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Nutrients limit photosynthesis in seedlings of a lowland tropical forest tree species

S. C. Pasquini · L. S. Santiago

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Abstract We investigated how photosynthesis by understory seedlings of the lowland tropical tree species *Alseis blackiana* responded to 10 years of soil nutrient fertilization with N, P and K. We ask whether nutrients are limiting to light and CO₂ acquisition in a low light understory environment. We measured foliar nutrient concentrations of N, P and K, isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), and light response curves of photosynthesis and chlorophyll fluorescence. Canopy openness was measured above each study seedling and included in statistical analyses to account for variation in light availability. Foliar N concentration increased by 20% with N addition. Foliar P concentration increased by 78% with P addition and decreased by 14% with N addition. Foliar K increased by 8% with K addition. Foliar $\delta^{13}\text{C}$ showed no significant responses, and foliar $\delta^{15}\text{N}$ decreased strongly with N addition, matching the low $\delta^{15}\text{N}$ values of applied fertilizer. Canopy openness ranged from 0.01 to 6.71% with a mean of 1.76 ± 0.14 ($\pm 1\text{SE}$). Maximum photosynthetic CO₂ assimilation rate increased by 9% with N addition. Stomatal conductance increased with P addition and with P and K in combination. Chlorophyll fluorescence measurements revealed that quantum yield of photosystem II increased

with K addition, maximum electron transport rate trended 9% greater with N addition ($p = 0.07$), and saturating photosynthetically active radiation increased with N addition. The results demonstrate that nutrient addition can enhance photosynthetic processes, even under low light availability.

Keywords Fertilization · Nitrogen isotope · Panama · Phosphorus · Potassium

Introduction

The understory environment in forests with tall trees and a thick canopy are among the darkest habitats occupied by vascular plants. Within these habitats, light availability is limited to low levels of diffuse light most of the time, interspersed by short-duration, high-intensity lightflecks (Chazdon 1988; Pearcy 1988). Because the energy provided by lightflecks is one to two orders of magnitude greater than the predominant diffuse light conditions, up to 65% of the total photosynthetic carbon gain of understory plants can be achieved during lightflecks (Pfitsch and Pearcy 1989). As a result, photosynthesis in forest understory environments is considered to be primarily limited by light (Pearcy 1988). However, plant allocation to carbon gain capacity reflects total resource availability (Field 1991), so belowground resources could co-limit photosynthesis, even in low light. If nutrients were limiting to plant growth, and limitation were alleviated by frequent fertilization, investment in plant organs to acquire limiting resources could change, favoring allocation to enhance the light and CO₂ acquisition capacity of leaves (Bloom et al. 1985; Giardina et al. 2003; Bucci et al. 2006). This study addresses photosynthetic responses of seedlings of the shade-tolerant

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pioneer tropical tree species, *Alseis blackiana*, to long-term factorial fertilization with nitrogen (N), phosphorus (P) and potassium (K) in a lowland tropical forest.

Leaf N is a strong determinant of photosynthesis (Field and Mooney 1986; Wright et al. 2004), primarily because the proteins of the Calvin cycle and thylakoids make up the majority of N in leaves. N addition in shade plants is expected to increase N allocation toward proteins of the thylakoid membrane of the chloroplast, including the pigment–protein complexes and components of the electron transport chain (Evans 1989). N addition could also increase the maximum rate of CO₂ assimilation ($A_{\max}$), because carboxylation capacity and electron transport are tightly coupled (Wullschlegel 1993). Leaf P is a component of polyphosphates and phospholipids and is necessary for activity of the Calvin cycle and rubisco regeneration (Marschner 1995), and thus P addition has been shown to stimulate $A_{\max}$, but reduce $A_{\max}$ per unit leaf P (Cordell et al. 2001), because of the ability of leaves to sequester large amounts of P in vacuoles (Sinclair and Vadez 2002; Ostertag 2009). K⁺ is the most abundant ion in plant cells and is critical for numerous biochemical functions including osmoregulation, photosynthesis, cell extension, oxidative phosphorylation and protein activation (Evans and Sorger 1966; Morgan 1984; Marschner 1995; Santiago and Wright 2007). Therefore, K addition has the potential to increase photosynthesis through efficient biochemistry and stomatal control. In addition, enhanced N, P and K supply have all been linked to increased water use efficiency of plant productivity (Raven et al. 2004). Therefore, nutrient addition has the potential to increase the carbon gain capacity of understory plants by increasing efficient use of limited light resources.

Limitation of plant growth by N and P is widespread, and has most often been demonstrated experimentally as a positive growth response to nutrient addition (Vitousek 2004). Relative to N and P, less is known regarding K-limitation, but recent explorations suggest that the role of K in forest ecosystems is unique, and has closer similarity to N than other base cations (Tripler et al. 2006). At the site of the study reported in this paper, seedlings primarily increase height growth in response to K addition, and secondarily with N and P added in combination (Santiago, unpublished data). Saplings (10–25 mm diameter) increase diameter growth with N and K added in combination (Wright et al. 2011). The growth response of seedlings and saplings to added nutrients suggests two possibilities. The first possibility is that these plants grow more in response to added nutrients by allocating less photosynthate to root growth which causes an increase in above-ground growth. Consistent with this hypothesis, at the site of this study, seedling root-to-shoot biomass ratio decreases with K addition (Santiago, unpublished data),

and K addition decreases stand-level fine root biomass (Yavitt et al. 2010; Wright et al. 2011). The second possibility is that increased nutrient supply provides a resource that may be used to increase the uptake of another limiting resource such as light (Fahey et al. 1998).

In this paper, we investigate the second possibility. We present photosynthesis data in response to long-term N, P and K factorial fertilization in lowland Panama to test the question of whether nutrient addition increases acquisition of light and CO₂, which are also limiting in the low light environment of the tropical forest understory. We hypothesized that N, P and K fertilization all had the potential to limit aspects of photosynthetic carbon gain. The alternative hypothesis is that plants would be unresponsive to nutrient addition in the understory because there is not enough light energy to physiologically take advantage of the increase in nutrient availability.

Materials and methods

Study site and species

The study was conducted in mature (~200 years) lowland tropical forest on the Gigante Peninsula (9°06'31"N, 79°50'37"W), within the Barro Colorado Nature Monument (BCNM) in Central Panamanian moist tropical forest. Annual precipitation averages 2,600 mm, less than 10% of which falls during the 4-month dry season. The soils consist of Endogleyic Cambisols and Acric Nitisols (Koehler et al. 2009), and are relatively fertile for lowland tropical forest soils (Fyllas et al. 2009; Dieter et al. 2010; Wright et al. 2011).

The study species was *A. blackiana* Hemsl. (Rubiaceae), one of the most common tree species at the site. *Alseis* is a medium-sized forest tree growing just into the canopy and is one of the most common canopy species in seasonally moist tropical forest in central Panama (Croat 1978). *Alseis* recruits from tiny, wind-dispersed seeds exclusively in canopy gaps, but unlike other pioneer tree species, it is able to persist in the shaded understory (Dalling et al. 2001).

Experimental design

The eight treatments of a 2 × 2 × 2 factorial NPK experiment were replicated four times. The four replicates were arranged perpendicular to the 36-m topographic gradient. Within each replicate, we blocked the N, P, K and NPK treatments versus the NP, NK, PK and control treatments. 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

et al. 1991). The 32 experimental plots were 40×40 m and the minimum distance between plots was 40 m, with the exception of two plots separated by 20 m and located on opposite sides of a stream.

Stand-level fertilization was initiated in 1998, with fertilizer added by hand in four equal doses each wet season with 6–8 weeks between applications (May 15–30, July 1–15, September 1–15 and October 15–30). Fertilizers are applied at annual dosages of $125 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (as urea), $50 \text{ kg P ha}^{-1} \text{ year}^{-1}$ (as triple super-phosphate) and $50 \text{ kg K ha}^{-1} \text{ year}^{-1}$ (as KCl).

Photosynthetic traits

During the wet season of 2008 (July–August), photosynthetic gas exchange and chlorophyll fluorescence were measured on 1–9 (median = 5) individuals in each plot for a total of 146 plants. A total of 16–20 seedlings <0.5 m height in each of the eight treatment combinations were measured. We characterized photosynthesis with fluorescence-light curves (White and Critchley 1999; Rascher et al. 2000) simultaneously measured with CO_2 assimilation using a portable infrared gas analyzer (Model LI-6400; Li-Cor Biosciences, Lincoln, NE, USA) equipped with a leaf chamber chlorophyll fluorescence meter and red/blue light source (Model LI-6400-40; Li-Cor Biosciences). Leaves were dark adapted for 30 s before an initial measurement was taken at $0 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photon flux density (PFD). Irradiance was then increased in a stepwise fashion (10, 50, 70, 100, 250, 500, 750, $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PFD). At each PFD, photosynthetic CO_2 assimilation (A), stomatal conductance to water vapor (g_s), leaf transpiration (E), and chlorophyll fluorescence were recorded once leaves reached steady-state values, defined as coefficients of variation of CO_2 and water vapor within the chamber of $<0.25\%$ (Caruso et al. 2005).

Electron transport rate (ETR) was calculated based on chlorophyll fluorescence data using the equation:

$$\text{ETR} = (\Phi_{\text{PSII}}) \cdot (\text{PFD}) \cdot (f) \cdot (\alpha) \quad (1)$$

where ETR is electron transport rate, Φ_{PSII} is the effective quantum yield of PSII measured during a 0.8-s saturating flash ($2,000\text{--}3,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$), f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, indicating that excitation energy is distributed equally between the two photosystems (Maxwell and Johnson 2000), and α is the proportion of light absorptance by photosynthetic tissue in decimal format and was assumed to be 0.84 as a mean value for a wide variety of leaves (Björkman and Demmig 1987; Stenke and Santiago 2011).

Following photosynthetic measurements, leaves were harvested, sealed in plastic bags and returned to the

laboratory on Barro Colorado Island where their area was recorded with an area meter (Model LI-3100; Li-Cor Biosciences). Leaves were then dried for 48 h at 60°C and weighed to determine specific leaf area (SLA; $\text{cm}^2 \text{ g}^{-1}$).

Leaf and fertilizer chemistry

Dried leaf samples were ground in a ball mill (8000D; Spex Sample Prep, Stanmore, UK) to a fine powder. Samples of each of the four applications of urea nitrogen fertilizer per year from 2004 to 2008 were pooled and ground with a mortar and pestle. N concentration, as well as stable isotopic composition of N ($\delta^{15}\text{N}$) and C ($\delta^{13}\text{C}$) were determined with an elemental analyzer (ECS 4010; Costech, Valencia, CA, USA) interfaced with an isotope ratio mass spectrometer (Delta V Advantage; Thermo Scientific, Bremen, Germany) at the University of California Facility for Isotope Ratio Mass Spectrometry (FIRMS), Riverside, CA, USA. Leaf P and K were determined on pooled samples from each plot using inductively coupled plasma atomic emission spectroscopy after nitric acid digestion at the University of California Analytical Laboratory, Davis, CA, USA.

Light availability

Hemispherical photographs were used to quantify the light environment for each plant measured for fluorescence-light response curves. Photographs were taken using a digital camera (Coolpix 4500; Nikon, Japan) and a fisheye converter lens (FC-E8; Nikon) with an exposure time of $1/125$ s and aperture of 2.8 (Engelbrecht and Herz 2001). The camera was positioned at each measurement plant at approximately the height of the measured leaf and oriented toward magnetic north using a compass. All photographs were taken with overcast skies, common during the wet season in Panama, to maximize contrast between foliage and sky. Determination of percent direct and diffuse light transmittance from hemispherical photographs was accomplished with the Gap Light Analyzer program (Frazer et al. 1999) and used to calculate total light transmittance (T_{tot}).

Data analyses

The maximum A for each curve (A_{max}), the g_s and E at A_{max} , the maximum observed ETR (ETR_{max}), and Φ_{PSII} at $1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ PFD were used to evaluate the effects of nutrient fertilization on photosynthesis. Saturating photosynthetically active radiation (PFD_{sat}) was calculated by evaluating ETR as a function of PFD in Photosynthesis Assistant (ver. 1.1; Dundee Scientific, Dundee, UK). Multiple regression analysis of photosynthetic parameters

Table 1 Multiple regression results of nitrogen (N), phosphorus (P) and potassium (K) fertilization effects on leaf physiological parameters for *A. blackiana* in lowland tropical forest in Panama

	Overall model	T_{tot}	N	P	K	N $\times$ P	N $\times$ K	P $\times$ K
SLA ($\text{cm}^2 \text{g}^{-1}$)	≤ 0.001	0.021	0.039	0.920	0.364	0.043	0.645	0.810
A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.022	0.042	0.054	0.091	0.241	0.511	0.193	0.214
g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	≤ 0.005	0.217	0.512	≤ 0.001	0.075	0.121	0.615	≤ 0.005
c_i ($\mu\text{mol mol}^{-1}$)	≤ 0.001	0.419	0.242	0.077	≤ 0.005	0.879	0.150	0.022
E ($\text{mmol m}^{-2} \text{s}^{-1}$)	≤ 0.001	0.040	0.916	0.018	0.696	0.014	0.553	0.293
ETR_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	≤ 0.001	≤ 0.001	0.070	0.852	0.951	0.375	0.482	0.768
PFD_{sat}	≤ 0.001	≤ 0.001	0.054	0.741	0.946	0.225	0.606	0.619
Φ_{PSII}	≤ 0.001	≤ 0.001	0.706	0.961	0.038	0.088	0.053	0.848

p values are presented for the overall model, as well as main effects and interactions. Total canopy light transmission above seedling (T_{tot}), specific leaf area (SLA), maximum photosynthetic CO_2 assimilation rate per area (A_{max}), stomatal conductance at maximum photosynthesis (g_s), internal CO_2 concentration (c_i), transpiration at maximum photosynthesis (E), maximum electron transport rate (ETR_{max}), saturating photon flux density (PFD_{sat}), and quantum yield of photosystem II (Φ_{PSII}). Bold values are significant at $p \leq 0.05$

for individual leaves was used to analyze responses to nutrient addition. Total transmittance of each measurement leaf was included in the multiple regression model to account for variation in canopy openness above each leaf because allocation to photosynthetic capacity responds strongly to light availability (Montgomery and Givnish 2008) and because light is extremely heterogeneous in tropical forest understory environments (Montgomery and Chazdon 2001). Responses of foliar chemistry to nutrient addition were analyzed using ANOVA on mean values for plots with nutrient main effects, two-way interactions, and blocking effects.

Results

Photosynthetic traits

The multiple regression analysis revealed that all photosynthetic traits except for g_s and c_i were significantly affected by T_{tot} (Table 1). Mean SLA decreased with N addition and increased with addition of N and P in combination (Tables 1 and 2). Values of A_{max} increased significantly by 9% with N addition (Fig. 1a; Table 1). Values of g_s increased significantly by 14% with P addition (Fig. 1b) and trended 9% greater with K addition (Table 1). There was also a significant P $\times$ K interaction for g_s (Table 1), indicating an increase in g_s when P and K were added in combination. There was a significant increase in c_i with K addition, and with P and K in combination (Table 1). P addition caused a significant increase in E , but there was also an N $\times$ P interaction, indicating that N and P in combination caused a decrease in E (Fig. 1c). The mean PFD at which A_{max} was observed was $832 \mu\text{mol m}^{-2} \text{s}^{-1}$, but there was no effect of nutrient addition ($p = 0.14$).

ETR_{max} trended 9% greater with N addition ($p = 0.07$) (Fig. 2a; Table 1). The mean PFD at which ETR_{max} was observed was $436 \mu\text{mol m}^{-2} \text{s}^{-1}$, but there was no effect of nutrient addition ($p = 0.24$). PFD_{sat} showed a significant increase with N addition (Fig. 2b; Table 1). There was a significant increase in Φ_{PSII} with K addition (Table 1), and a significant N $\times$ K interaction on Φ_{PSII} because N and K added in combination caused a decrease in Φ_{PSII} (Fig. 2c). There was significant correlation between A_{max} and ETR_{max} across all seedlings demonstrating the functional interdependence of these two independently measured variables (Fig. 3).

Leaf structure and chemistry

Foliar nutrient concentration responded strongly to nutrient addition. Foliar N concentration increased by 20% with N addition (Fig. 4a) and was not affected by other elements (Table 3). Foliar P concentration increased by 78% with P addition and decreased by 14% with N addition (Table 3; Fig. 4b). There was also a significant N $\times$ K interaction on foliar P concentration (Table 3), indicating that N and K in combination decreased foliar P concentration by 11% below control values (Fig. 4b). Foliar K increased 8% with K addition (Table 3; Fig. 4c). Foliar $\delta^{13}\text{C}$ showed no significant responses to nutrient addition (Fig. 4d). Foliar $\delta^{15}\text{N}$ decreased strongly with N fertilizer addition (Table 3; Fig. 4e), consistent with the low urea fertilizer $\delta^{15}\text{N}$ values of $-1.86\text{‰} \pm 0.10$ ($\pm 1\text{SE}$), relative to soil $\delta^{15}\text{N}$ values of $+4.86\text{‰} \pm 0.52$ at 0–5 cm depth in control plots (Koehler et al. 2009).

Discussion

The results of this long-term factorial nutrient fertilization experiment indicate that multiple elements limit

Table 2 Summary of significant main effects of fertilization with N, P and K on specific leaf area (SLA) for seedlings of *A. blackiana* in lowland tropical forest in Panama

Treatment	SLA (cm ² g ⁻¹)
+N	431.5 ± 6.8
–N	451.5 ± 6.8
+N and P	459.5 ± 16.1
–N and P	438.3 ± 9.4

Treatments include all plots receiving N addition (+N), no N (–N), N and P in combination (+N and P), and plots that did not receive N and P in combination (–N and P). See Table 1 for complete statistical results

Fig. 1 Responses of **a** maximum CO_2 assimilation per leaf area ($A_{\max}$), **b** stomatal conductance to water vapor at $A_{\max}$ (g_s) and **c** leaf transpiration at $A_{\max}$ (E) of seedlings of the tree species *A. blackiana* to factorial addition of nitrogen (N), phosphorus (P) and potassium (K) and control (C) treatments in the understory of lowland tropical forest in Panama

photosynthetic processes in seedlings of a tropical tree species. There is a clear effect of added N on photosynthesis that includes increased $A_{\max}$ and PFD_{sat} , and a

Fig. 2 Responses of **a** maximum electron transport rate ($\text{ETR}_{\max}$), **b** saturating photosynthetic photon flux density (PFD_{sat}) and **c** effective quantum yield of photosystem II at $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD (Φ_{PSII}) of seedlings of the tree species *A. blackiana* to factorial addition of nitrogen (N), phosphorus (P) and potassium (K) and control (C) treatments in the understory of lowland tropical forest in Panama

parallel trend in $\text{ETR}_{\max}$. These results suggest that increased N supply is allocated to enzymes of the light harvesting complex and carboxylation reactions. Other significant responses of photosynthetic processes to nutrient addition include a strong effect of P, and to a lesser extent K, on g_s indicating that stomatal function may be limited by nutrient availability and important for maximizing carbon gain in light-limited environments with rapidly changing light conditions (Chazdon and Pearcy 1986; Valladares et al. 1997). Our results also indicate that enhanced photosynthesis may play a role in the increased growth of tree seedlings, saplings and poles in response to fertilization measured at this study site (Wright et al. 2011; Santiago, unpublished data). The increase in height growth of seedlings in response to K addition, and to N and P added in combination is consistent with limitation of

Fig. 3 Maximum photosynthetic CO_2 assimilation per area (A_{max}) as a function of maximum electron transport rate (ETR_{max}) for 146 seedlings of the tree species *A. blackiana* in a factorial fertilization experiment with nitrogen (N), phosphorus (P) and potassium (K) and control (C) treatments in the understory of lowland tropical forest in Panama

photosynthesis by multiple elements measured in this study. In addition to decreased root biomass with K addition, the increased A_{max} with N addition and increased g_s with P addition may contribute to positive effects of N and P on seedling height growth.

The larger increase in foliar P compared to foliar N with fertilization is consistent with other fertilization experiments in tropical forest (Cordell et al. 2001; Campo and Dirzo 2003). In addition to increases in foliar nutrients that are allocated to metabolic function, Chapin et al. (1990) recognize three types of storage: *accumulation*, in which compounds do not directly promote growth, *reserve formation*, which involves synthesis of storage compounds from resources that might otherwise promote growth, and *recycling*, in which compounds are broken down and may be mobilized for later growth. A greater degree of accumulation is expected for elements stored primarily as inorganic compounds such as P and K (Chapin et al. 1990). In contrast, reserve formation is likely for elements stored primarily as organic compounds such as N, which is not commonly stored as inorganic N in leaves but rather stored in forms such as amino acids or proteins, thus incurring a metabolic cost for storage (Chapin et al. 1990; Ostertag 2010). Inorganic P on the other hand can be stored in vacuoles and can make up substantial fractions of total plant P (Sinclair and Vadez 2002; Ostertag 2009). Our results are consistent with these predictions for N and P; increases in foliar P were much greater than increases in foliar N with addition of the same element (Fig. 4a, b). Foliar P also decreased with N addition. N addition in the same long-term fertilization experiment reduced soil pH by 0.5 pH units (Yavitt et al. 2010), which may have affected

Fig. 4 Responses of foliar concentrations of **a** nitrogen (N), **b** phosphorus (P), **c** potassium (K), and isotopic composition of **d** carbon ($\delta^{13}\text{C}$) and **e** nitrogen ($\delta^{15}\text{N}$) of seedlings of the tree species *A. blackiana* to factorial addition of N, P and K and control (C) treatments in the understory of lowland tropical forest in Panama

Table 3 Results of analysis of variance for foliar nitrogen (N), phosphorus (P), potassium (K), carbon stable isotopic composition ($\delta^{13}\text{C}$) and nitrogen stable isotopic composition ($\delta^{15}\text{N}$) for seedlings of *A. blackiana* in response to fertilization with N, P and K

Source	df	Foliar N		Foliar P		Foliar K		Foliar $\delta^{13}\text{C}$		Foliar $\delta^{15}\text{N}$	
		F	P	F	P	F	P	F	P	F	P
Between blocks											
Rep	3	2.28	0.114	0.79	0.513	0.89	0.465	8.45	0.001	2.48	0.094
Block (Rep)	4	1.43	0.266	1.76	0.182	2.34	0.094	0.7	0.601	1.07	0.402
Within blocks											
N	1	16.26	0.0008	17.04	0.0006	3.41	0.081	0.68	0.420	22.95	<0.0001
P	1	1.52	0.233	246.24	<0.0001	0.04	0.852	0	0.958	1.48	0.24
K	1	0.26	0.616	1.3	0.27	5.21	0.035	0.25	0.623	1.82	0.195
N $\times$ P	1	0.4	0.536	1.79	0.198	0.14	0.714	2.09	0.165	0.95	0.344
N $\times$ K	1	0.25	0.623	7.31	0.015	0.5	0.487	0	0.962	0.93	0.348
P $\times$ K	1	0.37	0.552	2.29	0.147	0.85	0.37	0.1	0.756	0.06	0.808
Error	18										

Bold type indicates statistical significance of $p \leq 0.05$

the solubility of P or other elements. Foliar K increased by 8%, which was more modest than expected for an element that can be stored inorganically. Yet the significant effect of K on Φ_{PSII} (Fig. 2c), and the combined effect with P on g_s (Table 1), suggest that increased foliar K was allocated to metabolic function. Our results indicate that substantial physiological changes accompany increases in foliar nutrient concentration and that altered physiological function may be related to previously reported growth responses of seedlings, saplings and poles, as well as changes in litterfall productivity at this study site (Kaspari et al. 2008; Wright et al. 2011; Santiago, unpublished data).

The response of A_{max} to nutrient addition is consistent with other studies investigating fertilization effects on tropical tree seedlings in low light. For example, seedlings of *Flindersia brayleyana*, an Australian tropical rainforest tree species with broad tolerance to sun and shade, increase A_{max} and PFD_{sat} in response to nutrient addition (Thompson et al. 1988). In addition, nutrient addition causes an increase in A_{max} in seedlings of two out of three shade-tolerant tree species and one shade-intolerant tree species (Thompson et al. 1992). Furthermore, nutrient addition causes an increase in A_{max} in West African tree seedlings (Riddoch et al. 1991). Although the studies by Thompson et al. (1988, 1992) and Riddoch et al. (1991) showed similar results to our data, their low light treatments ($25\text{--}35 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD) likely had greater light availability than our study plants, whose ambient irradiance during our measurements, as recorded by the external PFD sensor on our infrared gas analyzer, was $6.7 \mu\text{mol m}^{-2} \text{s}^{-1} \pm 0.5$ ($\pm 1\text{SE}$). Another difference between the studies by Thompson et al. (1988, 1992) and Riddoch et al. (1991) and the current study is that they added all elements together in nutrient treatments, limiting the potential to

determine which elements affected photosynthetic processes. Our factorial design allowed us to distinguish elemental effects and interactions.

We expected allocation of increased N supply to thylakoids to increase ETR in N addition treatments (Evans 1989). Indeed, ETR_{max} trended 9% greater with N addition (Fig. 2a; Table 1), but the most noteworthy response of photosynthesis to N addition was the increase in A_{max} (Fig. 1a). The positive linear relationship between A_{max} and foliar N concentration has been well studied (Field and Mooney 1986; Wright et al. 2004), and our measured response of A_{max} to N addition demonstrates that even in a tropical forest with relatively high N availability (Yavitt et al. 2009), plants can use more N to enhance photosynthetic rates. The strong increase in g_s in response to P addition and interaction with K addition was also striking (Fig. 1b; Table 1), and suggests that the efficiency of stomatal responses to changing light conditions are co-limited by P and K. The role of K in guard cell control and osmoregulation is well documented (Morgan 1984; Marschner 1995), and consistent with increased g_s and increased carbon gain during short-duration high-intensity light-flecks. Detailed molecular physiology studies are needed to sort out the precise mechanisms for the observed photosynthetic responses to nutrient addition.

The responses of photosynthetic processes to N, P and K are consistent with the multiple limiting nutrients hypothesis (Bloom et al. 1985), which postulates that the addition of any resource should increase plant growth because plants adjust allocation of resources until growth is equally limiting by all resources. Interestingly, we observed relatively small effects of the +NPK treatment, suggesting that although multiple resources limit photosynthetic processes in this study system, individual processes often respond to

specific elements. Numerous studies have investigated the effects of nutrient addition on ecosystem net primary productivity (Vitousek 2004 and references therein). Incorporating a physiologically based view into these processes will improve our ability to predict the effects of environmental change on ecosystem function. Nutrient limitation of photosynthetic processes could be important for mediating plant responses to global change and may represent an important constraint for plant responses to increasing CO₂ concentration and alterations of temperature and precipitation regimes.

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