyses with SYSTAT 11.0 (Richmond, California, USA).

Results

Fine-root biomass responded to fertilization (Fig. 1). The addition of K significantly reduced total fine-root biomass (Fig. 1a; $F_{1,18} = 5.11$, $P = 0.036$) and marginally reduced biomass of the individual size classes (Appendix A; $F_{1,18} = 3.75$, $P = 0.069$ for 0–1 mm roots; $F_{1,18} = 3.99$, $P = 0.061$ for 1–2 mm roots). We also observed a significant interaction between N and P, where the addition of both elements together reduced total fine-root biomass (Fig. 1b; for N × P interaction, $F_{1,18} = 6.31$, $P = 0.009$) and the biomass of 1–2 mm diameter roots (Appendix A; $F_{1,18} = 12.97$, $P = 0.002$). For the smaller size class of roots (0–1 mm), N alone reduced root biomass (Appendix A; $F_{1,18} = 4.76$, $P = 0.043$). Total fine-root biomass declined by 50% in response to all three nutrients combined (Fig. 1c).

Root tissue density also responded to fertilization (Fig. 2). Root tissue density declined with the addition of K (Fig. 2a; $F_{1,18} = 5.88$, $P = 0.026$), with similar responses for the individual size classes (Appendix B; for 0–1 mm roots, $F_{1,18} = 3.85$, $P = 0.065$; for 1–2 mm roots, $F_{1,18} = 5.28$, $P = 0.034$). Tissue density also declined with the addition of N and P combined for all fine roots (Fig. 2b; for N × P interaction, $F_{1,18} = 7.07$, $P = 0.016$) and for individual size classes (Appendix B; for 0–1 mm roots, N × P interaction, $F_{1,18} = 4.62$, $P = 0.045$; for 1–2 mm roots, N × P interaction, $F_{1,18} = 5.31$, $P = 0.033$). Tissue density decreased by 25% in response to all three nutrients combined (Fig. 2c). The mean diameter of fine roots did not respond to N, P, or K addition (not shown; $F_{1,18} = 0.627$, $P = 0.439$; $F_{1,18} = 2.354$, $P = 0.142$; $F_{1,18} = 0.328$, $P = 0.574$, respectively), nor to any interaction between nutrients.

The responses of fine-root length depended on the nutrient added. There were no significant responses to K addition for all fine roots (Fig. 3a; $F_{1,18} = 2.19$, $P = 0.156$) nor for the 0–1 and 1–2 mm size classes (Appendix C). In contrast, N addition led to significant decreases in total fine-root length (Fig. 3b; $F_{1,18} = 5.37$, $P = 0.033$) and the length of 0–1 mm roots (Appendix C; $F_{1,18} = 4.76$, $P = 0.043$). There was also a significant N × P interaction for the length of 1–2 mm fine roots, with the lowest values when both nutrients were added together (Appendix C; N × P interaction, $F_{1,18} = 7.12$, $P = 0.016$). Total fine-root length declined by 20% in response to all three nutrients combined (Fig. 3c).

SRL tended to increase in response to fertilization (Fig. 4). SRL did not respond to K addition for all fine roots (Fig. 4a; $F_{1,18} = 3.13$, $P = 0.09$), but increased in response to K addition for 1–2 mm roots (Appendix D; $F_{1,18} = 5.59$, $P = 0.030$). SRL increased in response to N and P combined for all fine roots (Fig. 4b; N × P interaction, $F_{1,18} = 11.32$, $P = 0.003$) and for 0–1 mm roots (Appendix D; $F_{1,18} = 6.03$, $P = 0.026$). SRL increased by 50–60% in response to all three nutrients combined (Fig. 4c).

The responses of root nutrient concentrations depended on the nutrient added (Table 1). N fertilization did not significantly change the N concentration of root tissue (for 0–1 mm roots, $F_{1,18} = 1.70$, $P = 0.21$; for 1–2 mm roots, $F_{1,18} = 3.71$, $P = 0.07$). In contrast, P addition strongly increased the P concentration of roots (for 0–1 mm roots, $F_{1,18} = 70.39$, $P < 0.0001$; for 1–2 mm roots, $F_{1,18} = 110.2$, $P < 0.0001$), and K addition strongly increased the K concentration of roots (for 0–1 mm roots, $F_{1,18} = 12.72$, $P = 0.002$; for 1–2 mm roots, $F_{1,18} = 19.46$, $P < 0.0001$) (Table 1).

The responses of AM fungi also depended on the nutrient added (Fig. 5). Mycorrhizal colonization (percentage of root length) was not significantly affected by N or P addition for arbuscules and vesicles (Fig. 5a–d); however, N addition led to declines in colonization of hyphae (Fig. 5e; $F_{1,18} = 5.83$, $P = 0.026$) and all AM structures (Fig. 5g; $F_{1,18} = 5.27$, $P = 0.034$), and P addition led to increases in hyphae (Fig. 5f; $F_{1,18} = 5.46$, $P = 0.031$) and all AM structures (Fig. 5h; $F_{1,18} = 9.98$, $P = 0.005$).

Mycorrhizal root density (length of root colonized per core) consistently declined in response to the addition of N across all mycorrhizal structures, including arbuscules ($F_{1,18} = 10.12$, $P = 0.005$), vesicles ($F_{1,18} = 6.75$, $P = 0.018$), hyphae ($F_{1,18} = 10.3$, $P = 0.005$), and all structures ($F_{1,18} = 9.92$, $P = 0.006$), and increased in response to the addition of P for arbuscules ($F_{1,18} = 5.59$, $P = 0.029$), hyphae ($F_{1,18} = 7.01$, $P = 0.016$), and all structures ($F_{1,18} = 7.73$, $P = 0.012$) (data not shown). In sum, the addition of N reduced mycorrhizae, the addition of P increased mycorrhizae, and the addition of K had no significant effect on mycorrhizae.

The responses of fine-root biomass and fine-root traits to nutrient addition can be summarized as follows:

1) Fine-root biomass (M) declined substantially in response to K addition and to N plus P addition (Fig. 1; Appendix A).
2) Fine-root length (L) tended to decline (but insignificantly) with K addition and declined significantly with N addition and N plus P addition (Fig. 3; Appendix C).

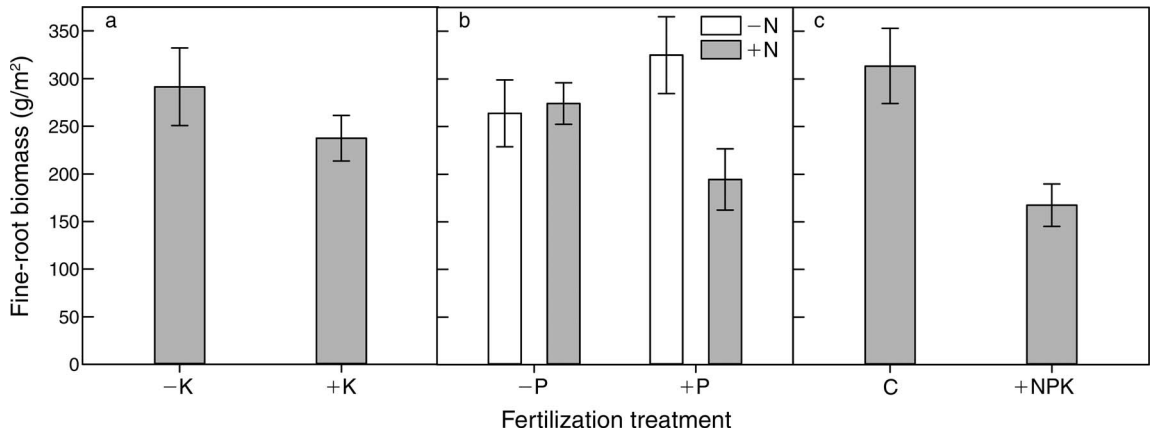

FIG. 1. Total fine-root (0–2 mm) biomass (mean ± SE) in surface soils (0–10 cm depth) in fertilization plots in lowland tropical forest in the Barro Colorado Nature Monument, Panama, (a) without or with the addition of K, (b) without or with the addition of N and P, and (c) without or with the addition of NPK. Panel (a) contrasts 16 –K plots vs. 16 +K plots. Panel (b) contrasts eight −N−P, eight −N+P, eight +N−P, and eight +N+P plots. Panel (c) contrasts four control (C) vs. four +N+P+K plots.

3) Average diameter ($\bar{D}$) was largely unaffected by nutrient addition (not shown).
4) The decreases in M were quantitatively larger than the decreases in L (cf, Figs. 1 and 3) so that (a) TD, which is proportional to M and inversely proportional to L, tended to decrease with nutrient addition (Fig. 2; Appendix B), while (b) SRL, which is proportional to L and inversely proportional to M, tended to increase with nutrient addition (Fig. 4; Appendix D).

DISCUSSION

We evaluated fine-root responses after 14 years of factorial N, P, and K addition in a lowland tropical forest growing on relatively fertile soils in central Panama. Long-lived (decades to centuries) trees and lianas dominate plant biomass in lowland tropical forests, and species composition did not change in response to 14 years of fertilization (S. J. Wright, *unpublished data*). Therefore, stand-level fine-root measurements integrate the responses of many long-lived individuals of many species. Nonetheless, we predicted that fine-root abundance (biomass and length), morphological and chemical traits, and colonization by symbionts (AM fungi) would respond to nutrient addition. We found support for this hypothesis because fertilization reduced fine-root biomass, tissue density, and nutrient content, and altered mycorrhizal colonization. Although the specific way that N-, P-, and K-induced root responses varied, our results demonstrate that the alleviation of multiple nutrient limitation affects fine roots in a species-rich lowland tropical forest.

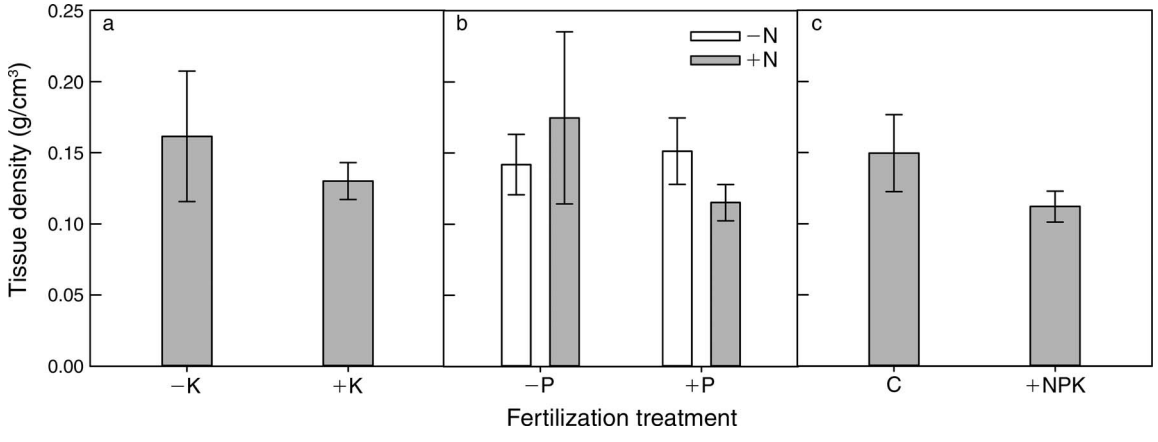

FIG. 2. Total fine-root (0–2 mm) tissue density (mean ± SE) in surface soils (0–10 cm depth) in fertilization plots (a) without or with the addition of K, (b) without or with the addition of N and P, and (c) without or with the addition of NPK. Panel (a) contrasts 16 −K vs. 16 +K plots. Panel (b) contrasts eight −N−P, eight −N+P, eight +N−P and eight +N+P plots. Panel (c) contrasts four control (C) vs. four +N+P+K plots.

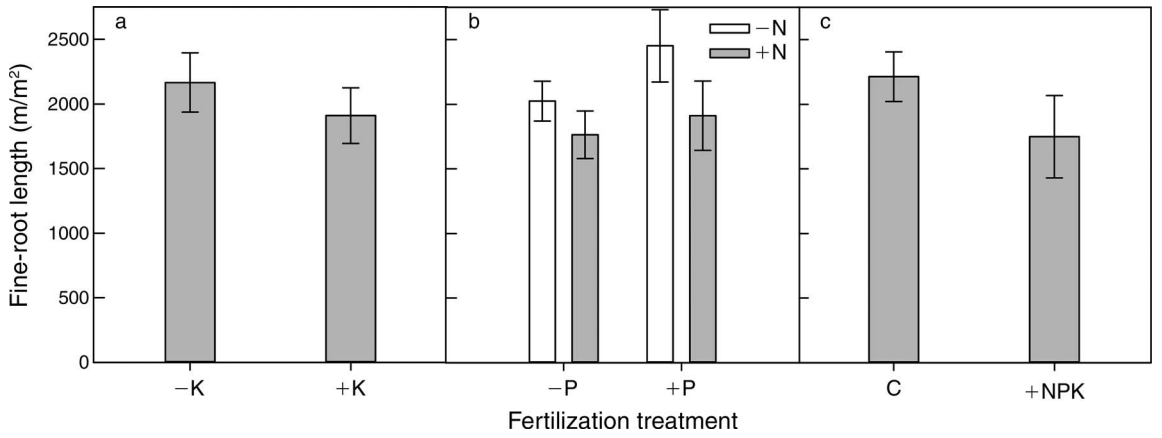

FIG. 3. Total fine-root (0–2 mm) length (mean ± SE) in surface soils (0–10 cm depth) in fertilization plots (a) without or with the addition of K, (b) without or with the addition of N and P, and (c) without or with the addition of NPK. Panel (a) contrasts 16 −K vs. 16 +K plots. Panel (b) contrasts eight −N–P, eight −N+P, eight +N–P, and eight +N+P plots. Panel (c) contrasts four control (C) vs. four +N+P+K plots.

Root responses

Fine-root biomass and length reflect plant investments in nutrient acquisition and tend to be negatively associated with soil fertility (Aerts and Chapin 2000). In tropical forests, standing root biomass declines along natural gradients of increasing soil fertility (Ostertag 2001, Powers et al. 2005, Espeleta and Clark 2007, Jiménez et al. 2009, Powers and Peréz-Aviles 2012, Kochsiek et al. 2013) as well as in response to experimental nutrient addition (Fig. 1; Appendix A; Ostertag 2001). These responses suggest that tropical trees reduce the partitioning of biomass to fine roots as nutrient limitation is alleviated. At our site, N, P, and K addition reduced standing fine-root biomass by 50% and fine-root length by 20% (Figs. 1c and 3c, respectively). This is consistent with our previous finding that all three nutrients limit some component of aboveground net primary production (Wright et al. 2011).

We calculated stand-level mean values for three morphological functional traits (TD, $\bar{D}$, and SRL) of fine roots. Structural integrity increases with TD, and low root TD is associated with greater susceptibility to herbivory and shorter root life spans (Aerts and Chapin 2000). Thus, the reductions in TD associated with nutrient additions (Fig. 2; Appendix B) are consistent with the more rapid root turnover rates observed with K addition during the first four years of our study (Yavitt et al. 2011). These results suggest that fertilization is shifting the expression of root functional traits toward short-lived roots suited for rapid resource acquisition and that multiple soil nutrients regulate root TD in this tropical forest. In contrast, stand level $\bar{D}$ was insensitive to fertilization. There is limited information about root diameter responses to nutrient availability. For individual tree species, fine-root diameter varies little along gradients of soil fertility (Eissenstat et al. 2000), and is

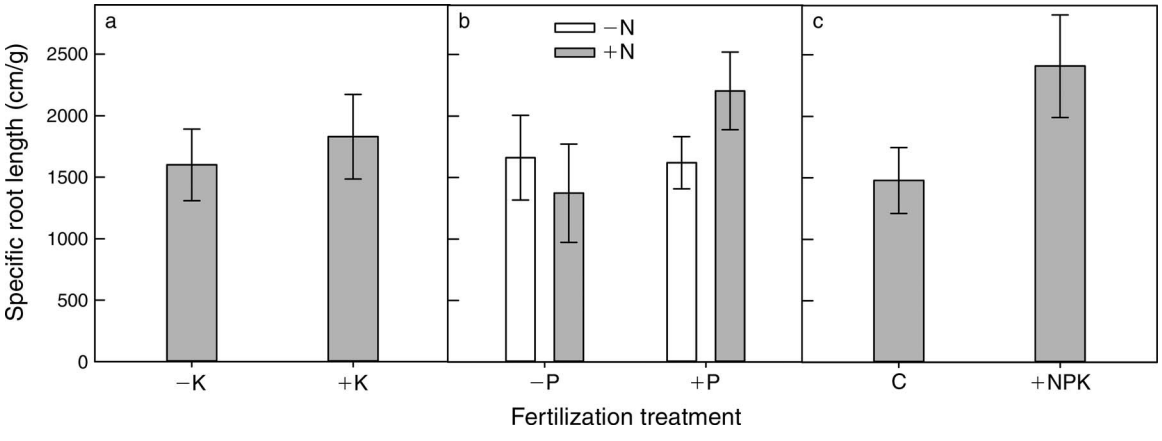

FIG. 4. Total fine-root (0–2 mm) specific root length (mean ± SE) in surface soils (0–10 cm depth) in fertilization plots, (a) without or with the addition of K, (b) without or with the addition of N and P, and (c) without or with the addition of NPK. Panel (a) contrasts 16 −K vs. 16 +K plots. Panel (b) contrasts eight −N–P, eight −N+P, eight +N–P, and eight +N+P plots. Panel (c) contrasts four control vs. four +N+P+K plots.

TABLE 1. Elemental concentration of fine-root tissue from the Gigante fertilization experiment in a lowland tropical forest on Barro Colorado Island, Panama.

Treatment by root size class	C (%)	N (%)	C:N	P (ppm)	K (ppm)
0–1 mm root tissue					
Control	46.9 (0.4)	1.68 (0.06)	28.1 (1.2)	0.68 (0.02)	4.69 (0.28)
N	48.0 (1.3)	1.79 (0.28)	27.2 (3.6)	0.56 (0.05)	4.08 (0.46)
P	47.6 (1.2)	1.62 (0.06)	29.4 (1.2)	1.52 (1.2)	4.76 (0.78)
K	46.9 (0.8)	1.78 (0.19)	26.5 (3.1)	0.66 (0.09)	5.48 (0.78)
NP	46.9 (1.3)	1.85 (0.20)	25.6 (2.8)	1.58 (0.36)	4.42 (0.71)
NK	47.4 (1.5)	1.77 (0.06)	26.8 (1.2)	0.62 (0.08)	5.07 (0.42)
KP	46.9 (1.2)	1.85 (0.22)	25.6 (3.7)	1.58 (0.38)	4.42 (1.4)
NPK	46.4 (1.2)	1.72 (0.18)	27.2 (3.4)	1.37 (0.52)	5.35 (0.69
1–2 mm root tissue					
Control	47.6 (0.39)	1.19 (0.04)	40.2 (1.6)	0.47 (0.03)	4.20 (0.48)
N	47.9 (1.5)	1.24 (0.11)	38.8 (2.5)	0.38 (0.01)	3.86 (0.37)
P	47.6 (0.79)	1.07 (0.08)	44.6 (4.2)	1.87 (0.36)	4.31 (1.4)
K	48.1 (4.1)	1.16 (0.07)	41.6 (3.8)	0.47 (0.05)	5.66 (0.47)
NP	48.9 (2.4)	1.25 (0.32)	40.5 (7.4)	1.29 (0.31)	4.03 (0.74)
NK	47.3 (1.6)	1.30 (0.20)	36.3 (1.8)	0.48 (0.05)	6.18 (2.2)
KP	48.9 (6.9)	1.25 (0.19)	40.5 (6.5)	1.29 (0.44)	4.03 (0.89)
NPK	47.1 (1.7)	1.34 (0.47)	38.1 (11)	1.67 (0.69	5.55 (1.9)

Notes: Values are means with SE in parentheses. P addition increased the P concentration of roots (for 0–1 mm roots, $P < 0.0001$; for 1–2 mm roots, $P < 0.0001$) and K addition increased the K concentration of roots (for 0–1 mm roots, $P = 0.002$; for 1–2 mm roots, $P < 0.0001$). The 0–1 mm size class generally represented first- to third-order roots and the 1–2 mm size class represented third- or fourth-order roots.

unresponsive or minimally responsive to fertilization (Tingey et al. 1997, Ostonen et al. 2007).

Mathematical relationships among TD, $\bar{D}$, and SRL (Eqs. 1 and 2) complicate the interpretation of our findings and may explain inconsistent responses of SRL to nutrient availability in the literature. SRL increases along gradients of increasing nutrient availability (Holdaway et al. 2011, Freschet et al. 2013), increases with fertilization in two experiments (Bakker et al. 2009), but decreases with N fertilization in a meta-analysis of 54 European experiments (Ostonen et al. 2007). Our understanding of SRL responses to nutrients could be improved with concurrent measures of TD and root diameter measurements on individual roots rather than the stand-level mean values provided by measurements pooled over all roots from soil cores.

We predicted that N, P, and K addition would increase concentrations of those elements in fine-root tissues. N was the only nutrient that did not trigger the predicted increase. In our study system, N addition increases N concentrations in fine litter (Kaspari et al. 2008), in seedling tissues including root tissues (Santiago et al. 2012), and in sapling leaf tissue with consequences for photosynthetic and stomatal physiology (Pasquini and Santiago 2012; Pasquini et al., *in press*). The lack of a stand-level response of fine-root tissue N concentrations is therefore surprising. We speculate that fine-root tissues are maintained at optimal N concentrations in non-fertilized conditions, and that additional N made available by fertilization is allocated to aboveground tissues. The interpretation of responses to N addition is complicated because N addition acidified the soil by about 0.7 pH units (Turner et al. 2013). Acidification was ameliorated when N was applied in conjunction with P (Turner et al. 2013). An inhibitory effect of acidification on tissue N concentrations should therefore be associated with a significant N × P interaction. The N × P interaction was insignificant for root tissue N concentrations (Table 1), but significant for fine-root abundance and morphological traits (Figs. 1–4).

Although our study focuses on soil nutrients as limiting belowground resources, water availability can also regulate belowground allocation and the expression of root traits (Metcalfe et al. 2008). In our seasonally dry tropical forest, as nutrient additions have alleviated constraints on plant growth and reduced root biomass, the demand for water may become relatively more important and change the expression of root functional traits.

Mycorrhizal responses

We observed mycorrhizal responses to the addition of N and P, but not to the addition of K or the addition of combinations of nutrients. Nitrogen addition reduced AM colonization (Fig. 5a, c, e, g). Similar reductions have been documented in several terrestrial ecosystems (Treseder 2004, van Diepen et al. 2007), which suggests that plants regulate investment in AM fungi as a function of soil N availability or plant N demand. The possibility that soil acidification (Turner et al. 2013) might affect AM fungi should be considered as well; however, it is unclear what type of response to expect. AM colonization can decline with soil acidification, particularly below a pH of 4 (Hutchinson et al. 1999), but colonization can also be unchanged at low soil pH and provide enhanced benefit to ameliorating plant stress (Heijne et al. 1996). Soil pH in water averaged 4.5

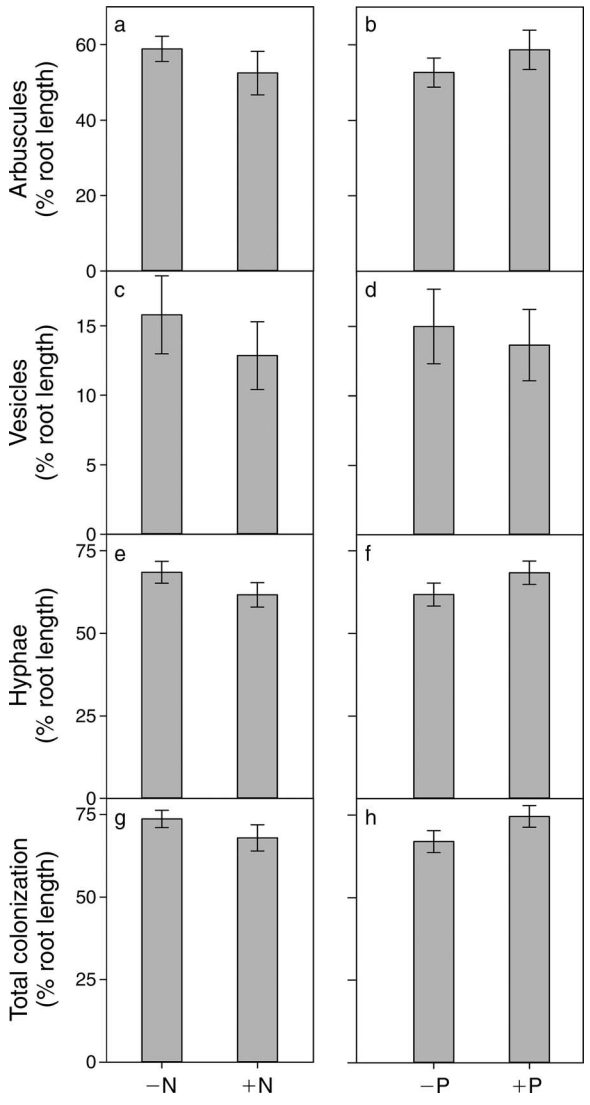

FIG. 5. Arbuscular mycorrhizal root colonization (percentage of root length colonized by mycorrhizal structures, mean ± SE) in fertilization plots: (a and b) arbuscules, (c and d) vesicles, (e and f) hyphae, (g and h) total colonization, without or with the addition of N (left-hand panels, for 16 −N vs. 16 +N plots) and without or with the addition of P (right-hand panels, for 16 −P vs. 16 +P plots).

after a decade of N (only) addition in our study system (Turner et al. 2013).

Our finding that P addition stimulated AM colonization was unexpected. Across many ecosystem types, P fertilization tends to reduce mycorrhizal colonization (Treseder 2004), but this response may depend on the P status of the ecosystem (Treseder and Allen 2002). In our study system, P regulates microbial biomass; microbial C, N, and P; and soil phosphatase activity (Turner and Wright 2014). Nonetheless, the addition of P was associated with a significant increase in mycorrhizal colonization. Host plants select for fungal community assemblages based on local constraints of soil nutrients (Johnson et al. 2010), and because of this, fertilization can alter the structure and composition of the AM fungal community (Egerton-Warburton and Allen 2000, van Diepen et al. 2011) and can even lead to a change in fungal composition from mutualistic to parasitic forms (Johnson et al. 1997). Therefore, changes in AM colonization after 14 years of N or P addition could be the result of complex biotic interactions between plants and a modified assemblage of AM fungal taxa.

The statistically significant response of mycorrhizal colonization to P and N addition was modest in comparison to the response of root biomass. Mycorrhizal colonization increased by 8% and declined by 6% in response to P and N addition (Fig. 5), respectively, whereas root biomass decreased by 30% in response to K and N plus P and by 50% in response to N, P, and K combined (Fig. 1). The lowest level of AM colonization observed in any treatment was ~60% of fine-root length (Fig. 5g, h). The relative abundance of AM fungi after 14 years of fertilization suggests that plants have limited control over their investment in AM fungi, or alternatively, that AM fungi are maintained because they provide benefits other than nutrient acquisition (Herre et al. 2007).

Conclusions

Tropical forest responses to soil nutrients are diverse (e.g., Mirmanto et al. 1999, Newbery et al. 2002, Wright et al. 2011, Baribault et al. 2012, Alvarez-Clare et al. 2013, Condit et al. 2013, Kochsiek et al. 2013), reflecting the heterogeneity in soils across the biome (Quesada et al. 2010) as well as the variety of biological processes regulated by soil nutrients. The latter is captured well in our experiment in an old-growth lowland tropical forest in Panama. Fertilization has stimulated a wide range of microbial processes, including microbial biomass and enzyme production, decomposition, N_2 fixation, and N-oxide emissions (Kaspari et al. 2008, Barron et al. 2009, Koehler et al. 2009, Turner and Wright 2014). Fertilization has also stimulated stand-level plant responses, including increased litter production with P addition and increased wood production with N plus K addition (Wright et al. 2011). After 14 years of fertilization, fine-root biomass is the only stand-level plant tissue pool to decline in response to the addition of N, P, and K. The addition of N, P, and K also induced a shift, at the stand level, toward the production of fine roots that are less dense, more nutrient rich, and have modified interactions with mycorrhizal fungi. Our study demonstrates that fine roots respond strongly to the alleviation of multiple nutrient limitations in this lowland tropical forest.

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Supplemental Material

Ecological Archives

Appendices A–D and the Supplement are available online: http://dx.doi.org/10.1890/14-1362.1.sm