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# Nutrient acquisition strategies augment growth in tropical $N_2$-fixing trees in nutrient-poor soil and under elevated $CO_2$

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*Abstract.* Tropical forests play a dominant role in the global carbon (C) cycle, and models predict increases in tropical net primary productivity (NPP) and C storage in response to rising atmospheric carbon dioxide ($CO_2$) concentrations. The extent to which increasing $CO_2$ will enhance NPP depends in part on the availability of nitrogen (N) and phosphorus (P) to support growth. Some tropical trees can potentially overcome nutrient limitation by acquiring N via symbiotic dinitrogen ($N_2$) fixation, which may provide a benefit in acquiring P via investment in N-rich phosphatase enzymes or arbuscular mycorrhizal (AM) fungi. We conducted a seedling experiment to investigate the effects of elevated $CO_2$ and soil nutrient availability on the growth of two $N_2$-fixing and two non-$N_2$-fixing tropical tree species. We hypothesized that under elevated $CO_2$ and at low nutrient availability (i.e., low N and P), $N_2$ fixers would have higher growth rates than non-$N_2$ fixers because $N_2$ fixers have a greater capacity to acquire both N and P. We also hypothesized that differences in growth rates between $N_2$ fixers and non-$N_2$ fixers would decline as nutrient availability increases because $N_2$ fixers no longer have an advantage in nutrient acquisition. We found that the $N_2$ fixers had higher growth rates than the non-$N_2$ fixers under elevated $CO_2$ and at low nutrient availability, and that the difference in growth rates between the $N_2$ and non-$N_2$ fixers declined as nutrient availability increased, irrespective of $CO_2$. Overall, $N_2$ fixation, root phosphatase activity, and AM colonization decreased with increasing nutrient availability, and increased under elevated $CO_2$ at low nutrient availability. Further, AM colonization was positively related to the growth of the non-$N_2$ fixers, whereas both $N_2$ fixation and root phosphatase activity were positively related to the growth of the $N_2$ fixers. Though our results indicate all four tree species have the capacity to up- or down-regulate nutrient acquisition to meet their stoichiometric demands, the greater capacity for the $N_2$ fixers to acquire both N and P may enable them to overcome nutritional constraints to NPP under elevated $CO_2$, with implications for the response of tropical forests to future environmental change.

*Key words: arbuscular mycorrhizal fungi; elevated $CO_2$; nitrogen fixation; nutrient acquisition strategies; phosphatase enzymes; tropical trees.*

## Introduction

Tropical forests are a critical component of the global carbon (C) cycle. They contain ~25% of the world's terrestrial C in their biomass and soil (Jobbagy and Jackson 2000, Schlesinger and Bernhardt 2013), exchange more carbon dioxide ($CO_2$) with the atmosphere than any other biome (Foley et al. 2005), and account for at least one-third of terrestrial net primary productivity (NPP; e.g., Roy et al. 2001, Beer et al. 2010, Saatchi et al. 2011). In addition, many Earth system models predict substantial increases in terrestrial NPP in response to increasing atmospheric $CO_2$ concentrations (Todd-Brown et al. 2014, Wieder et al. 2015). Given the large influence of tropical forests on the global C cycle, increases in tropical NPP could translate into a larger terrestrial C sink, thus enhancing a critical climate benefit provided by tropical forests (Ahlström et al. 2012, Todd-Brown et al. 2014).

Despite model predictions, the extent to which increasing $CO_2$ will enhance terrestrial NPP is likely to depend on the availability of two key nutrients: nitrogen (N) and phosphorus (P) (Hungate et al. 2003, de Graaff et al. 2006, Cernusak et al. 2013). Though N and P are both essential to all forms of life, multiple lines of evidence suggest tropical NPP may already be limited by

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both N and P, as well as other critical micronutrients like potassium (K). In a fertilization experiment in a lowland tropical forest in Panama, Wright et al. (2011) reported increased wood production with N and K addition and increased litter production with P addition. Wurzburger and Wright (2015) also reported a stand level shift toward the production of fine-roots that are less dense and more nutrient rich with N, P, and K addition. In a similar experiment in Costa Rica, Alvarez-Clare et al. (2013) reported that only small trees with a diameter of 5–10 cm increased in stem growth with P addition. Underscoring the potential importance of nutrients and heterogeneous nutrient limitation, Wieder et al. (2015) demonstrated that current rates of new inputs of N and P may be insufficient to meet the demands of enhanced productivity with elevated $CO_2$, and highlight the potential for P to ultimately limit terrestrial NPP and C storage not only in the tropics, but globally.

Nevertheless, evidence suggests that some tropical forest tree species have the capacity to meet the nutritional demands of increasing NPP predicted with higher atmospheric $CO_2$. For example, some members of the Fabaceae family, common canopy trees throughout the Neotropics and African tropics (Gentry 1988, Losos and Leigh 2004, ter Steege et al. 2006), form a symbiotic relationship with bacteria that are capable of acquiring N from the atmosphere in exchange for C (Sprent 2009). This C costly process, known as symbiotic dinitrogen ($N_2$) fixation, may be promoted under elevated $CO_2$ as the photosynthetic capacity of trees increase, alleviating N limitation to tree growth (Hungate et al. 1999, Cernusak et al. 2011). Moreover, the ability to fix atmospheric $N_2$ may also improve P acquisition by enhancing the production of N-rich phosphatase enzymes to hydrolyze organic phosphates (Houlton et al. 2008), or by allowing greater investment of C in arbuscular mycorrhizal (AM) fungi to scavenge greater soil area for inorganic P (Nasto et al. 2014). Together, these two mechanisms may confer a competitive advantage to $N_2$-fixing trees over non-$N_2$-fixing trees (Nasto et al. 2017), and elicit greater growth responses to elevated $CO_2$ in nutrient-poor soils.

Though no study has explicitly addressed the N and P acquisition strategies of $N_2$ and non-$N_2$ fixers across a gradient of soil nutrient availability and under elevated $CO_2$, some previous work highlights the potential for $N_2$ fixers to regulate nutrient acquisition to maintain high growth responses to elevated $CO_2$ regardless of the availability of soil nutrients. For example, Cernusak et al. (2011) showed that on N-poor soils, the growth of $N_2$-fixing tropical tree seedlings responded more strongly than non-$N_2$ fixers to elevated $CO_2$, and the growth of the $N_2$-fixing seedlings was closely related to their mass of nodules, the location of symbiotic $N_2$ fixation. However, when soil N availability was high, the $N_2$-fixing and non-$N_2$-fixing seedlings had similar growth responses to elevated $CO_2$. The large nodule biomass of the $N_2$-fixing seedlings at low N availability suggests that they fixed $N_2$ at relatively high rates, allowing a greater growth response to elevated $CO_2$. By contrast, when soil N was plentiful, they likely down-regulated $N_2$ fixation, as indicated by small nodule biomass, while the non-$N_2$-fixing seedlings now had equal access to N, reducing their advantage over the non-$N_2$-fixing seedlings (Thomas et al. 1991, Tissue et al. 1996). In addition, previous research has shown that root phosphatase activity and AM colonization tend to decrease with increasing soil P (Treseder 2004; Marklein and Houlton 2011), suggesting that $N_2$ fixers would similarly maintain high investment in both P acquisition strategies at low soil P to elicit a greater growth response to elevated $CO_2$, but down-regulate them at high soil P. Thus, $N_2$ fixers, through a greater investment in N and P acquisition, may have the capacity to offset nutrient limitation of tropical NPP predicted by some global models.

Here, we examined the role of N and P acquisition strategies (i.e., symbiotic $N_2$ fixation, root phosphatase activity, and AM colonization) as potential drivers of tropical tree seedling growth under differing soil nutrient availabilities and $CO_2$. To do this, we grew two $N_2$-fixing and two non-$N_2$-fixing tree species in pots and exposed them to three different levels of nutrient availability (0NP, +NP, ++NP) and two different levels of $CO_2$ (ambient and elevated) in a full-factorial design. We explored how relative growth rate (RGR) and nutrient acquisition strategies of the $N_2$- and non-$N_2$-fixing seedlings varied by functional group and/or species, and in response to increased nutrient availability and elevated $CO_2$ in a high-light, high-water environment. In addition, we determined the strength of the relationships between growth and nutrient acquisition for both functional groups. We hypothesized that, at low soil nutrient availability and under elevated $CO_2$, $N_2$ fixers would grow more rapidly than non-$N_2$ fixers because $N_2$ fixers have a greater capacity to acquire both N (via $N_2$ fixation) and P (via phosphatase and/or AM fungi). We also hypothesized that these differences in growth rates between $N_2$ fixers and non-$N_2$ fixers would decline as soil nutrient availability increased because $N_2$ fixers no longer have an advantage in nutrient acquisition.

## Methods

### *Study site*

We conducted this experiment at the Santa Cruz Experimental Field Facility of the Smithsonian Tropical Research Institute (STRI), Gamboa, Panama (9°07′ N, 79°42′ W). The study site is located within the Panama Canal Watershed at 28 m above sea level. Two glasshouses were used: one with $CO_2$ similar to ambient conditions (400 ppm) and the other with elevated $CO_2$ (800 ppm). The elevated $CO_2$ glasshouse was maintained by releasing $CO_2$ gas from a high-pressure cylinder when $CO_2$ declined below 800 ppm. The glasshouses were air-conditioned automatically so that air temperature did not exceed 30°C. In addition, 20%

shade cloths were placed over the glasshouses to reduce surface temperatures of the plant foliage without greatly limiting light.

### *Species and seedling preparation*

We used seedlings of four pioneer tree species that coexist in and are common to lowland moist tropical forests: two $N_2$ fixers (*Adenanthera pavonina* L. [Fabaceae] and *Inga spectabilis* (Vahl) Willd. [Fabaceae]) and two non-$N_2$ fixers (*Swietenia macrophylla* King [Meliaceae] and *Tabebuia guayacan* (Seem.) Hemsl. [Bignoniaceae]; Appendix S1: Table S1). Recently, *T. guayacan* has been recognized as a synonym of *Handroanthus guayacan* (Seem.) S.O.Grose [Bignoniaceae] (Grose and Olmstead 2007). These species were chosen because they have a strong tendency to recruit into gaps, and high rates of growth, mortality, and dispersal (Condit et al. 1996). Seeds were collected from mature trees growing in the Panama Canal watershed and germinated in 1.25-L tree pots (Stuewe & Sons, Corvallis, Oregon, USA) containing a 95:5 mixture of silica sand and sieved unsterilized forest soil. The soil was collected from the Santa Rita ridge, is classified as an Oxisol (Typic Kandiudox), and has some of the lowest resin-extractable P (<0.2 mg P/kg) in central Panama (Condit et al. 2013). During the germination phase, tree pots were on plastic benches 1 m above a concrete surface and underneath a rain shelter with a glass roof. The shelter had no sidewalls, so air temperature, wind speed, and relative humidity were similar to ambient conditions. Once each species displayed fully expanded cotyledons, five seedlings per species were harvested for initial seedling mass and leaf area. An additional 15–24 seedlings per species were randomly chosen to be moved into each glasshouse (ambient and elevated $CO_2$) for the duration of the experiment.

### *Experimental treatments*

At the beginning of the experiment, five to eight seedlings per species in each glasshouse (ambient and elevated $CO_2$) were randomly chosen to receive a 0NP, +NP, or a ++NP nutrient treatment corresponding to 0, 30, and 60 kg $N \cdot ha^{-2} \cdot yr^{-1}$ as calcium nitrate ($Ca(NO_3)_2$) and 0, 20, 40 kg $P \cdot ha^{-2} \cdot yr^{-1}$ as potassium phosphate ($KH_2PO_4$). The N and P fertilizer solution, as well as 75 mL of a N and P free Hoagland's solution (one-fifth strength; to ensure that all other macro- and micronutrients were balanced and non-limiting), were added to each seedling on a weekly basis. Seedlings were watered daily. The nutrient treatments were chosen to represent a wide gradient of nutrient availability and are similar to other seedling studies (e.g., Batterman et al. 2013).

### *Harvest measurements*

We harvested the seedlings of each species in an effort to optimize biomass while avoiding the negative effect of pot size (Poorter et al. 2012). Seedlings within a species were harvested when the dry plant biomass to pot volume ratios were, on average, <1 g/L in the elevated $CO_2$, ++NP treatment. Thus, seedlings of different species grew for different periods of time (e.g., 44–60 d), but all species were harvested with similar biomass among the seedlings in the elevated $CO_2$, ++NP treatment (Appendix S1: Table S1). At the time of harvest, seedlings were separated into root, stem, and leaf fractions. Roots were gently washed with tap water and if the $N_2$-fixing seedlings contained nodules, they were excised for immediate $N_2$ fixation analysis using the acetylene reduction assay (ARA). The roots of all seedlings were subsampled and stored overnight at 4°C for phosphatase assays and AM colonization. Total plant dry biomass was measured after drying for 72 h at 60°C.

Plant dry biomass was used to calculate the RGR of each seedling,

$$\mathrm{RGR} = \frac{\ln(M_f) - \ln(M_i)}{dt}$$

where $M_f$ is the final plant dry biomass, $M_i$ is the initial plant dry biomass, and $dt$ is the duration of the experiment in days for each species. The trait is log-transformed as seedling growth is assumed to be exponential and for the convenience of comparing across species (Hunt and Cornelissen 1997; Rees et al. 2010). However, interspecific comparisons may be hampered if growth is size dependent and if size distributions vary among species. We, thus, compared absolute growth rate (AGR) with RGR across all seedlings and found a strong correlation ($r = 0.93$, $P < 0.001$), indicating size-dependent growth does not bias our analyses nor that RGR yields different results than AGR (Poorter et al. 2008).

Dry leaves of each seedling were ground and analyzed for foliar C, N, and P concentrations. Foliar C and N were measured by automated combustion and thermal conductivity detection on a Thermo Flash EA 1112 analyzer (CE Elantech, Lakewood, New Jersey, USA). Foliar P was determined by ignition (550°C, 1 h) and dissolution of the ash in 1 mol/L hydrochloric acid (HCl), with phosphate detection by automated molybdovanadate colorimetry on a Lachat Quikchem 8500 (Hach, Loveland, Colorado, USA).

Root phosphatase activity was determined using *para*-nitrophenyl phosphate (*p*NPP) as an analogue substrate for phosphomonoesterase (Turner et al. 2001). Briefly, ~500 mg of fresh fine roots per seedling were placed in glass vials and immersed in 9 mL of 50 mmol/L sodium acetate-acetic acid buffer (pH 5). The vials were placed in a water bath at 26°C (mean soil temperature in lowland tropical forests of Panama) and shaken for 5 min to equilibrate. While still in the shaking water bath, 1 mL of 50 mmol/L *p*NPP was added to each vial to initiate the assay. After 30 min, the vials were taken out of the shaking water bath and 0.5 mL of solution was

removed and added to 4.5 mL of 0.11 mol/L sodium hydroxide to terminate the assay. The absorbance of each sample was measured at 405 nm against a standard curve of *para*-nitrophenol (*p*NP). Each sample included a negative control for sample and substrate absorbance. Root phosphatase activity was calculated as μmol *p*NPP·(g root dry mass) $^{-1}$·h$^{-1}$.

Arbuscular mycorrhizal colonization was measured by staining roots with trypan blue (Koske and Gemma 1989). Roots of each seedling were cleared in 10% potassium hydroxide for 3 d, rinsed with tap water, and placed in 3% HCl for 12 h for acidification. The HCl solution was replaced with trypan blue for 24 h and roots were de-stained in water for 12 h. Ten cleared and uniformly stained root pieces per seedling were mounted on slides and root colonization was quantified using the magnified intersection method (McGonigle et al. 1990) on ~50 intersections. Colonization was calculated as the percentage of root length colonized by AM fungi.

Symbiotic $N_2$ fixation was measured using the ARA method (Hardy et al. 1968). All nodules from each $N_2$-fixing seedling (if nodules were present) were excised and incubated in 50-mL clear acrylic tubes with a 10% acetylene (made from reacting calcium carbide with water) atmosphere. After 1 h, 14 mL of headspace gas was removed from each tube with a syringe, injected into 10-mL glass vials, and returned to the laboratory for analysis by gas chromatography using a Shimadzu GC-2014 equipped with a flame ionization detector (Shimadzu, Kyoto, Japan). Background ethylene was accounted for from nodules without acetylene exposure, ethylene produced from tubes and glass vials, ethylene within our acetylene, and ethylene lost as a result of photodegradation during transport. To convert acetylene reduction rates into $N_2$ fixation rates, an ethylene : N conversion ratio of 2.8:1 was applied (Sullivan et al. 2014). Though this ratio was determined by sampling nodules across a presumably wide variety of $N_2$-fixing species in a nearby rain forest, the ratio can vary by species (Batterman et al. 2018). We, thus, urge caution when making inferences about our $N_2$ fixation rates. Rates of $N_2$ fixation were calculated on a whole plant basis as μg N/h.

### *Statistical analyses*

Data were tested for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene test, and data that did not satisfy those assumptions were log-transformed (e.g., root : shoot, foliar C:N and C:P, $N_2$ fixation, root phosphatase activity, and AM colonization). We used a nested analysis of variance (ANOVA) to test for effects of $CO_2$ and nutrient treatments on all harvest measurements. Species were nested into functional groups to test whether the species and/or the functional groups differed in their responses to the $CO_2$ and nutrient treatments. Multiple linear models were used to determine the influence of nutrient acquisition strategies (root phosphatase activity, AM colonization, and $N_2$ fixation) on the RGR of seedlings from each functional group growing at 0NP. All statistical analyses were performed using the open source R software v.2.15.3 (R Development Core Team 2015). For all data, significance was determined when $P < 0.05$.

## Results

### *Growth*

Relative growth rates varied across the experimental treatments but were not driven by any specific species (Table 1). While the $N_2$ fixers had a higher average RGR than the non-$N_2$ fixers, overall ($P < 0.001$), the difference between the two functional groups depended on nutrient availability (functional group by nutrient treatment effect, $P < 0.001$; Fig. 1a). Specifically, the RGR of the $N_2$ fixers showed a slight decrease from the low (0NP) to high (++NP) nutrient treatments while the RGR of the non-$N_2$ fixers increased. As such, the $N_2$ fixers and non-$N_2$ fixers had different average RGRs in the low (0NP) and intermediate (+NP) nutrient treatments, but not in the high (++NP). Also, average RGR was higher under elevated $CO_2$ than under ambient $CO_2$, overall ($P < 0.001$; Fig. 1b). There were no interactive effects between the $CO_2$ treatment and functional group, species, or the nutrient treatment.

### *Root : shoot allocation and foliar stoichiometry*

The variation in root : shoot allocation depended on the combination of the grouping variables and experimental treatments (Table 1), but the variation was primarily due to a single species. While the non-$N_2$ fixers had a higher average root : shoot than the $N_2$ fixers, overall ($P = 0.03$), the difference between the two functional groups depended on nutrient availability (functional group by nutrient treatment effect, $P < 0.001$), as well as $CO_2$ (functional group by $CO_2$ treatment effect, $P = 0.03$; Fig. 2a). While average root : shoot decreased across the nutrient treatments ($P < 0.001$), it was higher in the non-$N_2$ fixers only in the low (0NP) nutrient treatment, and this difference was driven by the non-$N_2$ fixer *Tabebuia guayacan* (species by nutrient treatment effect, $P < 0.001$). Also, while average root : shoot decreased under elevated $CO_2$ ($P < 0.001$), it was higher in the non-$N_2$ fixers than the $N_2$ fixers only under ambient $CO_2$, and this difference was driven, again, by *Tabebuia guayacan* (species by $CO_2$ treatment effect, $P < 0.001$).

The variation in foliar stoichiometry also depended on the combination of the grouping variables and experimental treatments (Table 1). First, while the non-$N_2$ fixers had a higher average foliar C:N than the $N_2$ fixers overall ($P < 0.001$), the difference depended on the interaction between nutrient availability and $CO_2$ (functional group by nutrient by $CO_2$ treatment effect, $P < 0.001$; Fig. 2b). For example, average foliar C:N was higher in

TABLE 1. *F* table for the nested ANOVAs of the two $N_2$ fixers and two non-$N_2$ fixers grown under ambient (400 ppm) or elevated (800 ppm) $CO_2$, and at low (0NP), intermediate (+NP), and high (++NP) nutrient availabilities.

| Parameter | Functional group | Nutrient | $CO_2$ | Species | Functional group × Nutrient | Functional group × $CO_2$ | Nutrient × $CO_2$ | Species × Nutrient | Species × $CO_2$ | Functional group × Nutrient × $CO_2$ | Species × Nutrient × $CO_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| RGR | **43.9*** ** | **6.0** ** | **23.6*** ** | 0.3 | **10.9*** ** | <0.1 | 1.9 | 0.5 | 2.4 | 0.2 | 0.5 |
| Root : shoot | **5.0* ** | **60.5*** ** | **14.6*** ** | 2.3 | **8.2*** ** | **5.1* ** | 1.0 | **19.6*** ** | **8.4*** ** | 1.2 | **7.7*** ** |
| Foliar C:N | **27.5*** ** | **484.0*** ** | **127.5*** ** | **76.2*** ** | **5.8** ** | <0.1 | 0.7 | **5.8*** ** | **7.7*** ** | **20.2*** ** | **5.8*** ** |
| Foliar C:P | **20.2*** ** | **153.1*** ** | **80.0*** ** | **146.5*** ** | **34.5*** ** | 2.6 | 2.5 | 2.1 | **4.0* ** | 2.4 | **5.6*** ** |
| Foliar N:P | 3.4 | **5.6** ** | **11.2** ** | **68.1*** ** | **39.7*** ** | 2.3 | 1.8 | 2.0 | **5.6** ** | **9.9*** ** | **3.8** ** |
| Root phosphatase activity | **217.4*** ** | **50.0*** ** | **18.7*** ** | 1.7 | 3.0 | <0.1 | 0.8 | **3.7** ** | 3.0 | 1.0 | 1.5 |
| AM colonization | **61.1*** ** | **64.5*** ** | **45.2*** ** | 1.9 | 0.8 | 0.2 | **3.4* ** | **2.8* ** | 0.6 | 0.1 | 0.4 |
| $N_2$ fixation | | **593.4*** ** | **56.2*** ** | **8.3** ** | | | **14.9*** ** | 2.2 | 0.3 | | 0.2 |

*Notes:* Statistically significant results are shown in boldface type (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Relative growth rate, RGR; arbuscular mycorrhizal (AM) colonization.

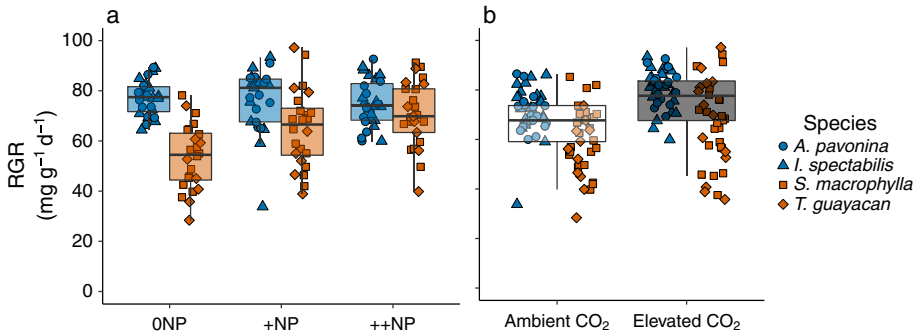


FIG. 1. Relative growth rate (RGR) of (a) the $N_2$-fixing and non-$N_2$-fixing seedlings across the low (0NP), intermediate (+NP), and high (++NP) nutrient treatments and (b) all of the seedlings under ambient and elevated $CO_2$. Box plot mid lines represent medians, boxes represent the 25th and 75th quartile values, whiskers represent 1.5 × the interquartile range, and points represent individual seedlings. Species are *Adenanthera pavonina*, *Inga spectabilis*, *Swietenia macrophylla*, and *Tabebuia guayacan*.

the non-$N_2$ fixers than the $N_2$ fixers across all nutrient treatments under elevated $CO_2$, but only in the intermediate (+NP) and high (++NP) nutrient treatments under ambient $CO_2$. These differences, however, were driven primarily by the non-$N_2$ fixer *Swietenia macrophylla* (species by nutrient by $CO_2$ treatment effect, $P < 0.001$). Despite these differences, average foliar C:N, overall, decreased across the nutrient treatments ($P < 0.001$) and increased under elevated $CO_2$ ($P < 0.001$).

Second, average foliar C:P of the non-$N_2$ fixers was higher than the $N_2$ fixers overall ($P < 0.001$), but the difference between the two functional groups depended on nutrient availability (functional group by nutrient treatment effect, $P < 0.001$; Fig. 2c). Specifically, the average foliar C:P of the non-$N_2$ fixers decreased more strongly across the nutrient treatments than the $N_2$ fixers. As such, average foliar C:P in the non-$N_2$ fixers was higher than the $N_2$ fixers only in the low (0NP) treatment. Also, while average foliar C:P increased under elevated $CO_2$ overall ($P < 0.001$), it was driven by both $N_2$ fixers in the low (0NP) and intermediate (+NP) nutrient treatments and the non-$N_2$ fixer *Swietenia macrophylla* in the low nutrient treatment (species by nutrient by $CO_2$ treatment effect, $P < 0.001$; Fig. 2c).

Last, while average foliar N:P differed across the nutrient ($P < 0.01$) and $CO_2$ ($P < 0.01$) treatments, this variation was driven by the high foliar N:P of the non-$N_2$ fixer *Swietenia macrophylla* in the low nutrient treatment under ambient $CO_2$ (species by nutrient by $CO_2$ treatment effect, $P < 0.01$; Fig. 2d). As a result, there was no general difference in average foliar N:P between the two functional groups ($P = 0.07$).

### *Nutrient acquisition strategies*

We found little variation among the three nutrient acquisition strategies across the grouping variables and experimental treatments (Table 1). With respect to N acquisition, average whole plant $N_2$ fixation (μg N/h) differed between the two $N_2$ fixer species overall and was highest in *Inga spectabilis* ($P < 0.01$; Fig. 3). Also, average whole plant $N_2$ fixation decreased to zero across the nutrient treatments ($P < 0.001$) and increased under elevated $CO_2$ ($P < 0.001$). There was a nutrient by $CO_2$

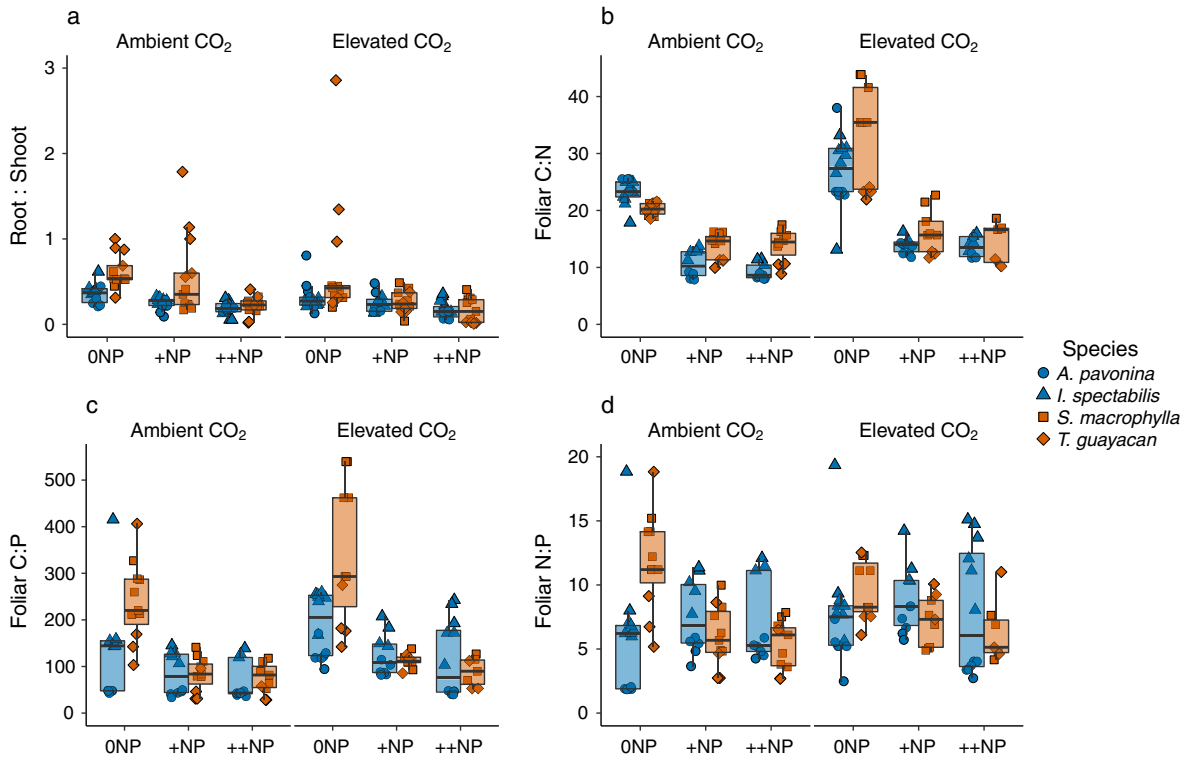


FIG. 2. (a) Root : shoot (a), (b) foliar C:N, (c) foliar C:P, and (d) foliar N:P of the $N_2$-fixing and non-$N_2$-fixing seedlings across the low (0NP), intermediate (+NP), and high (++NP) nutrient treatments, and under ambient and elevated $CO_2$. Box plot mid lines represent medians, boxes represent the 25th and 75th quartile values, whiskers represent 1.5 × the interquartile range, and points represent individual seedlings.

treatment effect, but this was driven by the absence of $N_2$ fixation in the high (++NP) nutrient treatment under both ambient and elevated $CO_2$.

Average root phosphatase activity of the $N_2$ fixers was higher than the non-$N_2$ fixers ($P < 0.001$), and decreased across the nutrient treatments ($P < 0.001$). However, the decrease in root phosphatase activity across the nutrient treatments was driven by the $N_2$ fixer *Adenanthera pavonina* and the non-$N_2$ fixer *Tabebuia guayacan* (species by nutrient treatment effect, $P < 0.01$; Fig. 4a.). Also, average root phosphatase activity was higher under elevated $CO_2$ overall ($P < 0.001$; Fig. 4b). There were no interactive effects between the $CO_2$ treatment and functional group, species, or the nutrient treatment (Table 1).

Finally, average AM colonization was higher on the roots of the non-$N_2$ fixers than the $N_2$ fixers ($P < 0.001$), decreased across the nutrient treatments ($P < 0.001$), and increased under elevated $CO_2$ overall ($P < 0.001$). However, the response in AM colonization to the $CO_2$ treatment depended on nutrient availability (nutrient by $CO_2$ treatment effect, $P = 0.04$; Fig. 5). Specifically, AM colonization, across all species, was only higher under elevated $CO_2$ in the intermediate (+NP) and high (++NP) nutrient treatments. There was an interaction between species and the nutrient treatment in which AM colonization of the non-$N_2$ fixer *Swietenia macrophylla* did not vary across the nutrient treatments.

### *Relationships between growth and nutrient acquisition strategies*

In the low (0NP) nutrient treatment and across both $CO_2$ treatments, the RGR of the $N_2$ fixers was positively correlated with whole plant $N_2$ fixation ($P = 0.03$, $r = 0.39$; Fig. 6a) and root phosphatase activity ($P = 0.03$, $r = 0.37$; Fig. 6b), but not with AM colonization ($P = 0.53$; Fig. 6c). However, an interactive model that included $N_2$ fixation and root phosphatase activity accounted for 60% of the variation in the RGR of the $N_2$ fixers ($P < 0.01$). In the low (0NP) nutrient treatment, and across both $CO_2$ treatments, the RGR of the non-$N_2$ fixers was not correlated with root phosphatase activity ($P = 0.06$, Fig. 6d), but was positively correlated with AM colonization ($P = 0.03$, $r = 0.40$; Fig. 6e).

## DISCUSSION

Our study demonstrates that N and P acquisition strategies of the two tropical $N_2$-fixing tree species studied here are capable of augmenting growth when

exposed to elevated $CO_2$, especially when nutrients are relatively scarce. We hypothesized that, in nutrient-poor soils and under elevated $CO_2$, $N_2$ fixers would have higher growth rates than non-$N_2$ fixers because $N_2$ fixers have a greater capacity to acquire both N and P. In our study, the non-$N_2$ fixers had higher levels of AM colonization than the $N_2$ fixers, but the $N_2$ fixers had higher root phosphatase activity and the ability to fix atmospheric $N_2$. Moreover, the latter two strategies were up-regulated in low nutrient soil and under elevated $CO_2$, which may have enabled higher growth than the non-$N_2$ fixers. We also hypothesized that the differences in growth between $N_2$ and non-$N_2$ fixers would decline as soil nutrient availability increased. Indeed, as soil nutrient availability increased, the difference between the $N_2$ and non-$N_2$ fixer growth declined, likely reflecting the observed decrease in nutrient acquisition strategies as the seedlings could invest less in these strategies and still acquire sufficient soil nutrients. Overall, our results provide compelling evidence that coupled N and P acquisition strategies may enable some tropical tree species to overcome the nutritional constraints on NPP that are likely to be exacerbated with future increases in $CO_2$.

The $N_2$ fixers had higher growth than the non-$N_2$ fixers, but this difference was driven by the disparate growth of the two functional groups in the low and intermediate nutrient soils (Fig. 1a). This implies, at least for the species studied here, that soil nutrients must be relatively scarce in order for the $N_2$ fixers to realize higher growth rates than the non-$N_2$ fixers. Previous work suggests that $N_2$ fixers may have a greater capacity than non-$N_2$ fixers to acquire both N and P (Houlton et al. 2008). This is especially relevant in the tropics, where $N_2$ fixers can potentially use fixed $N_2$ to invest in soil P acquisition (Nasto et al. 2014). A greater capacity to acquire both nutrients, especially P, would confer a competitive advantage in $N_2$ fixers over non-$N_2$ fixers in low nutrient soils but not necessarily in high nutrient soils. Our results provide multiple lines of evidence supporting this relationship between nutrient acquisition and the growth of $N_2$ fixers relative to non-$N_2$ fixers.

Whole-plant $N_2$ fixation increased as soil nutrient availability decreased (Fig. 3), reflecting similar patterns in number of nodules (Appendix S1: Fig. S1). Apparently, the $N_2$ fixers up-regulated their investment in $N_2$ fixation to meet the nutrient demands of high growth when soil N was insufficient, but down-regulated $N_2$ fixation when soil N was plentiful. This facultative strategy of $N_2$ fixation (Menge et al. 2009, Barron et al. 2011, Batterman et al. 2013) may enable $N_2$ fixer growth to benefit in low N soil and to avoid the C cost of fixation in high N soil as the cost of fixation is higher than direct uptake of soil N (Gutschick 1981, Vitousek et al. 2002, Vance 2008, Fisher et al. 2010). In addition, $N_2$ fixation was related to growth rates of $N_2$ fixers in low nutrient soil (Fig. 6a). Though we did not measure the $\delta^{15}N$ of the $N_2$ fixer biomass (which can be used to estimate the contribution of fixed $N_2$ to the overall N nutrition of the

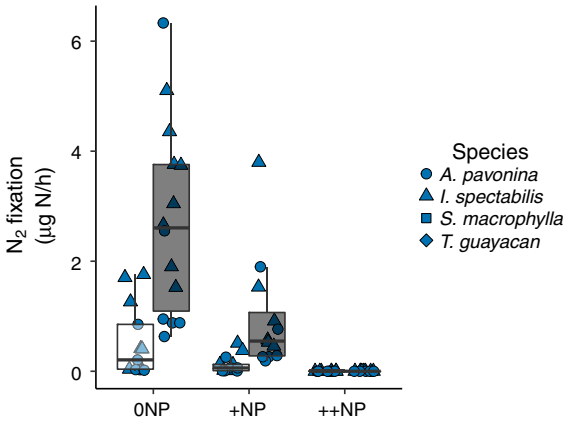


FIG. 3. Whole-plant symbiotic dinitrogen ($N_2$) fixation of the $N_2$-fixing seedlings across the low (0NP), intermediate (+NP), and high (++NP) nutrient treatments, and under ambient (white) and elevated (black) $CO_2$. Box plot mid lines represent medians, boxes represent the 25th and 75th quartile values, whiskers represent 1.5 × the interquartile range, and points represent individual seedlings.

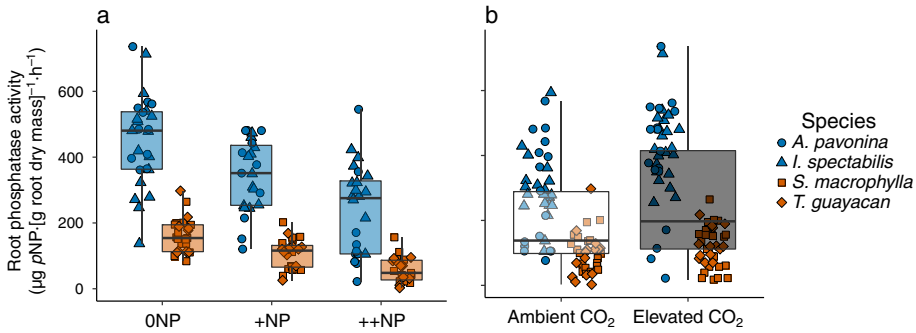


FIG. 4. Root phosphatase activity of the (a) $N_2$-fixing and non-$N_2$-fixing seedlings across the low (0NP), intermediate (+NP), and high (++NP) nutrient treatments and (b) all of the seedlings under ambient and elevated $CO_2$. Box plot mid lines represent medians, boxes represent the 25th and 75th quartile values, whiskers represent 1.5 × the interquartile range, and points represent individual seedlings. The measure *p*NP is *para*-nitrophenol.

$N_2$-fixing seedlings; Shearer et al. 1983), the flexibility of $N_2$ fixation may help explain, in part, consistent $N_2$ fixer growth rates across the wide range of soil nutrient availability in this experiment.

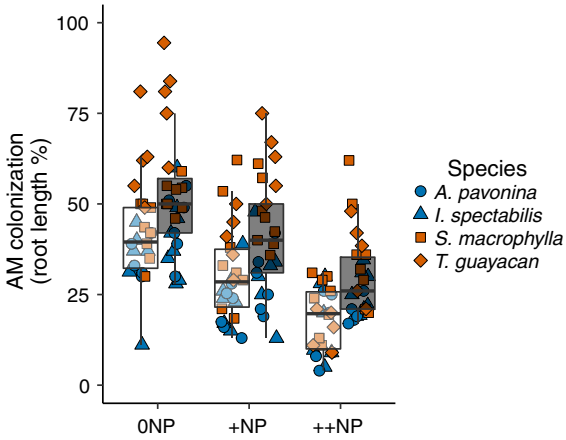


FIG. 5. Arbuscular mycorrhizal (AM) colonization of the $N_2$-fixing and non-$N_2$-fixing seedlings across the low (0NP), intermediate (+NP), and high (++NP) nutrient treatments, and under ambient (white) and elevated (black) $CO_2$. Box plot lines represent medians, boxes represent the 25th and 75th quartile values, whiskers represent 1.5 × the interquartile range, and points represent individual seedlings.

Root phosphatase activity and AM colonization also increased as soil nutrient availability decreased (Fig. 4a and 5, respectively). Such patterns have been reasonably well documented (Treseder 2004, Marklein and Houlton 2011, Png et al. 2017), and suggest an increased demand for P at low fertility. Though root phosphatase activity and AM colonization are not direct measures of P uptake, both strategies are important for P acquisition (Lambers et al. 2008, Smith and Read 2008), and have been linked to the capacity for P uptake (Sanders and Tinker 1971, Ortas et al. 1996, Khaliq and Sanders 2000, Treseder and Vitousek 2001). It is worth noting, however, that this pattern in P acquisition was driven by the responsiveness of one of the two species from each functional group: the $N_2$ fixer *Adenanthera pavonina* and the non-$N_2$ fixer *Tabebuia guayacan.* The latter species is a low-P specialist, occurring predominantly on low P soils in the central Panama region (Condit et al. 2013), suggesting that investment in AM fungi might explain its competitive ability at low fertility.

Despite this species-specific response to nutrient availability, root phosphatase activity was higher in the $N_2$ fixers than the non-$N_2$ fixers (Fig. 4), and was related to $N_2$ fixer growth in low nutrient soil (Fig. 6b). Even more, $N_2$ fixation was positively related to root phosphatase activity (Appendix S1: Fig. S2), and both nutrient acquisition strategies, together, were related to $N_2$ fixer growth in low nutrient soil. If $N_2$ fixers were indeed

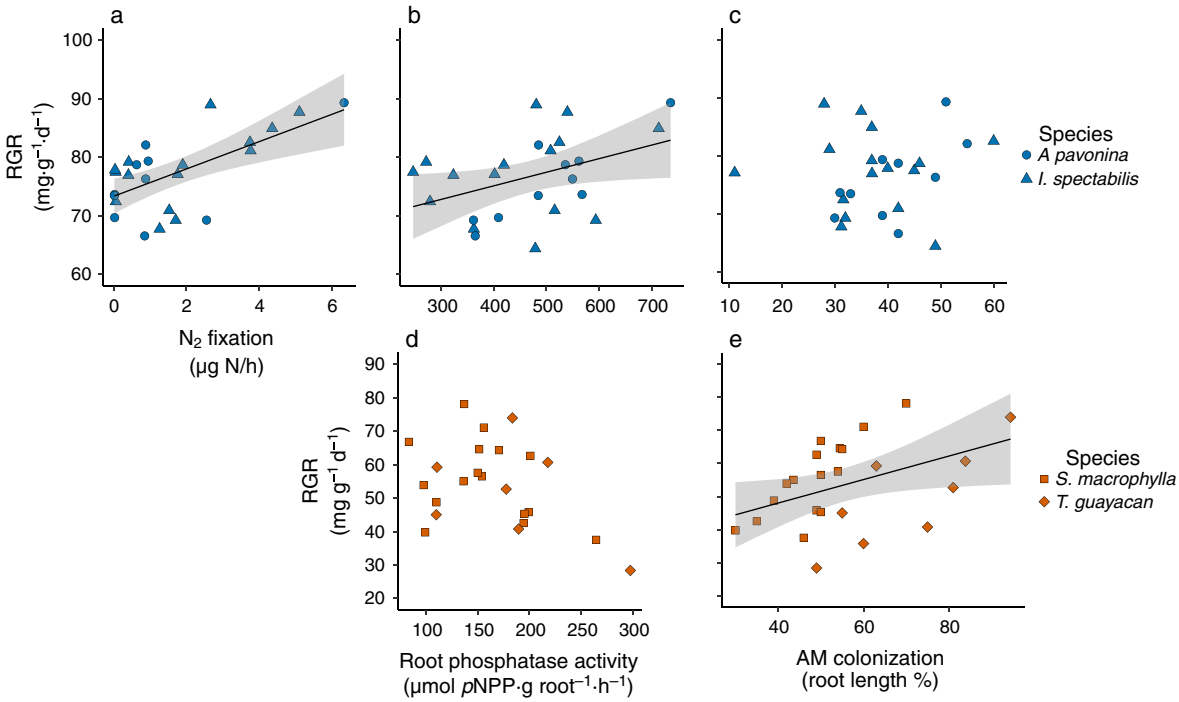


FIG. 6. Relationship between (a) $N_2$ fixation ($P$ = 0.03, $r$ = 0.39), (b) root phosphatase activity ($P$ = 0.03, $r$ = 0.37), and AM colonization ($P$ = 0.53), and (c) the relative growth rate (RGR) of the $N_2$-fixing seedlings grown in the low nutrient treatment and across both $CO_2$ treatments. Relationship between (d) root phosphatase activity ($P$ = 0.06) and (e) AM colonization ($P$ = 0.03, $r$ = 0.40), and the relative growth rate (RGR) of the non-$N_2$-fixing seedlings grown in the low nutrient treatment and across both $CO_2$ treatments.

able to invest fixed N into the production of root phosphatases (Houlton et al. 2008), it would help to explain not only the greater rates of root phosphatase activity in $N_2$ fixers relative to non-$N_2$ fixers (Nasto et al. 2017), but also their pivotal role in sustaining higher growth than non-$N_2$ fixers in low nutrient soil. Alternatively, a plant's investment into the production of root phosphatases may not be dependent on $N_2$ fixation, per se, but the internal N supply of the plant itself (Soper et al. 2018). However, we found no relationship between foliar N:P and root phosphatase activity (Appendix S1: Fig. S3), indicating that N status, in itself, is not a strong control on P acquisition.

By contrast, AM colonization was higher in the non-$N_2$ fixers than the $N_2$ fixers (Fig. 5), and was related to non-$N_2$ fixer growth in low nutrient soil (Fig. 6e). Though it is clear that the non-$N_2$ fixers (specifically, *Tabebuia guayacan*) were flexible in their ability to adjust levels of AM colonization to appropriately match the soil P conditions, it was not sufficient to allow them to maintain their highest levels of growth across the gradient of nutrient availability. The non-$N_2$ fixers had no discernible strategy to acquire enough N to keep pace with demand. Thus, it appears that the enhanced capacity for the $N_2$ fixers to acquire more N and P than the non-$N_2$ fixers conferred a competitive advantage in the $N_2$ fixers over the non-$N_2$ fixers, but only when soil nutrients were scarce.

Nutrient acquisition strategies are not the only mechanisms that could potentially enable the $N_2$ and non-$N_2$ fixers to overcome low soil nutrients. Two additional mechanisms could contribute to the growth of both $N_2$ and non-$N_2$ fixers in low nutrient soil: greater root : shoot allocation and/or a stoichiometric increase in tissue chemistry. Indeed, root : shoot and foliar C:N and C:P increased as nutrient availability decreased (Fig. 2), but the increases were largely attributable to the non-$N_2$ fixers (specifically *Swietenia macrophylla*), which also had higher overall root : shoot, foliar C:N, and foliar C:P than the $N_2$ fixers (Fig. 2). These patterns complement those of the nutrient acquisition strategies in the species from both functional groups in two ways. First, the $N_2$ fixers had a greater capacity than the non-$N_2$ fixers to acquire both N and P in low nutrient soil, likely resulting in the observed insensitivity of root : shoot and foliar stoichiometry to variations in soil nutrient availability. Second, the non-$N_2$ fixers had a relatively limited ability to acquire both N and P in low nutrient soil, and likely relied on the adjustments of root : shoot and foliar stoichiometry (especially for *Swietenia macrophylla* considering its lack of response in root phosphatase activity and AM colonization) to overcome nutrient-poor soil conditions. However, such adjustments were clearly not sufficient, as growth of the non-$N_2$ fixers declined in the low nutrient soil.

Though the growth of the $N_2$ and non-$N_2$ fixers were higher under elevated than ambient $CO_2$ (Fig. 1b), the effect of $CO_2$ was not dependent on functional group (Table 1). In other words, the $N_2$ and non-$N_2$ fixers had similar (and positive) growth responses to elevated $CO_2$. This is a surprising result given that $N_2$ fixers are thought to respond more strongly to elevated $CO_2$ than non-$N_2$ fixers because $N_2$ fixers have this greater capacity to acquire N and P in low nutrient soil (Thomas et al. 1991, Tissue et al. 1996, Cernusak et al. 2011). Indeed, $N_2$ fixation (Fig. 3), root phosphatase activity (Fig. 4), and AM colonization (Fig. 5) were all higher under elevated than ambient $CO_2$. Greater access to C (Tissue et al. 1996, Hungate et al. 1999; Fitter et al. 2000, de Graaff et al. 2006, Cernusak et al. 2011, Jakobsen et al. 2016, Terrer et al. 2016) and stoichiometric demand for N and P (Barrett et al. 1998, Niu et al. 2013) likely led to the observed increase in nutrient acquisition strategies under elevated $CO_2$. However, these increases were not dependent on functional group, either, and thus did not confer a greater response of one functional group over the other to elevated $CO_2$.

A greater benefit to the $N_2$ fixers over the non-$N_2$ fixers from elevated $CO_2$ might have appeared if our study had been longer. For example, exposure to elevated $CO_2$ often increases the photosynthetic C uptake of plants, which increases the availability of carbohydrate and can translate to increased growth (Leakey et al. 2009). However, an increase in carbohydrate alters the C and N metabolism of the plant. As a result, the plant eventually acclimates by reducing synthesis of Rubisco, which ultimately lowers photosynthetic C uptake (Long et al. 2004, Nowak et al. 2004, Ainsworth and Long 2005, Ainsworth and Rogers 2007). Though this also lowers the demand for N, and can constrain the extent to which plants can assimilate C for enhanced growth for prolonged periods of time under elevated $CO_2$ (Rogers et al. 2009). Dinitrogen fixers can allocate some of the additional carbohydrate gained from an increase in photosynthetic C uptake to their rhizobial partners to stimulate $N_2$ fixation, allowing $N_2$ fixers to maximize the stimulation of both photosynthetic C uptake and $N_2$ fixation to gain a greater benefit in enhanced growth from elevated $CO_2$ than non-$N_2$ fixers (Rogers et al. 2009). Thus, if we had conducted a longer study we may have detected not only a competitive advantage of the $N_2$ fixers over the non-$N_2$ fixers in nutrient acquisition in low nutrient soil, but also under elevated $CO_2$.

Finally, we note that broad inferences from our results should be made with caution given the limited number of study species, and in light of a growing number of studies pointing to species-specific results. For example, Cernusak et al. (2011) showed variation in the capacity for nodulation among tropical $N_2$ fixers, Nasto et al. (2017) showed that differences in nutrient acquisition strategies between non-$N_2$ and $N_2$ fixers were driven by individual species belonging to each functional group, Zalamea et al. (2016) and Png et al. (2017) suggested that root phosphatase activity is phylogenetically constrained and not related to functional group or $N_2$ fixation, and Soper et al. (2018) demonstrated a lack of a

relationship between $N_2$ fixation, foliar N, and P acquisition among a variety of $N_2$-fixing legumes, non-$N_2$-fixing legumes, and non-legumes. Taken together, these studies suggest that larger numbers of species occupying multiple genera and families are needed to generate more robust relationships between nutrient acquisition strategies and growth, and to conclude fundamental differences between $N_2$ and non-$N_2$ fixers as functional groups.

Nonetheless, our results provide evidence that at least two tropical $N_2$-fixing trees have the capacity to maintain relatively high growth in nutrient-poor soil, and augment growth under elevated $CO_2$, because of their enhanced capacity to acquire N and P. If the mechanisms we invoked here hold true to a wider set of $N_2$-fixing trees, $N_2$ fixers could become increasingly important members of tropical forest communities, both in terms of abundance and function. While we note that the experimental conditions in our study replicated a range of conditions likely to be encountered by these species in the rain forest (e.g., the low nutrient treatment simulated a gap environment, or conditions akin to a secondary rain forest, with relatively high irradiance), it remains unclear whether the results from our seedling experiment reflect nutrient acquisition by mature canopy trees in natural forest or even trees of different life history traits. Additionally, it remains unclear if the higher growth rates realized by $N_2$ fixers are equivocal to higher NPP as respiration and other pathways of C loss may be increasing concurrently. However, it appears that at least two $N_2$-fixing trees may have the capacity to overcome the nutritional constraints to NPP with predicted increases in atmospheric $CO_2$, which could help sustain the C sink strength of tropical rain forests.

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M. K. Nasto, K. Winter, B. L. Turner, and C. C. Cleveland conceived of and designed the study. M. K. Nasto and K. Winter conducted the experiment. M. K. Nasto and B. L. Turner conducted the laboratory analyses. M. K. Nasto wrote the manuscript and K. Winter, B. L. Turner, and C. C. Cleveland contributed to revisions.

## Literature Cited

Ahlström, A., G. Schurgers, A. Arnet, and B. Smith. 2012. Robustness and uncertainty in terrestrial ecosystem carbon response to CMIP5 climate change projections. Environmental Research Letters 7:044008.

Ainsworth, E. A., and S. P. Long. 2005. What have we learned from 15 years of free-air $CO_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising $CO_2$. New Phytologist 165:351–372.

Ainsworth, E. A., and A. Rogers. 2007. The response of photosynthesis and stomatal conductance to rising [$CO_2$]: mechanisms and environmental interactions. Plant, Cell, and Environment 30:258–270.

Alvarez-Clare, S., M. C. Mack, and M. Brooks. 2013. A direct test of nitrogen and phosphorus limitation to net primary productivity in a lowland tropical wet forest. Ecology 94:1540–1551.

Barrett, D. J., A. E. Richardson, and R. M. Gifford. 1998. Elevated atmospheric $CO_2$ concentrations increase wheat root phosphatase activity when growth is limited by phosphorus. Australian Journal of Plant Physiology 25:87–94.

Barron, A. R., D. W. Purves, and L. O. Hedin. 2011. Facultative nitrogen fixation by canopy legumes in a lowland tropical forest. Oecologia 165:511–520.

Batterman, S. A., N. Wurzburger, and L. O. Hedin. 2013. Nitrogen and phosphorus interact to control tropical symbiotic $N_2$ fixation: a test in *Inga punctata*. Journal of Ecology 101:1400–1408.

Batterman, S. A., J. S. Hall, B. L. Turner, L. O. Hedin, J. K. LaHaela Walter, P. Sheldon, and M. van Breugel. 2018. Phosphate activity and nitrogen fixation reflect species differences, not nutrient trading or nutrient balance, across tropical rainforest trees. Ecology Letters 21:1486–1495.

Beer, C., et al. 2010. Terrestrial gross carbon dioxide uptake: global distribution and covariation with climate. Science 329:834–838.

Cernusak, L. A., K. Winter, C. Martínez, E. Correa, J. Aranda, M. Garcia, C. Jaramillo, and B. L. Turner. 2011. Responses of legume versus nonlegume tropical tree seedlings to elevated $CO_2$ concentration. Plant Physiology 157:372–385.

Cernusak, L. A., et al. 2013. Tropical forest responses to increasing atmospheric $CO_2$: current knowledge and opportunities for future research. Functional Plant Biology 40:531–551.

Condit, R., S. P. Hubbell, and R. B. Foster. 1996. Changes in tree species abundance in a Neotropical forest: impact of climate change. Journal of Tropical Ecology 12:231–256.

Condit, R., B. M. J. Engelbrecht, D. Pino, R. Pérez, and B. L. Turner. 2013. Species distributions in response to individual soil nutrients and seasonal drought across a community of tropical trees. Proceedings of the National Academy of Sciences USA 110:5064–5068.

de Graaff, M.-A., K.-J. van Groenigen, J. Six, B. Hungate, and C. van Kessel. 2006. Interactions between plant growth and soil nutrient cycling under elevated $CO_2$: a meta-analysis. Global Change Biology 12:2077–2091.

Fisher, J. B., S. Sitch, Y. Malhi, R. A. Fisher, C. Huntingford, and S. Y. Tan. 2010. Carbon cost of plant nitrogen acquisition: a mechanistic, globally applicable model of plant nitrogen uptake, retranslocation, and fixation. Global Biogeochemical Cycles 24:1–17.

Fitter, A. H., A. Heinemeyer, and P. L. Staddon. 2000. The impact of elevated $CO_2$ and global climate change on arbuscular mycorrhizas: a mycocentric approach. New Phytologist 147:179–187.

Foley, J., et al. 2005. Global consequences of land use. Science 309:570–574.

Gentry, A. H. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. Annals of the Missouri Botanical Garden 75:1–34.

Grose, S. O., and R. G. Olmstead. 2007. Taxonomic revisions in the polyphyletic genus *Tabebuia* s. l. (Bignoniaceae). Systematic Botany 32:660–670.

Gutschick, V. P. 1981. Evolved strategies in nitrogen acquisition by plants. American Naturalist 118:607–637.

Hardy, R. W. F., R. D. Holsten, E. K. Jackson, and R. C. Burns. 1968. The acetylene-ethylene assay for $N_2$ fixation: laboratory and field evaluation. Plant Physiology 43:1185–1207.

Houlton, B. Z., Y. P. Wang, P. M. Vitousek, and C. B. Field. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. Nature 454:327–330.

Hungate, B. A., P. Dijkstra, D. W. Johnson, C. R. Hinkle, and B. G. Drake. 1999. Elevated $CO_2$ increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. Global Change Biology 7:781–790.

Hungate, B. A., J. S. Dukes, R. Shaw, Y. Lou, and C. B. Field. 2003. Nitrogen and climate change. Science 302: 1512–1513.

Hunt, R., and J. H. C. Cornelissen. 1997. Components of relative growth rate and their interrelations in 59 temperate plant species. New Phytologist 135:395–417.

Jakobsen, I., S. E. Smith, A. Smith, S. J. Watts-Williams, S. S. Clausen, and M. Grønlund. 2016. Plant growth responses to elevated atmospheric $CO_2$ are increased by phosphorus sufficiency but not by arbuscular mycorrhizas. Journal of Experimental Botany 67:6173–6186.

Jobbagy, E. G., and R. B. Jackson. 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications 10:423–436.

Khaliq, A., and F. E. Sanders. 2000. Effects of vesicular-arbuscular mycorrhizal inoculation on the yield and phosphorus uptake of field-grown barley. Soil Biology and Biochemistry 32:1691–1696.

Koske, R. E., and J. N. Gemma. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92:486–505.

Lambers, H., J. Raven, G. Shaver, and S. Smith. 2008. Plant nutrient-acquisition strategies change with soil age. Trends in Ecology and Evolution 23:95–103.

Leakey, A. D. B., E. A. Ainsworth, C. J. Bernacchi, A. Rogers, S. P. Long, and D. R. Ort. 2009. Elevated $CO_2$ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany 60:2859–2876.

Long, S. P., E. A. Ainsworth, A. Rogers, and D. R. Ort. 2004. Rising atmospheric carbon dioxide: plants FACE the future. Annual Review of Plant Biology 55:591–628.

Losos, E. C., and E. G. Leigh. 2004. Tropical forest diversity and dynamics. University of Chicago Press, Chicago, Illinois, USA.

Marklein, A. R., and B. Z. Houlton. 2011. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytologist 193:696–704.

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115:495–501.

Menge, D. N. L., S. A. Levin, and L. O. Hedin. 2009. Facultative versus obligate nitrogen fixation strategies and their ecosystem consequences. American Naturalist 174: 465–477.

Nasto, M. K., S. Alvarez-Clare, Y. Lekberg, B. W. Sullivan, A. R. Townsend, and C. C. Cleveland. 2014. Interactions among nitrogen fixation and soil phosphorus acquisition in lowland tropical rain forests. Ecology Letters 17:1282–1289.

Nasto, M. K., B. B. Osborne, Y. Lekberg, G. P. Asner, C. S. Balzotti, S. Porder, P. G. Taylor, A. R. Townsend, and C. C. Cleveland. 2017. Nutrient acquisition, soil phosphorus partitioning, and competition among trees in a lowland tropical rain forest. New Phytologist 3:4243–4255.

Niu, Y., R. Chai, H. Dong, H. Wang, C. Tang, and Y. Zhang. 2013. Effect of elevated $CO_2$ on phosphorus nutrition of phosphate-deficient *Arabidopsis thaliana* (L.) Heynh under different nitrogen forms. Journal of Experimental Botany 64:355–367.

Nowak, R. S., D. S. Ellsworth, and S. D. Smith. 2004. Functional responses of plants to elevated atmospheric $CO_2$ – do photosynthetic and productivity data from FACE experiments support early predictions? New Phytologist 162:253–280.

Ortas, I., P. J. Harris, and D. L. Rowell. 1996. Enhanced uptake of phosphorus by mycorrhizal sorghum plants as influenced by forms of nitrogen. Plant and Soil 184:255–262.

Png, G. K., B. L. Turner, F. E. Albornoz, P. E. Hayes, H. Lambers, and E. Laliberté. 2017. Greater root phosphatase activity in nitrogen-fixing rhizobial but not actinorhizal plants with declining phosphorus availability. Journal of Ecology 38:1537–1547.

Poorter, H., J. Bühler, D. van Dusschoten, J. Climent, and J. A. Postma. 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology 39:839–850.

Poorter, L., et al. 2008. Are functional traits good predictors of demographic rates? Evidence from five neotropical forests. Ecology 89:1908–1920.

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rees, M., C. P. Osborne, F. I. Woodward, S. P. Hulme, L. A. Turnbull, and S. H. Taylor. 2010. Partitioning the components of relative growth rate: how important is plant size variation? The American Naturalist 176:152–161.

Rogers, A., E. A. Ainsworth, and A. D. B. Leakey. 2009. Will elevated carbon dioxide concentration amplify the benefits of nitrogen fixation in legumes? Plant Physiology 3:1009–1016.

Roy, J., B. Saugier, and H. A. Mooney. 2001. Terrestrial global productivity. Academic Press, San Diego, California, USA.

Saatchi, S. S., et al. 2011. Benchmark map of forest carbon stocks in tropical regions across three continents. Proceedings of the National Academy of Sciences USA 108:9899–9904.

Sanders, F. E., and P. B. Tinker. 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature 233:278–279.

Schlesinger, W. H., and E. S. Bernhardt. 2013. Biogeochemistry: an analysis of global change. Academic Press, Waltham, Massachusetts, USA.

Shearer, G., D. H. Kohl, R. A. Virginia, B. A. Bryan, J. L. Skeeters, E. T. Nilsen, M. R. Sharifi, and P. W. Rundel. 1983. Estimates of $N_2$ fixation from variation in the natural abundance of N-15 in Sonoran Desert ecosystems. Oecologia 56:365–373.

Smith, S. E., and D. J. Read. 2008. Mycorrhizal symbiosis. Third edition. Academic Press, San Diego, California, USA.

Soper, F. M., M. K. Nasto, B. B. Osborne, and C. C. Cleveland. 2018. Nitrogen fixation and foliar nitrogen do not predict phosphorus acquisition in tropical trees. Journal of Ecology 131:173–179.

Sprent, J. I. 2009. Legume nodulation: a global perspective. Wiley-Blackwell, Oxford, UK.

Sullivan, B. W., W. K. Smith, A. R. Townsend, M. K. Nasto, S. C. Reed, R. L. Chazdon, and C. C. Cleveland. 2014. Spatially robust estimates of biological nitrogen (N) fixation imply substantial human alteration of the tropical

N cycle. Proceedings of the National Academy of Sciences USA 111:8101–8106.

ter Steege, H., et al. 2006. Continental-scale patterns of canopy tree composition and function across Amazonia. Nature 443:444–447.

Terrer, C., S. Vicca, B. A. Hungate, R. P. Phillips, and I. C. Prentice. 2016. Mycorrhizal association as a primary control of the $CO_2$ fertilization effect. Science 353:72–74.

Thomas, R. B., D. D. Richter, H. Ye, P. R. Heine, and B. R. Strain. 1991. Nitrogen dynamics and growth of seedlings of an N-fixing tree (*Gliricidia sepium* (Jacq.) Walp.) exposed to elevated atmospheric carbon dioxide. Oecologia 88:415–421.

Tissue, D. T., J. P. Megonigal, and R. B. Thomas. 1996. Nitrogenase activity and $N_2$ fixation are stimulated by elevated $CO_2$ in a tropical $N_2$-fixing tree. Oecologia 109:28–33.

Todd-Brown, K. E. O., et al. 2014. Changes in soil organic carbon storage predicted by Earth system models during the 21st century. Biogeoscience 11:2341–2356.

Treseder, K. K. 2004. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric $CO_2$ in field studies. New Phytologist 164:347–355.

Treseder, K. K., and P. M. Vitousek. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. Ecology 82:1–9.

Turner, B. L., R. Baxter, N. Ellwood, and B. A. Whitton. 2001. Characterizations of the phosphatase activities of mosses in relation to their environment. Plant, Cell, and Environment 24:1165–1176.

Vance, C. P. 2008. Carbon and nitrogen metabolism in legume nodules. Pages 293–320 *in* M. J. Dilworth, E. K. James, J. I. Sprent, and W. E. Newton, editors. Nitrogen-fixing leguminous symbioses. Springer, Dordrecht, The Netherlands.

Vitousek, P. M., et al. 2002. Towards an ecological understanding of biological nitrogen fixation. Biogeochemistry 57:1–45.

Wieder, W. R., C. C. Cleveland, W. K. Smith, and K. Todd-Brown. 2015. Future productivity and carbon storage limited by terrestrial nutrient availability. Nature Geoscience 8:441–444.

Wright, S. J., et al. 2011. Potassium, phosphorus or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. Ecology 92:1616–1625.

Wurzburger, N., and J. S. Wright. 2015. Fine-root responses to fertilization reveal multiple nutrient limitation in a lowland tropical forest. Ecology 96:2137–2146.

Zalamea, P. C., B. L. Turner, K. Winter, A. Jones, C. Sarmiento, and J. W. Dalling. 2016. Seedling growth responses to phosphorus reflect adult distribution patterns of tropical trees. New Phytologist 212:400–408.

## Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.2646/suppinfo