

## RESEARCH ARTICLE

# Functional groups, species and light interact with nutrient limitation during tropical rainforest sapling bottleneck

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**Abstract**

1. Potential variability in nutrient limitation among tree size classes, functional groups and species calls for an integrated community- and ecosystem-level perspective of lowland tropical rainforest nutrient limitation. In particular, canopy trees determine ecosystem nutrient conditions, but competitive success for nutrients and light during the sapling bottleneck determines canopy composition.
2. We conducted an in situ multi-nutrient sapling fertilization experiment at La Selva Biological Station, Costa Rica, to determine how functional group identity, species identity and light availability can impact nutrient limitation of stem growth in three functional groups and nine species.
3. Despite high soil fertility, we found nutrient-light limitation in two functional groups and four species. Unexpectedly, the nitrogen-fixing ("N<sub>2</sub> fixers") and shade-tolerant functional groups were significantly nutrient limited, while the light-demanding functional group was not.
4. This was partially explained by species-level variation in nutrient limitation within these functional groups, with only some species conforming to the prediction of stronger nutrient limitation in light demanders compared to shade-tolerants.
5. Most surprisingly, we found strong nutrient limitation at low-light levels in the N<sub>2</sub> fixers (which were shade-tolerant), but not in the shade-tolerant non-fixers. We hypothesize that the N<sub>2</sub> fixers were actually nitrogen limited at low-light levels because of their nitrogen-rich leaves and the high carbon cost of their symbionts.
6. This finding suggests a highly shade-tolerant, N<sub>2</sub> fixation strategy, in addition to the perception that N<sub>2</sub> fixation is mostly advantageous in high-light environments during early and gap succession. The shade-tolerant, N<sub>2</sub> fixation strategy may be part of a sapling and canopy tree feedback, where the canopy N<sub>2</sub> fixers enrich the soil N, enhancing growth of their shade-tolerant saplings relative to non-fixing competitors, enabling further canopy domination by shade-tolerant N<sub>2</sub> fixers, as seen at La Selva.
7. *Synthesis.* The pervasiveness of functional group- and species-specific nutrient and light co-limitation in our saplings indicates that these interactions likely play an important role in the dynamics of lowland tropical rainforest nutrient limitation, potentially via other such sapling and canopy tree feedbacks as the one hypothesized.

**KEYWORDS**

co-limitation, Costa Rica, fertilization, gap succession, La Selva Biological Station, light limitation, lowland tropical rainforest, nitrogen fixation, plant–soil (below-ground) interactions, tropical trees

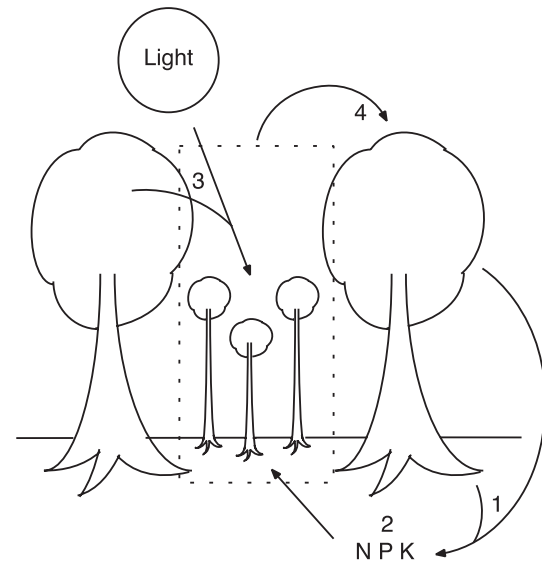
**1 | INTRODUCTION**

Understanding tropical tree growth limitation by nutrients such as nitrogen (N), phosphorus (P) and potassium (K) is fundamental for predicting the dynamic response of lowland tropical rainforests to future climatic conditions and their persistence as large carbon sinks (Huntingford et al., 2013; Körner, 2009; Santiago, 2015). A small number of in situ fertilization experiments have found mixed evidence of the extent to which nutrients limit tree growth in these forests and differences in the identity of the limiting nutrient(s) (Alvarez-Clare, Mack, & Brooks, 2013; Fisher et al., 2013; Mirmanto, Proctor, Green, Nagy, & Suriantata, 1999; Newbery et al., 2002; Wright et al., 2011). These inconsistencies may be due to the hypothesized “heterogeneous nutrient limitation” (sensu Alvarez-Clare et al., 2013) in lowland tropical rainforests, where variability in nutrient responses depends on differences among tree taxa and size classes, but these differences, especially among taxa, have yet to be comprehensively tested.

Although heterogeneity of nutrient limitation might be expected given the high diversity of lowland tropical rainforests, most of these previous in situ studies evaluated growth responses at the ecosystem scale. The potential that tree properties such as size class, taxonomic identity or functional group identity may complicate forest response to nutrients indicate the need to examine limitation also at the community, population and individual scales. At these scales, there is a central nutrient-light feedback between saplings and canopy trees, where success in competing for nutrients and light at the sapling stage determines which individuals survive the bottleneck passage into the canopy, and in turn these canopy trees determine ecosystem-level nutrient cycling and understorey light availability, influencing sapling success (Figure 1).

Therefore, to understand the dynamics of lowland tropical rainforest nutrient limitation, it is essential to examine how nutrients and light interact to determine the success of individual saplings as they experience the bottleneck transition to the canopy, with >90% of sapling mortality events occurring before they reach 4 cm in diameter (Clark & Clark, 1992). This transition is typically associated with treefall gaps, which provide the elevated light levels that a majority of species need at some point during their ontogeny in order to reach the canopy (Brokaw, 1985; Denslow, 1980, 1987). Due to the asymmetry of light availability from the top of the canopy to the shaded forest floor, compared to larger trees in the canopy and sub-canopy, saplings in the understorey experience a full range of light availabilities, from desirable gap environments to undesirable non-gap environments (Wright et al., 2010; Yoda, 1974).

This uncertain availability, but necessity, of gaps for individual success during the sapling bottleneck has selected for rapid sapling growth



**FIGURE 1** Sapling and canopy tree feedbacks are central to lowland tropical rainforest nutrient dynamics. Canopy trees dominate feedbacks to ecosystem-level nutrient cycling by providing a large proportion of ecosystem foliar, wood and root litter inputs with functional group- or species-specific nutrient concentrations (1). This in turn can result in functional group- or species-specific impacts on decomposition rates and total fluxes of nutrient inputs from litter pools to the soil (2). Additionally, these canopy trees may also impact understorey light availability in functional group- or species-specific ways based on their crown structure, and this light availability interacts with understorey nutrient dynamics as well (3). However, nutrient-light limitation of saplings during the bottleneck to reach the canopy (dotted box) determines which individuals become canopy trees (4). Sapling response to soil nutrients and light, and corresponding competitive success during this bottleneck, may also be functional group- or species-specific

rates under favourable high-light conditions (Clark & Clark, 1992; Denslow, 1987). Rapid growth and biomass accumulation increases plant nutrient demand (Montagnini, 2000), raising fundamental questions about the interaction between nutrient and light limitation at the sapling stage. Previous studies of understorey nutrient limitation and light interactions in lowland tropical rainforests focused on tree seedlings or shrub cuttings in shade houses and common gardens, and found either no response to nutrients (Denslow, Schultz, Vitousek, & Strain, 1990) or potentially species-specific responses (Fetcher et al., 1996; Palow & Oberbauer, 2009). More recently, in situ studies showed light but not nutrient limitation of understorey sapling growth (Magalhães, Marenco, & Camargo, 2014), nutrient limitation of low-light understorey tree seedling growth (Pasquini & Santiago, 2012; Santiago et al., 2012), and approximately equal contributions by light

and nutrients to understorey woody plant seedling growth (Holste, Kobe, & Vriesendorp, 2011). These studies suggest that saplings of at least some functional groups or species may be nutrient limited even in low-light understorey conditions, although the strength of this limitation likely increases as greater light availability elevates sapling growth rates and nutrient demand.

Furthermore, these studies indicate that nutrient and light limitation of sapling growth may differ across the wide array of tree strategies for resource acquisition (Reich, Walters, & Ellsworth, 1997), which can be observed at the level of species, or at a coarser scale, functional groups of species that respond to environmental variables similarly. A well known, but complicated gradient of resource acquisition strategies is tied to shade tolerance (Clark & Clark, 1992; Pacala et al., 1996), with a major trade-off between growth in high light and survival in low light (Wright et al., 2010). Species in the light-demanding functional group are less shade-tolerant and tend to have traits that allow for quick growth but lower nutrient use efficiency (NUE), such as short leaf life span, low leaf mass per area, high leaf nutrient concentration and low wood density (Poorter & Bongers, 2006; Swaine & Whitmore, 1988).

In contrast, shade-tolerant species tend to have traits at the opposite end of the spectrum that result in slower growth and higher NUE, with leaves that are well defended against herbivory and environmental stress. Thus, although saplings in both the light-demanding and shade-tolerant functional groups may be nutrient limited in the low-light understorey (and to an increasing degree with higher light availability), the strength of this limitation is likely greater in light-demanding saplings across all light levels due to their lower NUE.

A tree resource acquisition strategy with a direct impact on nutrient cycling is symbiotic  $N_2$  fixation, which in the tropics is largely carried out by species (hereafter " $N_2$  fixers") in the Fabaceae family that can host  $N_2$ -fixing rhizobial bacteria in root nodules to access atmospheric  $N_2$ . The ability to fix  $N_2$  gives  $N_2$  fixers a competitive advantage in environments where N demand is high relative to supply, such as during secondary or gap succession (Batterman et al., 2013; Menge & Chazdon, 2016). As  $N_2$  fixers are not directly constrained by soil N, they are likely limited by other nutrients, particularly P because the  $N_2$  fixation process raises demand for P (Vitousek & Howarth, 1991), and also molybdenum (Mo) which is a co-factor in the nitrogenase enzyme (Barron et al., 2009). Beyond their symbiotic relationship itself, other aspects of  $N_2$  fixer physiology that may be related to their  $N_2$  fixation lifestyle are distinctive compared to that of non-fixing trees. These characteristics include high leaf N concentrations and thus high N requirements, as well as greater water use efficiency (Adams, Turnbull, Sprent, & Buchmann, 2016; McKey, 1994). Although this suite of traits may complicate  $N_2$  fixer responses to nutrients, it is plausible that they are also nutrient limited in the low-light understorey (and to an increasing degree as higher light availability elevates growth and nutrient demand). However,  $N_2$  fixers may be less nutrient limited across all light levels than non-fixing saplings of similar shade tolerance due to their ability to fix  $N_2$  in response to N limitation.

We conducted an in situ multi-nutrient fertilization experiment (N, P, K and micronutrients) of naturally occurring saplings in a lowland

rainforest to directly test for interactions among nutrient limitation, light availability and functional group or species identities. To examine sapling responses to fertilization and light availability, we used stem growth, the most common metric of whole tree performance and also the most practical metric in this case, due to the challenge of measuring below-ground growth both at the individual scale and in an in situ experiment. Specifically, our experiment was designed to test the following hypotheses:  $H_1$ : Light-demanding saplings are more nutrient limited than shade-tolerant, non-fixing saplings across all light levels;  $H_2$ : Shade-tolerant, non-fixing saplings are more nutrient limited than shade-tolerant,  $N_2$ -fixing saplings across all light levels; and  $H_3$ : Nutrient-limited functional groups and species are co-limited by light, with greater light availability amplifying the degree of nutrient limitation.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

We conducted the experiment in the lowland tropical rainforest of northeastern Costa Rica at La Selva Biological Station (10°26'N, 83°59'W). The forest at La Selva is classified as tropical wet forest in the Holdridge life-zone system (Hartshorn, 1983; Holdridge, 1967). Mean annual temperature is 25.8°C and mean annual precipitation is 3,962 mm, with no true dry season as no months receive <100 mm rainfall (Sanford, Paaby, Luvall, & Phillips, 1994). Soils at La Selva are among the most fertile found in neotropical lowland rainforests in terms of N and P, but have lower base cation availability than many other tropical soils (Powers, Treseder, & Lerdau, 2005; Vitousek & Matson, 1988). We established the experiment on primarily residual ultisol soils that have consistent chemical and morphological characteristics (Sollins, Sancho, Mata, & Sanford, 1994), in a mix of old growth and regenerating forest, with an average elevation of approximately 100 m.

### 2.2 | Experimental design

We selected nine common species of canopy trees at La Selva belonging to three functional groups—light-demanding, shade-tolerant and  $N_2$ -fixing (O. Vargas, pers. comm.). Due to the complexity of the shade tolerance growth-mortality trade-off, we used a single trait, seed germination shade tolerance, to sort species into general shade tolerance categories (Clark & Clark, 1992; Swaine & Whitmore, 1988). We defined light-demanding species (*Casearia arborea*, *Laetia procera* and *Simarouba amara*) as requiring gap light conditions for seed germination and shade-tolerant species (*Hernandia didymantha*, *Protium pittieri*, *Virola koschnyi*) as capable of germinating in shaded understorey. As La Selva has an unusual abundance of shade-tolerant  $N_2$  fixers in the canopy (Hartshorn & Hammel, 1994; Lieberman & Lieberman, 1987), we chose three  $N_2$ -fixing species (*Inga pezizifera*, *Inga thibaudiana* and *Pentaclethra macroloba*) that were shade-tolerant by our classification scheme. Given that specific  $N_2$  fixation rates were not a focus of our study and that our species are known to nodulate and

actively fix  $N_2$  at La Selva and in the broader Central American region (Batterman et al., 2013; Carpenter, 1992), we did not measure  $N_2$  fixation rates. Doing so would have required repeated destructive root sampling throughout the study, which would have impacted the growth responses of interest.

In August and September 2012, we identified 235 naturally growing saplings in the forest, approximately 26 individuals per species, in a gradient of light conditions ranging from closed canopy to the largest canopy gaps we could find. Saplings ranged from 2.5 to 27 mm in diameter and 32 to 312 cm in height. We fertilized approximately half of the individuals of each species across the light gradient with a slow-release fertilizer (Miracle-Gro® Tree & Shrub Fertilizer Spikes; The Scotts Company, Marysville, OH, USA) containing N (15%), P (5%), K (10%) plus micronutrients: sulphur, iron and manganese, and repeated fertilization every 6 months during the 2.5 years of the experiment. Saplings received one fertilizer stake per application event, which was broken into four evenly sized pieces and buried 5 cm below the surface 0.6 m away from the stem in the cardinal directions. This resulted in the application of 0.0340 kg N, 0.0113 kg P, and 0.0227 kg K per sapling per year, and assuming the nutrients spread to 2 m<sup>2</sup> around each sapling, the application rate was approximately 170 kg N ha<sup>-1</sup> year<sup>-1</sup>, 57 kg P ha<sup>-1</sup> year<sup>-1</sup> and 114 kg K ha<sup>-1</sup> year<sup>-1</sup>, which scales to about 142% of N inputs, 1,256% of P inputs and 757% of K inputs from litterfall measured in this forest (Wood, Lawrence, & Clark, 2006).

### 2.3 | Census measurements

For every sapling, we measured stem diameter, stem height and light availability every 6 months over the 2.5 years of the study, thus six times total for each variable. We also measured foliar nutrient concentrations for each individual, but the responses were complex and we therefore treat them in a separate contribution. During each census, we measured stem diameter to the nearest 0.1 mm using callipers at a marked point of measurement below the lowest branch and away from stem irregularities at heights of 0, 40 or 130 cm when possible (Clark & Clark, 1992). For saplings >4 cm in diameter or for those that had highly non-cylindrical stems, we used a diameter tape to measure the stem to the nearest millimetre. We measured all stems of multi-stemmed saplings at the same point of measurement to calculate a diameter equivalent to that of a single-stemmed tree of equal basal area.

Additionally, during each census, we measured sapling height to the nearest millimetre using a folding 2-m ruler, or when necessary to the nearest centimetre using an extendable 3- or 15-m measuring pole. We defined height as the perpendicular distance between the ground and tallest meristem, except in approximately 3% of the saplings, where due to architectural form, growth was consistently in a bent direction throughout the study period, causing a sapling to become shorter with time absent any breakage. In these cases, we measured the bent stem length between the ground and furthest meristem, and found this to be an appropriate proxy for height growth, as inclusion or exclusion of these points did not fundamentally impact our results or conclusions.

Finally, we also quantified light availability for each sapling at each census by taking a hemispherical photograph at the height of its tallest leaf using a Nikon Coolpix 4500 camera equipped with the Nikon FC-E8 Fisheye Converter (Nikon, Tokyo, Japan), which was mounted on a gyroscopic pole to allow for level pictures at greater heights. Photos were taken pre-dawn or on uniformly cloudy days, and were analysed using Gap Light Analyzer Version 2.0 (Frazer, Canham, & Lertzman, 1999) to quantify total transmitted radiation.

### 2.4 | Tree growth analysis

We analysed tree growth responses to fertilization and light availability using total growth between the first and last census in the 2.5-year study period to capture the strongest signal of tree response to these resources while minimizing measurement errors that may be associated with the short census intervals. We also found similar results from a more complex repeated measures analysis that used the data from each census (see Appendix S1). Although we examined both diameter and height growth, we centre our interpretation on the diameter results as measurements of sapling diameter growth are inherently less variable than measurements of sapling height growth, which tend to include breakage and height loss.

For both diameter and height growth, we used relative growth rate (RGR) as the response variable in order to account for the effect of tree size on growth rate, where  $RGR = \ln(\text{size}_{\text{final}}/\text{size}_{\text{initial}})/(\text{number of study days}/365)$ . Individuals that did not survive the entire 2.5-year study period were excluded from all analyses, and individuals with multiple stems that had negative diameter growth due to stem death and individuals that had negative height growth due to observed stem breakage were excluded from the diameter ( $n = 202$ ) and height ( $n = 200$ ) growth analyses respectively.

We used stepwise linear regression to assess whether each functional group and each species was nutrient limited in its RGR and if this nutrient limitation interacted with light availability, which we calculated for each sapling as its mean light availability across the six censuses. For each functional group and species, we began with the maximal model, where  $RGR \sim \text{fertilization treatment} \times \text{light availability}$ , and simplified to the minimal adequate model, which contains only significant explanatory variables and interactions. We confirmed that regression assumptions were met in the residuals of each model and also tested for influential points using Cook's distance.

Functional groups or species that had a significant growth response to fertilization (with or without light interactions) in their minimal adequate model were considered nutrient limited. Although there was variability in the ranges of light availability among the functional groups and species due to the natural experimental design (light-demanding 7.62%–26.33%; shade-tolerant 5.70%–21.01%;  $N_2$ -fixing 5.83%–24.18%; *C. arborea* 7.62%–17.17%; *L. procera* 8.63%–22.29%; *S. amara* 7.84%–26.33%; *H. didymantha* 5.70%–12.83%; *P. pittieri* 6.62%–21.01%; *V. koschnyi* 7.44%–15.34%; *I. pezizifera* 5.83%–24.18%; *I. thibaudiana* 8.80%–17.09%; *P. macroloba* 8.31%–14.15%; all ranges in per cent total transmitted radiation), the linearity of the data reassures us that the linear regression models were suitable for

understanding the relative responses to fertilization and light availability among the functional groups and most species. All statistical analyses were performed in R 3.0.2 (R Core Team, 2013).

### 3 | RESULTS

#### 3.1 | Variable nutrient-light responses across functional groups

We found a unique diameter growth response to nutrients and light in each of the functional groups (Figure 2, see Table 1 for detailed results from all diameter RGR models). All groups responded significantly and positively to the effect of light alone ( $p < .001$ ) and in addition, some groups responded positively and some negatively to fertilization  $\times$  light interactions.

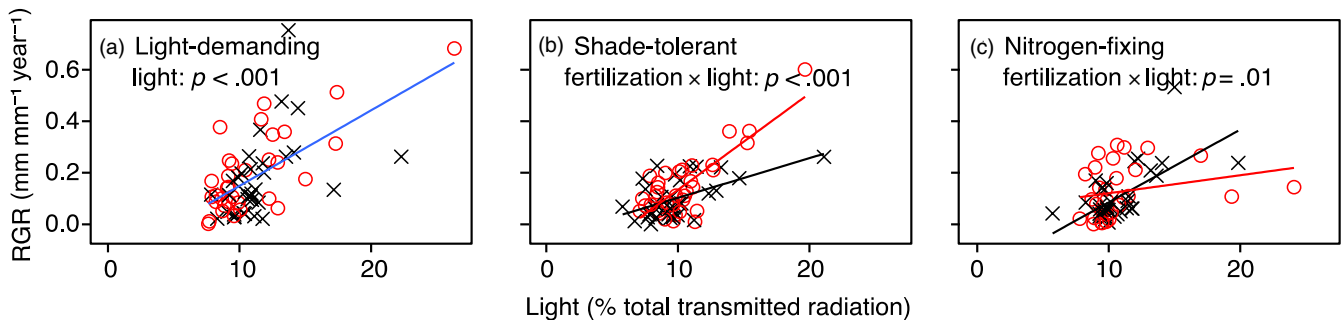
The light-demanding functional group did not respond significantly to fertilization (Figure 2a). In contrast, the shade-tolerant functional group showed a significant positive growth response to a fertilization  $\times$  light interaction ( $p < .001$ ; Figure 2b). As a result, fertilization

increased the slope of the positive relationship between RGR and light availability by 2.5 times, so that the response to fertilization increased with light availability. At very low light, there was a slight negative influence of fertilization on growth that was likely the result of the strong positive interaction term, although it is also possible that fertilization mildly suppressed growth in these conditions.

The  $N_2$ -fixing functional group also responded significantly to a fertilization  $\times$  light interaction, but differed from shade-tolerant group in that this interaction was negative ( $p = .01$ ; Figure 2c). For  $N_2$ -fixing saplings, fertilization decreased the slope of the positive relationship between RGR and light availability to one quarter of the unfertilized slope, with saplings responding positively to fertilization at low light and negatively at high light.

#### 3.2 | Variable nutrient-light responses across species

Although we did not find a response to fertilization in the light-demanding functional group as a whole, *C. arborea* did respond significantly and positively to a fertilization  $\times$  light interaction



**FIGURE 2** Relationship between diameter relative growth rate (RGR) and light availability by functional group and fertilization treatment (red circles = fertilized and black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each functional group (red = fertilized, black = unfertilized and blue = no significant fertilization treatment) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** Diameter relative growth rate regression table: sample size ( $n$ ), regression parameter estimates, adjusted multiple  $R^2$  and whole model  $p$ -value for the minimal adequate model of each functional group and species. Species are arranged by functional groups (LD = light-demanding, ST = shade-tolerant and NF =  $N_2$ -fixing). NA indicates that the factor or interaction was not included in the minimal adequate model.  $\dagger p < .1$ ;  $*p < .05$ ,  $**p < .01$ ;  $***p < .001$

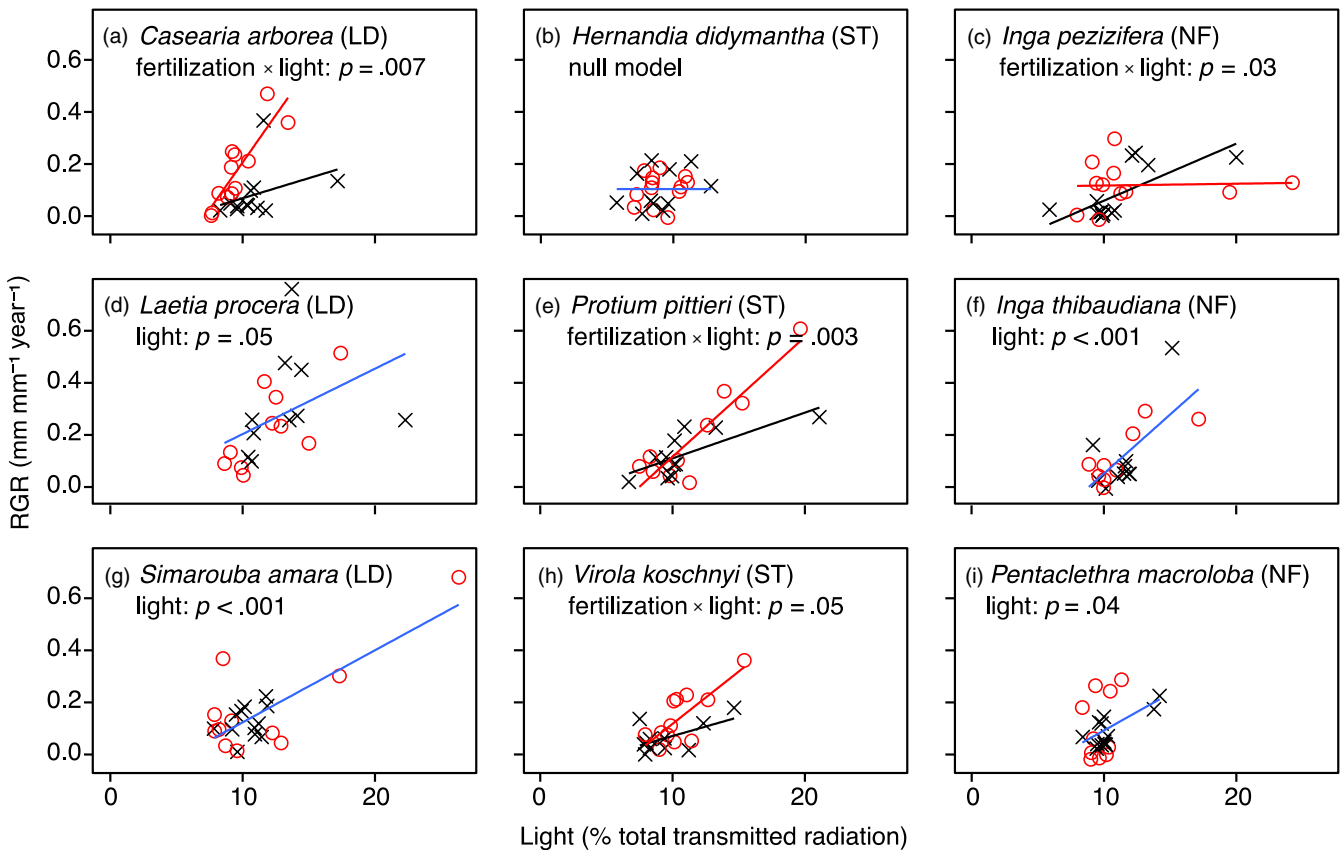
Functional group	$n$	Intercept	Light	Fertilization	Light $\times$ Fertilization	Adjusted $R^2$	Model $p$ -value
Light-demanding	67	-0.14	0.029***	NA	NA	.36	<.001
Shade-tolerant	69	-0.050	0.015***	-0.19**	0.022***	.56	<.001
Nitrogen-fixing	66	-0.20	0.028***	0.25**	-0.021*	.23	<.001
Species							
<i>Casearia arborea</i> (LD)	24	-0.086	0.015	-0.43*	0.057**	.55	<.001
<i>Laetia procera</i> (LD)	20	-0.034	0.024 $\dagger$	NA	NA	.15	.051
<i>Simarouba amara</i> (LD)	23	-0.13	0.027***	NA	NA	.56	<.001
<i>Hernandia didymantha</i> (ST)	24	0.12	NA	NA	NA	NA	NA
<i>Protium pittieri</i> (ST)	22	-0.071	0.018**	-0.28*	0.028**	.77	<.001
<i>Virosa koschnyi</i> (ST)	23	-0.073	0.015 $\dagger$	-0.21	0.026 $\dagger$	.57	<.001
<i>Inga pezizifera</i> (NF)	24	-0.14	0.021**	0.26*	-0.021*	.24	.038
<i>Inga thibaudiana</i> (NF)	19	-0.38	0.044***	NA	NA	.48	<.001
<i>Pentaclethra macroloba</i> (NF)	23	-0.17	0.028*	NA	NA	.15	.041

( $p = .007$ ; Figure 3a). Fertilization increased the response slope between RGR and light availability by 4.8 times, so that the growth increase from fertilization was greater with higher light availability, although (as discussed above) there was a slight negative response at very low light. In contrast, we did not find any significant responses to fertilization in *L. procera* or *S. amara*, the other two light-demanding species (Figure 3d,g). However, both *L. procera* ( $p = .05$ ) and *S. amara* ( $p < .001$ ) showed a significant positive growth response to light alone, while *C. arborea* did not.

In the shade-tolerant functional group, we found that *P. pittieri* and *V. koschnyi* responded significantly and positively to fertilization  $\times$  light interactions ( $p = .003$  and  $p = .05$ , respectively; Figure 3e,h), as observed for the functional group as a whole. Fertilization increased the RGR vs. light slope for *P. pittieri* by 2.6 times and for *V. koschnyi* by 2.7 times. For both species, there again was a slight negative fertilization effect at very low light. *Protium pittieri* also showed an additional, significant positive response to the effect of light alone ( $p = .007$ ), while *V. koschnyi* did not. In contrast, the third shade-tolerant species, *H. didymantha*, did not respond to either resource, although this species did have a restricted light availability range in our experiment that may have obstructed the observation of its complete response to these resources (Figure 3b).

Finally, as seen for the  $N_2$ -fixing functional group as a whole, the growth of *I. pezizifera* responded negatively to a fertilization  $\times$  light interaction ( $p = .03$ ; Figure 3c). Fertilization decreased the positive RGR vs. light slope to near zero, resulting in a positive fertilization response at low light and a negative response at high light. *Inga pezizifera* also responded positively in growth to light alone ( $p = .007$ ). In contrast, the growth of both *I. thibaudiana* ( $p < .001$ ) and *P. macroloba* ( $p = .04$ ) responded positively to light alone, but did not significantly respond to fertilization (Figure 3f,i). However, *P. macroloba* also had a restricted light availability range in our experiment, which may have obscured our understanding of its response to both resources.

The results for three species were sensitive to the influence of a single sapling in the highest light environment (Cook's distances of 1.53 for *C. arborea*, 5.8 for *S. amara* and 2.25 for *P. pittieri*), in that removing the influential point changed the minimal adequate model. However, each of these points is valuable for revealing the growth responses we are assessing, as high light can be critical for sapling success, but it can be exceedingly difficult to find naturally occurring saplings of certain species in very large forest gaps. In no case could we find a reason to exclude the points, even following a thorough examination of data accuracy and an evaluation of the biological feasibility of the observed growth rates.



**FIGURE 3** Relationship between diameter relative growth rate (RGR) and light availability by species and fertilization treatment (red circles = fertilized and black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each species (red = fertilized, black = unfertilized and blue = no significant fertilization treatment). Species are arranged by functional group columns (LD = light-demanding, ST = shade-tolerant and NF =  $N_2$ -fixing) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

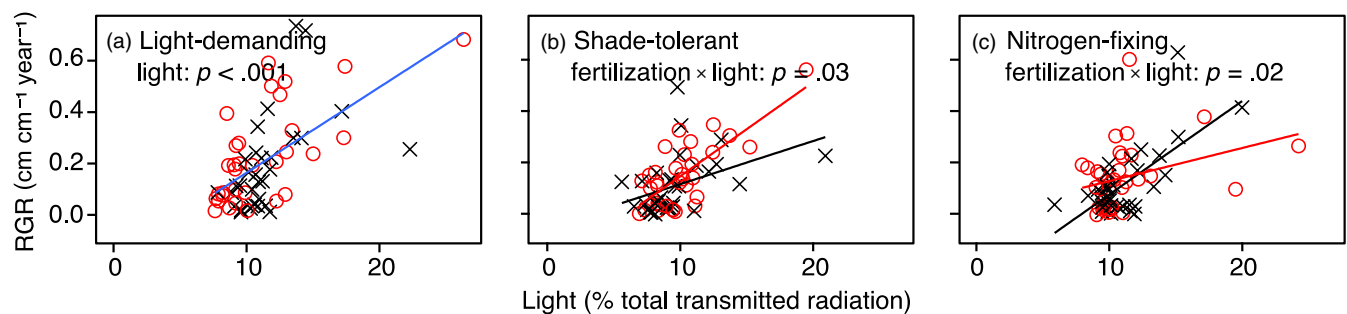


### 3.3 | Comparable height growth nutrient responses

Our results from the analyses of the height and diameter growth data were similar, despite the inherently larger variance of the height growth data: (1) significant responses to fertilization  $\times$  light interactions for the shade-tolerant and  $N_2$ -fixing functional groups (Figure 4, see Table 2 for detailed results from all height RGR models); (2) significant or near-significant responses to fertilization or fertilization  $\times$  light interactions in *C. arborea*, *P. pittieri*, *V. koschnyi* and *I. pezizifera* (Figure 5); and (3) significant or near-significant responses to fertilization  $\times$  light interactions in *P. pittieri* and *I. pezizifera* (Figure 5). However, the nature of the diameter and height growth responses to fertilization was dissimilar for *C. arborea* and *V. koschnyi*, because height growth responded to fertilization without any interactions with light availability. Finally, as with the diameter growth results, almost all functional groups and species showed a significant positive growth response light alone ( $p < .05$ ; Figures 4 and 5).

## 4 | DISCUSSION

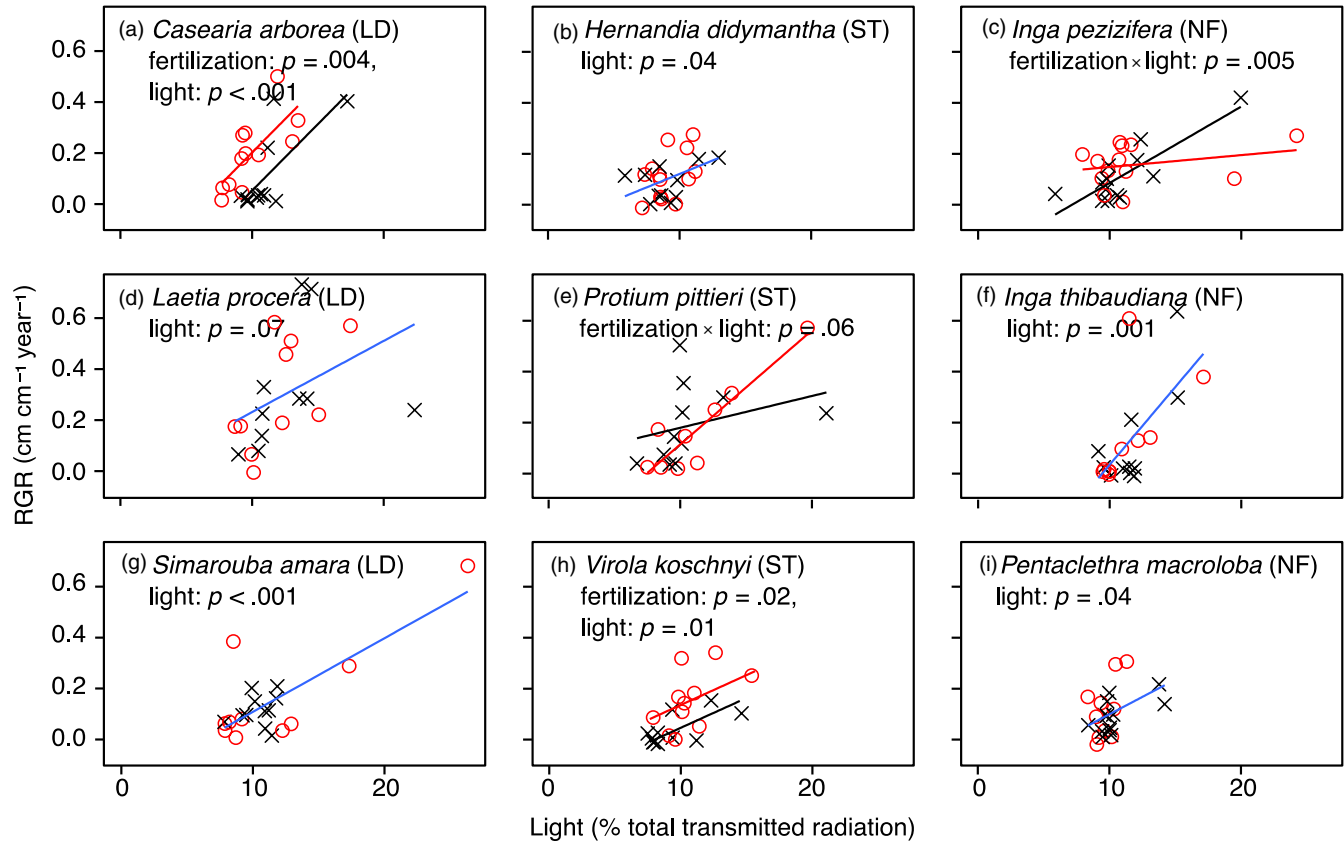
Lowland tropical rainforest saplings employ a variety of strategies to compete for nutrients and light during the sapling bottleneck. We found significant nutrient limitation in two out of the three functional groups and four out of the nine species we examined, as well as generally positive growth responses to increasing light availability, indicating that nutrient and light co-limitation may exist in many functional groups and species at La Selva. The pervasiveness of strong growth responses to nutrients in our saplings, even in a site as nutrient rich as La Selva, confirms the importance of nutrients in addition to light availability for sapling growth and emphasizes the significance of sapling nutrient and light co-limitation in lowland tropical rainforest nutrient dynamics. Additionally, this study revealed functional group- and species-specific interactions between nutrient limitation and light availability, some of which counter prevailing hypotheses of tree resource acquisition strategies and nutrient economies.



**FIGURE 4** Relationship between height relative growth rate (RGR) and light availability by functional group and fertilization treatment (red circles = fertilized and black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each functional group (red = fertilized, black = unfertilized and blue = no significant fertilization treatment) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 2** Height relative growth rate regression table: sample size ( $n$ ), regression parameter estimates, adjusted multiple  $R^2$  and whole model  $p$ -value for the minimal adequate model of each functional group and species. Species are arranged by functional groups (LD = light-demanding, ST = shade-tolerant and NF =  $N_2$ -fixing). NA indicates that the factor or interaction was not included in the minimal adequate model.  $\dagger p < .1$ ;  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$

Functional group	$n$	Intercept	Light	Fertilization	Light $\times$ Fertilization	Adjusted $R^2$	Model $p$ -value
Light-demanding	64	-0.17	0.033***	NA	NA	.34	<.001
Shade-tolerant	66	-0.050	0.017**	-0.17 $\dagger$	0.020*	.37	<.001
Nitrogen-fixing	70	-0.28	0.036***	0.29*	-0.024*	.27	<.001
Species							
<i>Casearia arborea</i> (LD)	23	-0.47	0.052***	0.15**	NA	.52	<.001
<i>Laetia procera</i> (LD)	20	-0.022	0.027 $\dagger$	NA	NA	.13	.070
<i>Simarouba amara</i> (LD)	21	-0.16	0.028***	NA	NA	.57	<.001
<i>Hernandia didymantha</i> (ST)	24	-0.067	0.020*	NA	NA	.14	.042
<i>Protium pittieri</i> (ST)	20	0.049	0.012	-0.39 $\dagger$	0.032 $\dagger$	.40	.011
<i>Virola koschnyi</i> (ST)	22	-0.16	0.023*	0.086*	NA	.46	.0011
<i>Inga pezizifera</i> (NF)	26	-0.22	0.030***	0.31**	-0.025**	.45	<.001
<i>Inga thibaudiana</i> (NF)	21	-0.55	0.060**	NA	NA	.40	.0012
<i>Pentaclethra macroloba</i> (NF)	23	-0.15	0.026*	NA	NA	.14	.043



**FIGURE 5** Relationship between height relative growth rate (RGR) and light availability by species and fertilization treatment (red circles = fertilized and black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each species (red = fertilized, black = unfertilized and blue = no significant fertilization treatment). Species are arranged by functional group columns (LD = light-demanding, ST = shade-tolerant and NF = N<sub>2</sub>-fixing) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

#### 4.1 | Counterintuitive functional group nutrient limitation

The functional group results falsified H<sub>1</sub>, the hypothesis that light-demanding saplings are more nutrient limited than shade-tolerant, non-fixing saplings. First, we found no evidence of nutrient limitation in the light-demanding saplings (Figure 2a), despite their propensity to have traits that lower their NUE (Poorter & Bongers, 2006; Swaine & Whitmore, 1988). In addition, we did find significant nutrient limitation in the shade-tolerant, non-fixing saplings (Figure 2b), although we expected these saplings to have traits that allow for greater NUE. The nutrient limitation in the shade-tolerant, non-fixing saplings did increase with light availability as hypothesized in H<sub>3</sub>, with fertilization more than doubling the slope of the positive relationship between diameter RGR and light availability.

A second surprise was that the functional group analysis also falsified H<sub>2</sub>. Although we found significant nutrient limitation in both the shade-tolerant, non-fixing saplings and shade-tolerant, N<sub>2</sub>-fixing saplings, the strength of nutrient limitation was not consistently greater in the shade-tolerant non-fixers, as predicted in H<sub>2</sub>. While the shade-tolerant, non-fixing saplings followed the pattern predicted in H<sub>3</sub>, unexpectedly, the N<sub>2</sub>-fixing saplings displayed the opposite

pattern, with fertilization increasing the RGR of saplings in low light but the strength of this nutrient limitation decreasing as light availability increased so that there was a negative response to fertilization at high light (Figure 2c). Although the shade-tolerant functional group appears more nutrient limited than the N<sub>2</sub>-fixing functional group at high-light levels, supporting H<sub>2</sub>, there were relatively few saplings in these light conditions. Thus, the difference in the response between the two groups is driven primarily by the lower light saplings, where the N<sub>2</sub> fixers were more nutrient limited than the non-fixers, falsifying H<sub>2</sub>.

We were surprised by these results because N<sub>2</sub> fixation is thought to provide the greatest competitive benefits either early in succession or during gap succession in mature forests, when rapid growth creates the highest N demand (Batterman et al., 2013; Menge & Chazdon, 2016). Thus, if N<sub>2</sub> fixers were nutrient limited, we would expect this limitation to be strongest at high-light levels (as predicted in H<sub>3</sub>), and that this limitation would be by P or Mo (Barron et al., 2009; Vitousek & Howarth, 1991). We would also expect that nutrient limitation of shade-tolerant N<sub>2</sub> fixers would be lower than shade-tolerant non-fixers (as predicted in H<sub>2</sub>), as non-fixers cannot fix their own N<sub>2</sub>. We explore the sapling and canopy tree feedbacks implied by this complex pattern of nutrient limitation in detail below.



## 4.2 | Variable species nutrient limitation within functional groups

When examining nutrient limitation by species, we found variable responses to nutrients within functional groups. The light-demanding functional group was not significantly nutrient limited as a whole, but one out of the three species, *C. arborea*, was significantly nutrient limited (Figure 3a). In the shade-tolerant functional group, two out of the three species, *P. pittieri* and *V. koschnyi*, were significantly nutrient limited, and their patterns of nutrient limitation were consistent with that observed in the functional group as a whole (Figure 3e,h). Finally, in the N<sub>2</sub>-fixing functional group, only one out of the three species, *I. pezizifera*, was significantly nutrient limited, and once again the pattern of nutrient limitation in this species was consistent with the functional group-level nutrient limitation (Figure 3c).

Notably, the patterns of nutrient limitation we found in *C. arborea* vs. *P. pittieri* and *V. koschnyi* were exactly what we expected for light-demanding saplings relative to shade-tolerant saplings, as predicted in H<sub>1</sub>. All three species had increasing nutrient limitation with light availability as predicted in H<sub>3</sub>, and the strength of nutrient limitation was much greater in the light-demanding *C. arborea* than in the shade-tolerant *P. pittieri* and *V. koschnyi* (Figure 3a vs. Figure 3e,h). Fertilization increased the slope of the positive relationship between diameter RGR and light by 4.8 times in *C. arborea*, compared to 2.6 times in *P. pittieri* and 2.7 times in *V. koschnyi*.

Although the restricted ranges of light availability for *H. didymantha* and *P. macroloba* may have limited a few species-level comparisons, it is clear in other cases, for example with the light-demanding *C. arborea* and *L. procera* (Figure 3a,d), that species within the same functional group can have entirely different responses to nutrients. Thus, although functional group classifications can be quite representative for some species, they are not for others.

## 4.3 | Nutrient limitation in shade-tolerant N<sub>2</sub> fixers: A case study of sapling-canopy feedbacks

In addition to our unexpected finding that shade-tolerant N<sub>2</sub> fixers were strongly nutrient limited at low-light levels while shade-tolerant non-fixers were not, the forest at La Selva has three other unusual characteristics: (1) Relative to other neotropical lowland rainforests, La Selva is known for its high abundance of and dominance by shade-tolerant, N<sub>2</sub>-fixing species, with *P. macroloba* alone accounting for 12.4%–13.7% of stems and 34.6%–36.0% of basal area in mature forest (Hartshorn & Hammel, 1994; Lieberman & Lieberman, 1987); (2) La Selva soils are known to be highly N rich relative to soils from other neotropical lowland rainforests (Powers et al., 2005; Vitousek & Matson, 1988); and (3) La Selva soils are also known to be highly P rich relative to soils from other neotropical lowland rainforests (Powers et al., 2005).

Together, these lines of evidence imply a niche for a shade-tolerant, N<sub>2</sub> fixer strategy that functions through a sapling and canopy tree feedback. Unlike the predominant perspective that N<sub>2</sub> fixation is most beneficial in high-light, successional environments where N demand

is high relative to supply (Batterman et al., 2013; Menge & Chazdon, 2016), N<sub>2</sub> fixation may also help shade-tolerant N<sub>2</sub> fixers in low-light environments, with the benefit seen not only within individuals fixing N<sub>2</sub> for their own gain but also across life-history stages with canopy N<sub>2</sub> fixers modifying the environment favourably for their saplings.

Consider this feedback at the ecosystem scale, where shade-tolerant N<sub>2</sub> fixers in the canopy are able to fix large quantities of N<sub>2</sub> and enrich soil N via their N-rich foliage and litterfall, which then helps their shade-tolerant, N<sub>2</sub>-fixing saplings grow faster than shade-tolerant, non-fixing competitors, which in turn increases the abundance of shade-tolerant, N<sub>2</sub>-fixing canopy trees (Figure 1). There is evidence for this feedback cycle at La Selva, as shade-tolerant, N<sub>2</sub>-fixing trees are dominant in the canopy, the N<sub>2</sub>-fixing functional group had significantly higher foliar N content in this experiment (ANOVA  $F_{2,179} = 51.08$ ,  $p < .001$ ; Tukey HSD  $p < .001$  for all comparisons; C.B. Chou, unpubl. data), there is high soil N and the N<sub>2</sub>-fixing saplings significantly increased growth rates in response to fertilization in this experiment. Additionally, a clue to how this feedback emerged at La Selva and not at other neotropical lowland rainforests may be the high soil P, which could potentially be one (but certainly not the only) factor that allowed for the selection of N<sub>2</sub> fixers with highly N-demanding lifestyles (Vitousek & Howarth, 1991).

Given the lines of evidence at La Selva supporting our hypothesized shade-tolerant, N<sub>2</sub> fixer niche where shade-tolerant, N<sub>2</sub>-fixing saplings benefit from high soil N, we hypothesize that the shade-tolerant, N<sub>2</sub>-fixing saplings in our study were likely co-limited by light and N, rather than light and P or Mo. Specifically, the addition of N from fertilizer may have down-regulated N<sub>2</sub> fixation in low-light saplings where the process was carbon costly, allowing them to shift the carbon they were using to feed their rhizobia to growth instead (Hedin, Brookshire, Menge, & Barron, 2009). As light limitation decreased, making fixation relatively less carbon costly, the N<sub>2</sub> fixers may have been able to meet the elevated N demand of their high-light growth rates themselves, diminishing the impact of the fertilizer N on growth. In addition, the discrete fertilization events may have unintentionally caused a negative growth response to fertilization at high light by triggering down-regulation of N<sub>2</sub> fixation without meeting the full N demand of these fast-growing individuals, while at low light, the entire N demand of the slower growing individuals was met by the fertilizer.

Alternatively, if the N<sub>2</sub> fixers were limited by P or Mo and light, the addition of P or trace amounts of Mo from fertilizer may have allowed low-light saplings to fix more N<sub>2</sub> and increase their light capture efficiency and RGR by growing more nutrient (especially N)-rich leaves or more leaves overall. In this case, the high-light, N<sub>2</sub>-fixing saplings were likely still P or Mo limited, but the lack of high-light individuals did not allow us to sufficiently test for a fertilization response. However, given the statistically significant divergent responses to fertilization between shade-tolerant non-fixers and shade-tolerant N<sub>2</sub> fixers (Figure 2b,c), and their similarly small numbers of high-light saplings, this explanation is less parsimonious.

In contrast, the non-fixing functional groups appeared purely light limited at low-light levels, likely because they had greater NUE and a less N-demanding lifestyle than N<sub>2</sub>-fixing species (McKey, 1994). This

result emphasizes the costliness of high leaf N concentrations in the N<sub>2</sub> fixers, which at low-light levels outweighed the growth advantage they should have seen from their ability to fix N<sub>2</sub>.

## 5 | CONCLUSIONS

This study revealed pervasive nutrient and light co-limitation of saplings growing in a lowland tropical rainforest with highly fertile soils, emphasizing the importance of sapling nutrient-light interactions in the nutrient dynamics of these ecosystems. Moreover, this co-limitation was functional group- and species-specific, providing evidence for “heterogeneous nutrient limitation” by tree taxonomic identity (Alvarez-Clare et al., 2013) as well as functional identity, although further studies can enhance our understanding of effective taxonomic or functional groupings for predicting nutrient responses. Within the functional groups we used, we found strong nutrient limitation at low-light levels in the shade-tolerant N<sub>2</sub> fixers, but not in the shade-tolerant non-fixers. This is evidence for a shade-tolerant, N<sub>2</sub> fixation niche through a sapling and canopy tree feedback cycle where shade-tolerant, N<sub>2</sub>-fixing canopy trees enrich soil N to the benefit of their saplings, allowing them to dominate forest canopy composition, as seen at La Selva. There are likely additional, varied sapling-canopy nutrient and light feedbacks in lowland tropical rainforests, and more studies of these feedbacks, combined with careful consideration of appropriate taxonomic or functional groupings, can aid our understanding of nutrient limitation dynamics in these ecosystems.

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## AUTHORS' CONTRIBUTIONS

C.B.C., L.O.H. and S.W.P. conceived the ideas and designed methodology; C.B.C. collected the data, analysed the data and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.k2152> (Chou, Hedin, & Pacala, 2017).

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