2

3   Functional groups, species, and light interact with nutrient limitation during tropical rainforest

4   sapling bottleneck

5   Cleo B. Chou[1*], Lars O. Hedin[1], Stephen W. Pacala[1]

6

7   [1]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544

8   USA

9   *Corresponding author: cleochou@princeton.edu; current address: Princeton Environmental

10 Institute, Princeton University, Princeton, NJ 08544 USA

11

12

13

14

15

16

17

18

19

20

21

22

23

Summary

24

25 1. Potential variability in nutrient limitation among tree size classes, functional groups, and

26 species calls for an integrated community- and ecosystem-level perspective of lowland tropical

27 rainforest nutrient limitation. In particular, canopy trees determine ecosystem nutrient conditions,

28 but competitive success for nutrients and light during the sapling bottleneck determines canopy

29 composition.

30 2. We conducted an *in situ* multi-nutrient sapling fertilization experiment at La Selva Biological

31 Station, Costa Rica, to determine how functional group identity, species identity, and light

32 availability can impact nutrient limitation of stem growth in three functional groups and nine

33 species.

34 3. Despite high soil fertility, we found nutrient-light limitation in two functional groups and four

35 species. Unexpectedly, the nitrogen-fixing ("N_2 fixers") and shade-tolerant functional groups

36 were significantly nutrient limited, while the light-demanding functional group was not.

37 4. This was partially explained by species-level variation in nutrient limitation within these

38 functional groups, with only some species conforming to the prediction of stronger nutrient

39 limitation in light demanders compared to shade tolerants.

40 5. Most surprisingly, we found strong nutrient limitation at low light levels in the N_2 fixers

41 (which were shade-tolerant), but not in the shade-tolerant non-fixers. We hypothesize that the N_2

42 fixers were actually nitrogen limited at low light levels because of their nitrogen rich leaves and

43 the high carbon cost of their symbionts.

44 6. This finding suggests a highly shade-tolerant N_2 fixation strategy, in addition to the perception

45 that N_2 fixation is mostly advantageous in high light environments during early and gap

46 succession. The shade-tolerant N_2 fixation strategy may be part of a sapling and canopy tree

47 feedback, where the canopy N_2 fixers enrich the soil N, enhancing growth of their shade-tolerant

48 saplings relative to non-fixing competitors, enabling further canopy domination by shade-

49 tolerant N_2 fixers, as seen at La Selva.

50 7. *Synthesis.* The pervasiveness of functional group- and species-specific nutrient and light co-

51 limitation in our saplings indicates that these interactions likely play an important role in the

52 dynamics of lowland tropical rainforest nutrient limitation, potentially via other such sapling and

53 canopy tree feedbacks as the one hypothesized.

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

90

91

92

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 (Körner 2009; Huntingford *et al.* 2013; 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) (Mirmanto *et al.* 1999; Newbery *et al.* 2002; Wright *et al.* 2011; Alvarez-Clare, Mack & Brooks 2013; Fisher *et al.* 2013). 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 understory light availability, influencing sapling success (Fig. 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

116   saplings as they experience the bottleneck transition to the canopy, with >90% of sapling

117   mortality events occurring before they reach 4 cm in diameter (Clark & Clark 1992). This

118   transition is typically associated with treefall gaps, which provide the elevated light levels that a

119   majority of species need at some point during their ontogeny in order to reach the canopy

120   (Denslow 1980, 1987; Brokaw 1985). Due to the asymmetry of light availability from the top of

121   the canopy to the shaded forest floor, compared to larger trees in the canopy and sub-canopy,

122   saplings in the understory experience a full range of light availabilities, from desirable gap

123   environments to undesirable non-gap environments (Yoda 1974; Wright *et al.* 2010).

124   This uncertain availability, but necessity, of gaps for individual success during the

125   sapling bottleneck has selected for rapid sapling growth rates under favourable high light

126   conditions (Denslow 1987; Clark & Clark 1992). Rapid growth and biomass accumulation

127   increases plant nutrient demand (Montagnini 2000), raising fundamental questions about the

128   interaction between nutrient and light limitation at the sapling stage. Previous studies of

129   understory nutrient limitation and light interactions in lowland tropical rainforests focused on

130   tree seedlings or shrub cuttings in shade houses and common gardens, and found either no

131   response to nutrients (Denslow *et al.* 1990) or potentially species-specific responses (Fetcher *et*

132   *al.* 1996; Palow & Oberbauer 2009). More recently, *in situ* studies showed light but not nutrient

133   limitation of understory sapling growth (Magalhães, Marenco & Camargo 2014), nutrient

134   limitation of low light understory tree seedling growth (Pasquini and Santiago 2012, Santiago et

135   al. 2012), and approximately equal contributions by light and nutrients to understory woody

136   plant seedling growth (Holste, Kobe & Vriesendorp 2011). These studies suggest that saplings of

137   at least some functional groups or species may be nutrient limited even in low light understory

138 conditions, although the strength of this limitation likely increases as greater light availability

139 elevates sapling growth rates and nutrient demand.

140   Furthermore, these studies indicate that nutrient and light limitation of sapling growth

141 may differ across the wide array of tree strategies for resource acquisition (Reich, Walters &

142 Ellsworth 1997), which can be observed at the level of species, or at a coarser scale, functional

143 groups of species that respond to environmental variables similarly. A well known, but

144 complicated gradient of resource acquisition strategies is tied to shade tolerance (Clark & Clark

145 1992; Pacala *et al.* 1996), with a major trade-off between growth in high light and survival in

146 low light (Wright *et al.* 2010). Species in the light-demanding functional group are less shade-

147 tolerant and tend to have traits that allow for quick growth but lower nutrient use efficiency

148 (NUE), such as short leaf lifespan, low leaf mass per area (LMA), high leaf nutrient

149 concentration, and low wood density (Swaine & Whitmore 1988; Poorter & Bongers 2006).

150 In contrast, shade-tolerant species tend to have traits at the opposite end of the spectrum that

151 result in slower growth and higher NUE, with leaves that are well defended against herbivory

152 and environmental stress. Thus, although saplings in both the light-demanding and shade-tolerant

153 functional groups may be nutrient limited in the low light understory (and to an increasing

154 degree with higher light availability), the strength of this limitation is likely greater in light-

155 demanding saplings across all light levels due to their lower NUE.

156   A tree resource acquisition strategy with a direct impact on nutrient cycling is symbiotic

157 N_2 fixation, which in the tropics is largely carried out by species (hereafter "N_2 fixers") in the

158 Fabaceae family that can host N_2-fixing rhizobial bacteria in root nodules to access atmospheric

159 N_2. The ability to fix N_2 gives N_2 fixers a competitive advantage in environments where N

160 demand is high relative to supply, such as during secondary or gap succession (Batterman *et al.*

161   2013; Menge & Chazdon 2016). Since N_2 fixers are not directly constrained by soil N, they are

162   likely limited by other nutrients, particularly P because the N_2 fixation process raises demand for

163   P (Vitousek & Howarth 1991), and also molybdenum (Mo) which is a co-factor in the

164   nitrogenase enzyme (Barron *et al.* 2009). Beyond their symbiotic relationship itself, other aspects

165   of N_2 fixer physiology that may be related to their N_2 fixation lifestyle are distinctive compared

166   to that of non-fixing trees. These characteristics include high leaf N concentrations and thus high

167   N requirements, as well as greater water use efficiency (McKey 1994; Adams *et al.* 2016).

168   Although this suite of traits may complicate N_2 fixer responses to nutrients, it is plausible that

169   they are also nutrient limited in the low light understory (and to an increasing degree as higher

170   light availability elevates growth and nutrient demand). However, N_2 fixers may be less nutrient

171   limited across all light levels than non-fixing saplings of similar shade tolerance due to their

172   ability to fix N_2 in response to N limitation.

173   We conducted an *in situ* multi-nutrient fertilization experiment (N, P, K, and

174   micronutrients) of naturally occurring saplings in a lowland rainforest to directly test for

175   interactions among nutrient limitation, light availability, and functional group or species

176   identities. To examine sapling responses to fertilization and light availability, we used stem

177   growth, the most common metric of whole tree performance and also the most practical metric in

178   this case, due to the challenge of measuring belowground growth both at the individual scale and

179   in an *in situ* experiment. Specifically, our experiment was designed to test the following

180   hypotheses: *H1*: Light-demanding saplings are more nutrient limited than shade-tolerant non-

181   fixing saplings across all light levels; *H2*: Shade-tolerant non-fixing saplings are more nutrient

182   limited than shade-tolerant N_2-fixing saplings across all light levels; and *H3*: Nutrient-limited

183   functional groups and species are co-limited by light, with greater light availability amplifying

184   the degree of nutrient limitation.

185

186

187

188

189

190

191

192

193

194

195

196

197

198

199

200

201

202

203

204

205

Materials and methods

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 (Holdridge 1967; Hartshorn 1983). 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 *et al.* 1994). Soils at La Selva are amongst 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 (Vitousek & Matson 1988; Powers, Treseder & Lerdau 2005). We established the experiment on primarily residual ultisol soils that have consistent chemical and morphological characteristics (Sollins *et al.* 1994), in a mix of old growth and regenerating forest, with an average elevation of approximately 100 m.

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 *personal communication*). 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 (Swaine & Whitmore 1988; Clark & Clark 1992). We defined light-demanding species (*Casearia arborea, Laetia procera, 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 understory. Since La Selva has an unusual abundance of shade-tolerant N_2 fixers in the canopy (Lieberman & Lieberman 1987; Hartshorn & Hammel 1994), we chose three N_2-fixing species (*Inga pezizifera, Inga thibaudiana,*

229   *Pentaclethra macroloba*) that were shade-tolerant by our classification scheme. Given that

230   specific N_2 fixation rates were not a focus of our study and that our species are known to

231   nodulate and actively fix N_2 at La Selva and in the broader Central American region (Carpenter

232   1992; Batterman *et al.* 2013), we did not measure N_2 fixation rates. Doing so would have

233   required repeated destructive root sampling throughout the study, which would have impacted

234   the growth responses of interest.

235   In August and September 2012, we identified 235 naturally growing saplings in the

236   forest, approximately twenty-six individuals per species, in a gradient of light conditions ranging

237   from closed canopy to the largest canopy gaps we could find. Saplings ranged from 2.5 mm to 27

238   mm in diameter and 32 cm to 312 cm in height. We fertilized approximately half of the

239   individuals of each species across the light gradient with a slow-release fertilizer (Miracle-Gro®

240   Tree & Shrub Fertilizer Spikes, The Scotts Company, Marysville, OH) containing N (15%), P

241   (5%), K (10%) plus micronutrients: sulfur, iron, and manganese, and repeated fertilization every

242   six months during the 2.5 years of the experiment. Saplings received one fertilizer stake per

243   application event, which was broken into four evenly sized pieces and buried 5 cm below the

244   surface 0.6 m away from the stem in the cardinal directions. This resulted in the application of

245   0.0340 kg N, 0.0113 kg P, and 0.0227 kg K per sapling per year, and assuming the nutrients

246   spread to 2 m^2 around each sapling, the application rate was approximately 170 kg N·ha^{-1}·yr^{-1}, 57

247   kg P·ha^{-1}·yr^{-1}, and 114 kg K·ha^{-1}·yr^{-1}, which scales to about 142% of N inputs, 1256% of P

248   inputs, and 757% of K inputs from litterfall measured in this forest (Wood, Lawrence & Clark

249   2006).

250   ***Census measurements***

251   For every sapling, we measured stem diameter, stem height, and light availability every

252   six months over the 2.5 years of the study, thus six times total for each variable. We also

253   measured foliar nutrient concentrations for each individual, but the responses were complex and

254   we therefore treat them in a separate contribution. During each census, we measured stem

255   diameter to the nearest 0.1 mm using callipers at a marked point of measurement below the

256   lowest branch and away from stem irregularities at heights of 0, 40, or 130 cm when possible

257   (Clark & Clark 1992). For saplings >4 cm in diameter or for those that had highly non-

258   cylindrical stems, we used a diameter tape to measure the stem to the nearest mm. We measured

259   all stems of multi-stemmed saplings at the same point of measurement to calculate a diameter

260   equivalent to that of a single-stemmed tree of equal basal area.

261   Additionally, during each census we measured sapling height to the nearest mm using a

262   folding 2 m ruler, or when necessary to the nearest cm using an extendable 3 m or 15 m

263   measuring pole. We defined height as the perpendicular distance between the ground and tallest

264   meristem, except in approximately 3% of the saplings, where due to architectural form, growth

265   was consistently in a bent direction throughout the study period, causing a sapling to become

266   shorter with time absent any breakage. In these cases we measured the bent stem length between

267   the ground and furthest meristem, and found this to be an appropriate proxy for height growth, as

268   inclusion or exclusion of these points did not fundamentally impact our results or conclusions.

269   Finally, we also quantified light availability for each sapling at each census by taking a

270   hemispherical photograph at the height of its tallest leaf using a Nikon Coolpix 4500 camera

271   equipped with the Nikon FC-E8 Fisheye Converter (Nikon, Tokyo, Japan), which was mounted

272   on a gyroscopic pole to allow for level pictures at greater heights. Photos were taken pre-dawn or

273 on uniformly cloudy days, and were analysed using Gap Light Analyzer Version 2.0 (Frazer,

274 Canham & Lertzman 1999) to quantify total transmitted radiation.

275 ***Tree growth analysis***

276   We analysed tree growth responses to fertilization and light availability using total

277 growth between the first and last census in the 2.5-study period to capture the strongest signal of

278 tree response to these resources while minimizing measurement errors that may be associated

279 with the short census intervals. We also found similar results from a more complex repeated

280 measures analysis that used the data from each census (see Appendix S1). Although we

281 examined both diameter and height growth, we centre our interpretation on the diameter results

282 since measurements of sapling diameter growth are inherently less variable than measurements

283 of sapling height growth, which tend to include breakage and height loss.

284   For both diameter and height growth, we used relative growth rate (RGR) as the response

285 variable in order to account for the effect of tree size on growth rate, where RGR =

286 $\ln(size_{final}/size_{initial})$/(number of study days/365). Individuals that did not survive the entire 2.5-

287 year study period were excluded from all analyses, and individuals with multiple stems that had

288 negative diameter growth due to stem death and individuals that had negative height growth due

289 to observed stem breakage were excluded from the diameter (n=202) and height (n=200) growth

290 analyses respectively.

291   We used stepwise linear regression to assess whether each functional group and each

292 species was nutrient limited in its RGR and if this nutrient limitation interacted with light

293 availability, which we calculated for each sapling as its mean light availability across the six

294 censuses. For each functional group and species, we began with the maximal model, where RGR

295 ~ fertilization treatment * light availability, and simplified to the minimal adequate model, which

13

296 contains only significant explanatory variables and interactions. We confirmed that regression

297 assumptions were met in the residuals of each model and also tested for influential points using

298 Cook's distance.

299 Functional groups or species that had a significant growth response to fertilization (with

300 or without light interactions) in their minimal adequate model were considered nutrient limited.

301 Although there was variability in the ranges of light availability among the functional groups and

302 species due to the natural experimental design (light-demanding 7.62-26.33%; shade-tolerant

303 5.70-21.01%; N_2-fixing 5.83-24.18%; *C. arborea* 7.62-17.17%; *L. procera* 8.63-22.29%; *S.*

304 *amara* 7.84-26.33%; *H. didymantha* 5.70-12.83%; *P. pittieri* 6.62-21.01%; *V. koschnyi* 7.44-

305 15.34%; *I. pezizifera* 5.83-24.18%; *I. thibaudiana* 8.80-17.09%; *P. macroloba* 8.31-14.15%; all

306 ranges in percent total transmitted radiation), the linearity of the data reassures us that the linear

307 regression models were suitable for understanding the relative responses to fertilization and light

308 availability among the functional groups and most species. All statistical analyses were

309 performed in R 3.0.2 (R Core Team 2013).

310

311

312

313

314

315

316

317

318

319   **Results**

320   *Variable nutrient-light responses across functional groups*

321   We found a unique diameter growth response to nutrients and light in each of the

322   functional groups (Fig. 2, see Table 1 for detailed results from all diameter RGR models). All

323   groups responded significantly and positively to the effect of light alone ($p<0.001$) and in

324   addition, some groups responded positively and some negatively to fertilization × light

325   interactions.

326   The light-demanding functional group did not respond significantly to fertilization (Fig.

327   2a). In contrast, the shade-tolerant functional group showed a significant positive growth

328   response to a fertilization × light interaction ($p<0.001$) (Fig. 2b). As a result, fertilization

329   increased the slope of the positive relationship between RGR and light availability by 2.5 times,

330   so that the response to fertilization increased with light availability. At very low light there was a

331   slight negative influence of fertilization on growth that was likely the result of the strong positive

332   interaction term, although it is also possible that fertilization mildly suppressed growth in these

333   conditions.

334   The N_2-fixing functional group also responded significantly to a fertilization × light

335   interaction, but differed from shade-tolerant group in that this interaction was negative ($p=0.01$)

336   (Fig. 2c). For N_2-fixing saplings, fertilization decreased the slope of the positive relationship

337   between RGR and light availability to one quarter of the unfertilized slope, with saplings

338   responding positively to fertilization at low light and negatively at high light.

339   *Variable nutrient-light responses across species*

340   Although we did not find a response to fertilization in the light-demanding functional

341   group as a whole, *Casearia arborea* did respond significantly and positively to a fertilization ×

342   light interaction (p=0.007) (Fig. 3a). Fertilization increased the response slope between RGR and

343   light availability by 4.8 times, so that the growth increase from fertilization was greater with

344   higher light availability, although (as discussed above) there was a slight negative response at

345   very low light. In contrast, we did not find any significant responses to fertilization in *Laetia*

346   *procera* or *Simarouba amara*, the other two light-demanding species (Figs 3d and g). However,

347   both *Laetia procera* (p=0.05) and *Simarouba amara* (p<0.001) showed a significant positive

348   growth response to light alone, while *Casearia arborea* did not.

349   In the shade-tolerant functional group, we found that *Protium pittieri* and *Virola koschnyi*

350   responded significantly and positively to fertilization × light interactions (p=0.003 and p=0.05,

351   respectively) (Figs 3e and h), as observed for the functional group as a whole. Fertilization

352   increased the RGR vs. light slope for *Protium pittieri* by 2.6 times and for *Virola koschnyi* by 2.7

353   times. For both species, there again was a slight negative fertilization effect at very low light.

354   *Protium pittieri* also showed an additional, significant positive response to the effect of light

355   alone (p=0.007) while *Virola koschnyi* did not. In contrast, the third shade-tolerant species,

356   *Hernandia didymantha*, did not respond to either resource, although this species did have a

357   restricted light availability range in our experiment that may have obstructed the observation of

358   its complete response to these resources (Fig. 3b).

359   Finally, as seen for the N_2-fixing functional group as a whole, the growth of *Inga*

360   *pezizifera* responded negatively to a fertilization × light interaction (p=0.03) (Fig. 3c).

361   Fertilization decreased the positive RGR vs. light slope to near zero, resulting in a positive

362   fertilization response at low light and a negative response at high light. *Inga pezizifera* also

363   responded positively in growth to light alone (p=0.007). In contrast, the growth of both *Inga*

364   *thibaudiana* (p<0.001) and *Pentaclethra macroloba* (p=0.04) responded positively to light alone,

365 but did not significantly respond to fertilization (Figs 3f and i). However, *Pentaclethra*

366 *macroloba* also had a restricted light availability range in our experiment, which may have

367 obscured our understanding of its response to both resources.

368   The results for three species were sensitive to the influence of a single sapling in the

369 highest light environment (Cook's distances of 1.53 for *Casearia arborea*, 5.8 for *Simarouba*

370 *amara*, and 2.25 for *Protium pittieri*), in that removing the influential point changed the minimal

371 adequate model. However, each of these points is valuable for revealing the growth responses we

372 are assessing, since high light can be critical for sapling success, but it can be exceedingly

373 difficult to find naturally occurring saplings of certain species in very large forest gaps. In no

374 case could we find a reason to exclude the points, even following a thorough examination of data

375 accuracy and an evaluation of the biological feasibility of the observed growth rates.

376 ***Comparable height growth nutrient responses***

377   Our results from the analyses of the height and diameter growth data were similar,

378 despite the inherently larger variance of the height growth data: 1) significant responses to

379 fertilization × light interactions for the shade-tolerant and N_2-fixing functional groups (Fig. 4, see

380 Table 2 for detailed results from all height RGR models); 2) significant or near-significant

381 responses to fertilization or fertilization × light interactions in *Casearia arborea*, *Protium*

382 *pittieri*, *Virola koschnyi*, and *Inga pezizifera* (Fig. 5); and 3) significant or-near significant

383 responses to fertilization × light interactions in *Protium pittieri* and *Inga pezizifera* (Fig. 5).

384 However, the nature of the diameter and height growth responses to fertilization was dissimilar

385 for *Casearia arborea* and *Virola koschnyi*, because height growth responded to fertilization

386 without any interactions with light availability. Finally, as with the diameter growth results,

387   almost all functional groups and species showed a significant positive growth response light

388   alone (p<0.05) (Figs 4 and 5).

389

390

391

392

393

394

395

396

397

398

399

400

401

402

403

404

405

406

407

408

409

Discussion

410

411   Lowland tropical rainforest saplings employ a variety of strategies to compete for

412   nutrients and light during the sapling bottleneck. We found significant nutrient limitation in two

413   out of the three functional groups and four out of the nine species we examined, as well as

414   generally positive growth responses to increasing light availability, indicating that nutrient and

415   light co-limitation may exist in many functional groups and species at La Selva. The

416   pervasiveness of strong growth responses to nutrients in our saplings, even in a site as nutrient

417   rich as La Selva, confirms the importance of nutrients in addition to light availability for sapling

418   growth and emphasizes the significance of sapling nutrient and light co-limitation in lowland

419   tropical rainforest nutrient dynamics. Additionally, this study revealed functional group- and

420   species-specific interactions between nutrient limitation and light availability, some of which

421   counter prevailing hypotheses of tree resource acquisition strategies and nutrient economies.

Counterintuitive functional group nutrient limitation

423   The functional group results falsified *H1*, the hypothesis that light-demanding saplings

424   are more nutrient limited than shade-tolerant non-fixing saplings. First, we found no evidence of

425   nutrient limitation in the light-demanding saplings (Fig. 2a), despite their propensity to have

426   traits that lower their NUE (Swaine & Whitmore 1988; Poorter & Bongers 2006). In addition, we

427   did find significant nutrient limitation in the shade-tolerant non-fixing saplings (Fig. 2b),

428   although we expected these saplings to have traits that allow for greater NUE. The nutrient

429   limitation in the shade-tolerant non-fixing saplings did increase with light availability as

430   hypothesized in *H3,* with fertilization more than doubling the slope of the positive relationship

431   between diameter RGR and light availability.

432   A second surprise was that the functional group analysis also falsified *H2*. Although we

433   found significant nutrient limitation in both the shade-tolerant non-fixing saplings and shade-

434   tolerant N_2-fixing saplings, the strength of nutrient limitation was not consistently greater in the

435   shade-tolerant non-fixers, as predicted in *H2*. While the shade-tolerant non-fixing saplings

436   followed the pattern predicted in *H3*, unexpectedly, the N_2-fixing saplings displayed the opposite

437   pattern, with fertilization increasing the RGR of saplings in low light but the strength of this

438   nutrient limitation decreasing as light availability increased so that there was a negative response

439   to fertilization at high light (Fig. 2c). Although the shade-tolerant functional group appears more

440   nutrient limited than the N_2-fixing functional group at high light levels, supporting *H2*, there

441   were relatively few saplings in these light conditions. Thus, the difference in the response

442   between the two groups is driven primarily by the lower light saplings, where the N_2 fixers were

443   more nutrient limited than the non-fixers, falsifying *H2*.

444   We were surprised by these results because N_2 fixation is thought to provide the greatest

445   competitive benefits either early in succession or during gap succession in mature forests, when

446   rapid growth creates the highest N demand (Batterman *et al.* 2013; Menge & Chazdon 2016).

447   Thus, if N_2 fixers were nutrient limited, we would expect this limitation to be strongest at high

448   light levels (as predicted in *H3*), and that this limitation would be by P or Mo (Vitousek &

449   Howarth 1991; Barron *et al.* 2009). We would also expect that nutrient limitation of shade-

450   tolerant N_2 fixers would be lower than shade-tolerant non-fixers (as predicted in *H2*), since non-

451   fixers cannot fix their own N_2. We explore the sapling and canopy tree feedbacks implied by this

452   complex pattern of nutrient limitation in detail below.

453   ***Variable species nutrient limitation within functional groups***

454   When examining nutrient limitation by species, we found variable responses to nutrients

455   within functional groups. The light-demanding functional group was not significantly nutrient

456   limited as a whole, but one out of the three species, *Casearia arborea*, was significantly nutrient

457   limited (Fig. 3a). In the shade-tolerant functional group, two out of the three species, *Protium*

458   *pittieri* and *Virola koschnyi*, were significantly nutrient limited, and their patterns of nutrient

459   limitation were consistent with that observed in the functional group as a whole (Figs 3e and 3h).

460   Finally, in the N_2-fixing functional group, only one out of the three species, *Inga pezizifera*, was

461   significantly nutrient limited, and once again the pattern of nutrient limitation in this species was

462   consistent with the functional group level nutrient limitation (Fig. 3c).

463   Notably, the patterns of nutrient limitation we found in *Casearia arborea* versus *Protium*

464   *pittieri* and *Virola koschnyi* were exactly what we expected for light-demanding saplings relative

465   to shade-tolerant saplings, as predicted in *H1*. All three species had increasing nutrient limitation

466   with light availability as predicted in *H3*, and the strength of nutrient limitation was much greater

467   in the light-demanding *Casearia arborea* than in the shade-tolerant *Protium pittieri* and *Virola*

468   *koschnyi* (Fig. 3a vs. 3e and 3h). Fertilization increased the slope of the positive relationship

469   between diameter RGR and light by 4.8 times in *Casearia arborea*, compared to 2.6 times in

470   *Protium pittieri* and 2.7 times in *Virola koschnyi*.

471   Although the restricted ranges of light availability for *Hernandia didymantha* and

472   *Pentaclethra macroloba* may have limited a few species-level comparisons, it is clear in other

473   cases, for example with the light-demanding *Casearia arborea* and *Laetia procera* (Figs 3a and

474   3d), that species within the same functional group can have entirely different responses to

475   nutrients. Thus, although functional group classifications can be quite representative for some

476   species, they are not for others.

Nutrient limitation in shade-tolerant N_2 fixers: A case study of sapling-canopy feedbacks

In addition to our unexpected finding that shade-tolerant N_2 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_2-fixing species, with *Pentaclethra macroloba* alone accounting for 12.4-13.7% of stems and 34.6-36.0% of basal area in mature forest (Lieberman & Lieberman 1987; Hartshorn & Hammel 1994); 2) La Selva soils are known to be highly N rich relative to soils from other neotropical lowland rainforests (Vitousek & Matson 1988; Powers *et al.* 2005); 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_2 fixer strategy that functions through a sapling and canopy tree feedback. Unlike the predominant perspective that N_2 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_2 fixation may also help shade-tolerant N_2 fixers in low light environments, with the benefit seen not only within individuals fixing N_2 for their own gain, but across life history stages with canopy N_2 fixers modifying the environment favourably for their saplings.

Consider this feedback at the ecosystem scale, where shade-tolerant N_2 fixers in the canopy are able to fix large quantities of N_2 and enrich soil N via their N-rich foliage and litterfall, which then helps their shade-tolerant N_2-fixing saplings grow faster than shade-tolerant non-fixing competitors, which in turn increases the abundance of shade-tolerant N_2-fixing canopy trees (Fig. 1). There is evidence for this feedback cycle at La Selva, since shade-tolerant N_2-fixing trees are dominant in the canopy, the N_2-fixing functional group had significantly

500 higher foliar N content in this experiment (ANOVA $F_{2,179} = 51.08$, p<0.001; Tukey HSD

501 p<0.001 for all comparisons; C.B. Chou *unpublished data*), there is high soil N, and the N_2-

502 fixing saplings significantly increased growth rates in response to fertilization in this experiment.

503 Additionally, a clue to how this feedback emerged at La Selva and not at other neotropical

504 lowland rainforests may be the high soil P, which could potentially be one (but certainly not the

505 only) factor that allowed for the selection of N_2 fixers with highly N demanding lifestyles

506 (Vitousek & Howarth 1991).

507   Given the lines of evidence at La Selva supporting our hypothesized shade-tolerant N_2

508 fixer niche where shade-tolerant N_2-fixing saplings benefit from high soil N, we hypothesize that

509 the shade-tolerant N_2-fixing saplings in our study were likely co-limited by light and N, rather

510 than light and P or Mo. Specifically, the addition of N from fertilizer may have down-regulated

511 N_2 fixation in low light saplings where the process was carbon costly, allowing them to shift the

512 carbon they were using to feed their rhizobia to growth instead (Hedin *et al.* 2009). As light

513 limitation decreased, making fixation relatively less carbon costly, the N_2 fixers may have been

514 able to meet the elevated N demand of their high-light growth rates themselves, diminishing the

515 impact of the fertilizer N on growth. In addition, the discrete fertilization events may have

516 unintentionally caused a negative growth response to fertilization at high light by triggering

517 down-regulation of N_2 fixation without meeting the full N demand of these fast-growing

518 individuals, while at low light, the entire N demand of the slower growing individuals was met

519 by the fertilizer.

520   Alternatively, if the N_2 fixers were limited by P or Mo and light, the addition of P or trace

521 amounts of Mo from fertilizer may have allowed low light saplings to fix more N_2 and increase

522 their light capture efficiency and RGR by growing more nutrient (especially N) rich leaves or

523   more leaves overall. In this case, the high light N_2-fixing saplings were likely still P or Mo

524   limited, but the lack of high light individuals did not allow us to sufficiently test for a

525   fertilization response. However, given the statistically significant divergent responses to

526   fertilization between shade-tolerant non-fixers and shade-tolerant N_2 fixers (Figs 2b and 2c), and

527   their similarly small numbers of high light saplings, this explanation is less parsimonious.

528   In contrast, the non-fixing functional groups appeared purely light limited at low light

529   levels, likely because they had greater NUE and a less N-demanding lifestyle than N_2-fixing

530   species (McKey 1994). This result emphasizes the costliness of high leaf N concentrations in the

531   N_2 fixers, which at low light levels outweighed the growth advantage they should have seen from

532   their ability to fix N_2.

533   ***Conclusions***

534   This study revealed pervasive nutrient and light co-limitation of saplings growing in a

535   lowland tropical rainforest with highly fertile soils, emphasizing the importance of sapling

536   nutrient-light interactions in the nutrient dynamics of these ecosystems. Moreover, this co-

537   limitation was functional group- and species-specific, providing evidence for "heterogeneous

538   nutrient limitation" by tree taxonomic identity (Alvarez-Clare *et al.* 2013) as well as functional

539   identity, although further studies can enhance our understanding of effective taxonomic or

540   functional groupings for predicting nutrient responses. Within the functional groups we used, we

541   found strong nutrient limitation at low light levels in the shade-tolerant N_2 fixers, but not in the

542   shade-tolerant non-fixers. This is evidence for a shade-tolerant N_2 fixation niche through a

543   sapling and canopy tree feedback cycle where shade-tolerant N_2-fixing canopy trees enrich soil

544   N to the benefit of their saplings, allowing them to dominate forest canopy composition, as seen

545   at La Selva. There are likely additional, varied sapling-canopy nutrient and light feedbacks in

546   lowland tropical rainforests, and more studies of these feedbacks, combined with careful

547   consideration of appropriate taxonomic or functional groupings, can aid our understanding of

548   nutrient limitation dynamics in these ecosystems.

549

550

551

552

553

554

555

556

557

558

559

560

561

562

563

564

565

566

567

568

569 **Author's contributions**

570 CBC, LOH and SWP conceived the ideas and designed methodology; CBC collected the data,

571 analysed the data, and led the writing of the manuscript. All authors contributed critically to the

572 drafts and gave final approval for publication.

573

574

575

576

577

578

579

580

581

582

583

584

585

586

587

588

589

590

591

615 **Data accessibility**

616 Data deposited in the Dryad repository: doi:10.5061/dryad.k2152 (Chou, Hedin & Pacala 2017).

617

618

619

620

621

622

623

624

625

626

627

628

629

630

631

632

633

634

635

636

637

638 **References**

639 Adams, M.A., Turnbull, T.L., Sprent, J.I. & Buchmann, N. (2016) Legumes are different: Leaf

640   nitrogen, photosynthesis, and water use efficiency. *Proceedings of the National Academy of*

641   *Sciences of the United States of America*, **113**, 4098–4103.

642 Alvarez-Clare, S., Mack, M.C. & Brooks, M. (2013) A direct test of nitrogen and phosphorus

643   limitation to net primary productivity in a lowland tropical wet forest. *Ecology*, **94**, 1540–

644   1551.

645 Barron, A.R., Wurzburger, N., Bellenger, J.P., Wright, S.J., Kraepiel, A.M.L. & Hedin, L.O.

646   (2009) Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils.

647   *Nature Geoscience*, **2**, 42–45.

648 Batterman, S.A., Hedin, L.O., van Breugel, M., Ransijn, J., Craven, D.J. & Hall, J.S. (2013) Key

649   role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature*, **502**,

650   224–7.

651 Brokaw, N.V.L. (1985) Gap-phase regeneration in a tropical forest. *Ecology*, **66**, 682–687.

652 Carpenter, E.J. (1992) Nitrogen fixation in the epiphyllae and root nodules of trees in the

653   lowland tropical rainforest of Costa Rica. *Acta Oecologica*, **13**, 153–160.

654 Chou, C.B., Hedin, L.O. & Pacala, S.W. (2017) Data from: Functional groups, species, and light

655   interact with nutrient limitation during tropical rainforest sapling bottleneck. *Journal of*

656   *Ecology*, doi:10.5061/dryad.k2152.

657 Clark, D.A. & Clark, D.B. (1992) Life history diversity of canopy and emergent trees in a

658   Neotropical rain forest. *Ecological Monographs*, **62**, 315–344.

659 Denslow, J.S. (1980) Gap partitioning among tropical rainforest trees. *Biotropica*, **12**, 47–55.

660 Denslow, J.S. (1987) Tropical rainforest gaps and tree species diversity. *Annual Review of*

661   *Ecology and Systematics*, **18**, 431–451.

662   Denslow, J.S., Schultz, J.C., Vitousek, P.M. & Strain, B.R. (1990) Growth responses of tropical

663   shrubs to treefall gap environments. *Ecology*, **71**, 165–179.

664   Fetcher, N., Haines, B.L., Cordero, R.A., Lodge, D.J., Walker, L.R., Fernandez, D.S. &

665   Lawrence, W.T. (1996) Responses of tropical plants to nutrients and light on a landslide in

666   puerto rico. , **84**, 331–341.

667   Fisher, J.B., Malhi, Y., Torres, I.C., Metcalfe, D.B., van de Weg, M.J., Meir, P., Silva-Espejo,

668   J.E. & Huasco, W.H. (2013) Nutrient limitation in rainforests and cloud forests along a

669   3,000-m elevation gradient in the Peruvian Andes. *Oecologia*, **172**, 889–902.

670   Frazer, G.W., Canham, C.D. & Lertzman, K.P. (1999) Gap Light Analzyer (GLA), Version 2.0:

671   imaging software to extract canopy structure and gap light indices from true-colour fisheye

672   photographs.

673   Hartshorn, G.S. (1983) Plants. *Costa Rican natural history* (ed D.H. Janzen), pp. 118–157.

674   University of Chicago Press, Chicago.

675   Hartshorn, G.S. & Hammel, B.E. (1994) Vegetation types and floristic patterns. *La Selva:*

676   *ecology and natural history of a neotropical rain forest* (eds L.A. McDade, S.B. Kamaljit,

677   H.A. Hespenheide & G.S. Hartshorn), pp. 73–89. Chicago, Illinois.

678   Hedin, L.O., Brookshire, E.N.J., Menge, D.N.L. & Barron, A.R. (2009) The nitrogen paradox in

679   tropical forest ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 613–

680   635.

681   Holdridge, L.R. (1967) *Life Zone Ecology*, Revised. Tropical Science Center, San Jose, Costa

682   Rica.

683   Holste, E.K., Kobe, R.K. & Vriesendorp, C.F. (2011) Seedling growth responses to soil

684   resources in the understory of a wet tropical forest. *Ecology*, **92**, 1828–38.

685   Huntingford, C., Zelazowski, P., Galbraith, D., Mercado, L.M., Sitch, S., Fisher, R., Lomas, M.,

686   Walker, A.P., Jones, C.D., Booth, B.B.B., Malhi, Y., Hemming, D., Kay, G., Good, P.,

687   Lewis, S.L., Phillips, O.L., Atkin, O.K., Lloyd, J., Gloor, E., Zaragoza-Castells, J., Meir, P.,

688   Betts, R., Harris, P.P., Nobre, C., Marengo, J. & Cox, P.M. (2013) Simulated resilience of

689   tropical rainforests to CO2-induced climate change. *Nature Geoscience*, **6**, 268–273.

690   Körner, C. (2009) Responses of humid tropical trees to rising CO2. *Annual Review of Ecology,*

691   *Evolution, and Systematics*, **40**, 61–79.

692   Lieberman, D. & Lieberman, M. (1987) Forest tree growth and dynamics at La Selva, Costa Rica

693   (1969-1982). *Journal of Tropical Ecology*, **3**, 347–358.

694   Magalhães, N.S., Marenco, R.A. & Camargo, M.A.B. (2014) Do soil fertilization and forest

695   canopy foliage affect the growth and photosynthesis of Amazonian saplings? *Scientia*

696   *Agricola*, **71**, 58–65.

697   McKey, D. (1994) Legumes and nitrogen: The evolutionary ecology of a nitrogen-demanding

698   lifestyle. *Advances in Legume Systematics 5. The Nitrogen Factor* (eds J.I. Sprent & D.

699   McKey), pp. 211–228. Royal Boatnic Gardens, Kew.

700   Menge, D.N.L. & Chazdon, R.L. (2016) Higher survival drives the success of nitrogen-fixing

701   trees through succession in Costa Rican rainforests. *New Phytologist*, **209**, 965–977.

702   Mirmanto, E., Proctor, J., Green, J., Nagy, L. & Suriantata. (1999) Effects of nitrogen and

703   phosphorus fertilization in a lowland evergreen rainforest. *Philosophical Transactions of*

704   *the Royal Society B*, **354**, 1825–1829.

705   Montagnini, F. (2000) Accumulation in above-ground biomass and soil storage of mineral

706   nutrients in pure and mixed plantations in a humid tropical lowland. *Forest Ecology and*

707   *Management*, **134**, 257–270.

708   Newbery, D.M., Chuyong, G.B., Green, J.J., Songwe, N.C., Tchuenteu, F. & Zimmermann, L.

709   (2002) Does low phosphorus supply limit seedling establishment and tree growth in groves

710   of ectomycorrhizal trees in a central African rainforest? *New Phytologist*, **156**, 297–311.

711   Pacala, S.W., Canham, C.D., Saponara, J., Silander Jr, J.A., Kobe, R.K. & Ribbens, E. (1996)

712   Forest models defined by field measurements: estimation, error analysis and dynamics.

713   *Ecological Monographs*, **66**, 1–43.

714   Palow, D.T. & Oberbauer, S.F. (2009) Soil type affects seedling shade response at low light for

715   two Inga species from Costa Rica. *Plant and Soil*, **319**, 25–35.

716   Pasquini, S.C. & Santiago, L.S. (2012) Nutrients limit photosynthesis in seedlings of a lowland

717   tropical forest tree species. *Oecologia*, **168**, 311–319.

718   Poorter, L. & Bongers, F. (2006) Leaf traits are good predictors of plant performance across 53

719   rain forest species. *Ecology*, **87**, 1733–1743.

720   Powers, J.S., Treseder, K.K. & Lerdau, M.T. (2005) Fine roots, arbuscular mycorrhizal hyphae

721   and soil nutrients in four neotropical rain forests: Patterns across large geographic distances.

722   *New Phytologist*, **165**, 913–921.

723   R Core Team. (2013) R: A language and environment for statistical computing.

724   Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997) From tropics to tundra : global convergence

725   in plant functioning. *Proceedings of the National Academy of Sciences of the United States*

726   *of America*, **94**, 13730–13734.

727   Sanford, R.L.J., Paaby, P., Luvall, J.C. & Phillips, E. (1994) Climate, geomorphology, and

728   aquatic systems. *La Selva: ecology and natural history of a neotropical rain forest* (eds

729   L.A. McDade, S.B. Kamaljit, H.A. Hespenheide & G.S. Hartshorn), pp. 19–33. University

730   of Chicago Press, Chicago, Illinois.

731   Santiago, L.S. (2015) Nutrient limitation of eco-physiological processes in tropical trees. *Trees*,

732   **29**, 1291–1300.

733   Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N. & Turner, B.L.

734   (2012) Tropical tree seedling growth responses to nitrogen, phosphorus and potassium

735   addition. *Journal of Ecology*, **100**, 309–316.

736   Sollins, P., Sancho, M.F., Mata, C.R. & Sanford, R.L.J. (1994) Soils and soil process research.

737   *La Selva: ecology and natural history of a neotropical rain forest* (eds L.A. McDade, S.B.

738   Kamaljit, H.A. Hespenheide & G.S. Hartshorn), pp. 34–53. University of Chicago Press,

739   Chicago, Illinois.

740   Swaine, M.D. & Whitmore, T.C. (1988) On the definition of ecological species groups in

741   tropical rain forests. *Vegetatio*, **75**, 81–86.

742   Vitousek, P.M. & Howarth, R.W. (1991) Nitrogen limitation on land and in the sea: How can it

743   occur? *Biogeochemistry*, **13**, 87–115.

744   Vitousek, P.M. & Matson, P.A. (1988) Nitrogen transformations in a range of tropical forest

745   soils. *Soil Biology & Biochemistry*, **20**, 361–367.

746   Wood, T.E., Lawrence, D. & Clark, D.A. (2006) Determinants of leaf litter nutrient cycling in a

747   tropical rain forest: soil fertility versus topography. *Ecosystems*, **9**, 700–710.

748   Wright, S.J., Kitajima, K., Kraft, N.J.B., Reich, P.B., Wright, I.J., Bunker, D.E., Condit, R.,

749   Dalling, J.W., Davies, S.J., Díaz, S., Engelbrecht, B.M.J., Harms, K.E., Hubbell, S.P.,

750   Marks, C.O., Ruiz-Jaen, M.C., Salvador, C.M. & Zanne, A.E. (2010) Functional traits and

751   the growth-mortality trade-off in tropical trees. *Ecology*, **91**, 3664–3674.

752   Wright, S.J., Yavitt, J.B., Wurzburger, N., Turner, B.I., Tanner, E.V.J., Sayer, E.J., Santiago,

753   L.S., Kaspari, M., Hedin, L.O., Harms, K.E., Garcia, M.N. & Corre, M.D. (2011)

754   Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in

755   a lowland tropical forest. *Ecology*, **92**, 1616–1625.

756 Yoda, K. (1974) Three-dimensional distribution of light intensity in a tropical rain forest of West

757   Malaysia. *Japanese Journal of Ecology*, **24**, 247–254.

758

759

760

761

762

763

764

765

766

767

768

769

770

771

772

773

774

775

776   **Tables**

777   **Table 1.** Diameter RGR regression table: sample size (n), regression parameter estimates,

778   adjusted multiple R^2, and whole model p-value for the minimal adequate model of each

779   functional group and species. Species are arranged by functional groups (LD = light-demanding,

780   ST = shade-tolerant, NF = N_2-fixing). NA indicates that the factor or interaction was not

781   included in the minimal adequate model.

782    $\cdot$ *P < 0.1; * P < 0.05, ** P<0.01; *** P <0.001*

Functional group	n	Intercept	Light	Fertilization	Light × Fertilization	Adjusted R^2	Model p-value
Light-demanding	67	-0.14	0.029***	NA	NA	0.36	<0.001
Shade-tolerant	69	-0.050	0.015***	-0.19**	0.022***	0.56	<0.001
Nitrogen-fixing	66	-0.20	0.028***	0.25**	-0.021*	0.23	<0.001
Species							
C. arborea (LD)	24	-0.086	0.015	-0.43*	0.057**	0.55	<0.001
L. procera (LD)	20	-0.034	0.024$\cdot$	NA	NA	0.15	0.051
S. amara (LD)	23	-0.13	0.027***	NA	NA	0.56	<0.001
H. didymantha (ST)	24	0.12	NA	NA	NA	NA	NA
P. pittieri (ST)	22	-0.071	0.018**	-0.28*	0.028**	0.77	<0.001
V. koschnyi (ST)	23	-0.073	0.015$\cdot$	-0.21	0.026$\cdot$	0.57	<0.001
I. pezizifera (NF)	24	-0.14	0.021**	0.26*	-0.021*	0.24	0.038
I. thibaudiana (NF)	19	-0.38	0.044***	NA	NA	0.48	<0.001
P. macroloba (NF)	23	-0.17	0.028*	NA	NA	0.15	0.041

783
784

785

786 **Table 2.** Height RGR regression table: sample size (n), regression parameter estimates, adjusted

787 multiple R^2, and whole model p-value for the minimal adequate model of each functional group

788 and species. Species are arranged by functional groups (LD = light-demanding, ST = shade-

789 tolerant, NF = N_2-fixing). NA indicates that the factor or interaction was not included in the

790 minimal adequate model.

791 ᐧ *P < 0.1; * P < 0.05, ** P<0.01; *** P <0.001*

| Functional group	n	Intercept	Light	Fertilization	Light × Fertilization	Adjusted R^2	Model p-value
Light-demanding	64	-0.17	0.033***	NA	NA	0.34	<0.001
Shade-tolerant	66	-0.050	0.017**	-0.17ᐧ	0.020*	0.37	<0.001
Nitrogen-fixing	70	-0.28	0.036***	0.29*	-0.024*	0.27	<0.001
Species							
C. arborea (LD)	23	-0.47	0.052***	0.15**	NA	0.52	<0.001
L. procera (LD)	20	-0.022	0.027ᐧ	NA	NA	0.13	0.070
S. amara (LD)	21	-0.16	0.028***	NA	NA	0.57	<0.001
H. didymantha (ST)	24	-0.067	0.020*	NA	NA	0.14	0.042
P. pittieri (ST)	20	0.049	0.012	-0.39ᐧ	0.032ᐧ	0.40	0.011
V. koschnyi (ST)	22	-0.16	0.023*	0.086*	NA	0.46	0.0011
I. pezizifera (NF)	26	-0.22	0.030***	0.31**	-0.025**	0.45	<0.001
I. thibaudiana (NF)	21	-0.55	0.060**	NA	NA	0.40	0.0012
P. macroloba (NF)	23	-0.15	0.026*	NA	NA	0.14	0.043

792

793

794

795 **Figures**
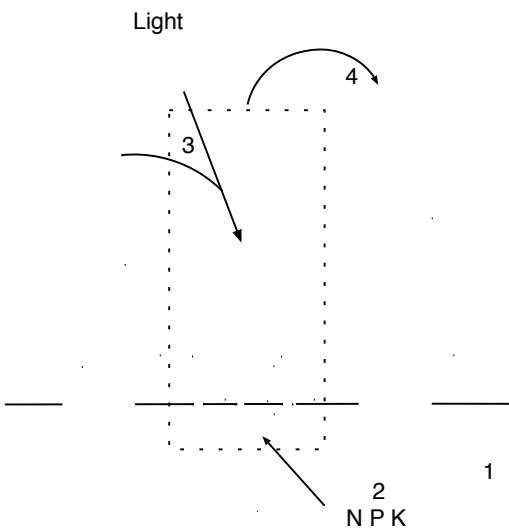
796

797 **Fig. 1.** Sapling and canopy tree feedbacks are central to lowland tropical rainforest nutrient

798 dynamics. Canopy trees dominate feedbacks to ecosystem level nutrient cycling by providing a

799 large proportion of ecosystem foliar, wood, and root litter inputs with functional group- or

800 species-specific nutrient concentrations (1). This in turn can result in functional group- or

801 species-specific impacts on decomposition rates and total fluxes of nutrient inputs from litter

802 pools to the soil (2). Additionally, these canopy trees may also impact understory light

803 availability in functional group- or species-specific ways based on their crown structure, and this

804 light availability interacts with understory nutrient dynamics as well (3). However, nutrient-light

805 limitation of saplings during the bottleneck to reach the canopy (dotted box) determines which

806 individuals become canopy trees (4). Sapling response to soil nutrients and light, and

807 corresponding competitive success during this bottleneck, may also be functional group- or

808 species-specific.

809

810
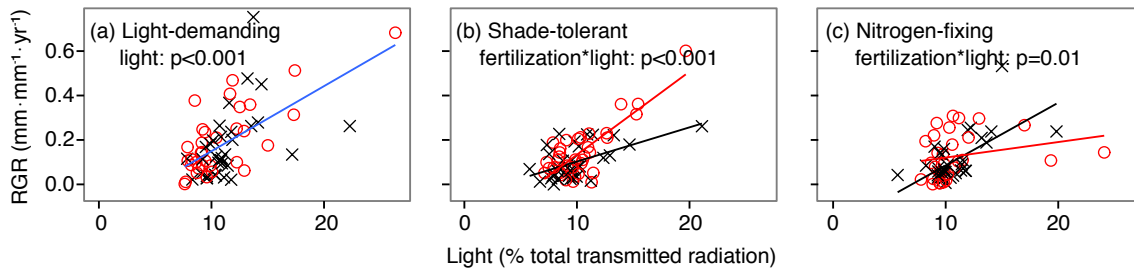
811

812 **Fig. 2.** Relationship between diameter RGR and light availability by functional group and

813 fertilization treatment (red circles = fertilized, black crosses = unfertilized). Significance values

814 and lines represent the minimal adequate model for each species (red = fertilized, black =

815 unfertilized, blue = no significant fertilization treatment).

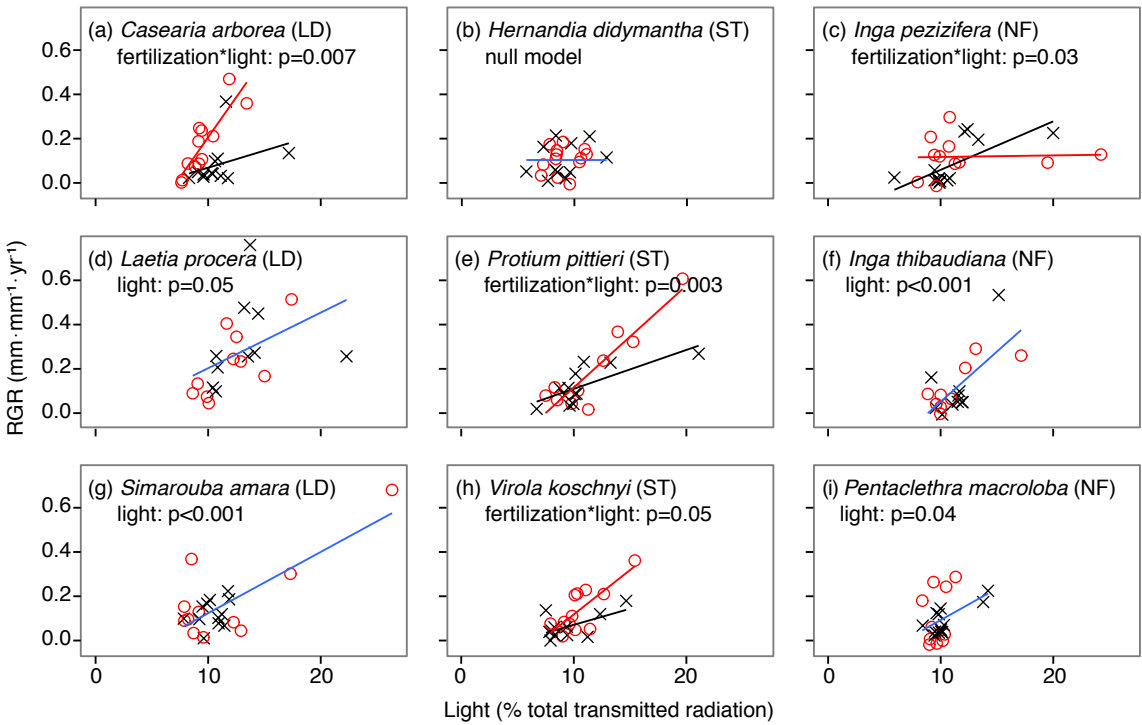816

817

818

819

820

821

822

823

824

825

826

827

Fig. 3. Relationship between diameter RGR and light availability by species and fertilization treatment (red circles = fertilized, black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each species (red = fertilized, black = unfertilized, blue = no significant fertilization treatment). Species are arranged by functional group columns (LD = light-demanding, ST = shade-tolerant, NF = N_2-fixing).
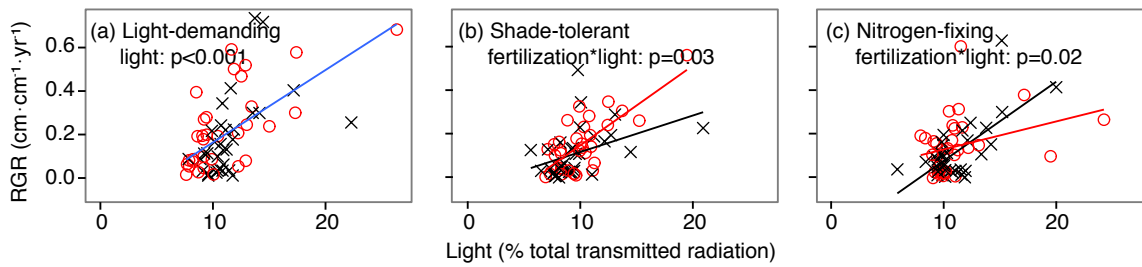
Fig. 4. Relationship between height RGR and light availability by functional group and fertilization treatment (red circles = fertilized, black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each species (red = fertilized, black = unfertilized, blue = no significant fertilization treatment).
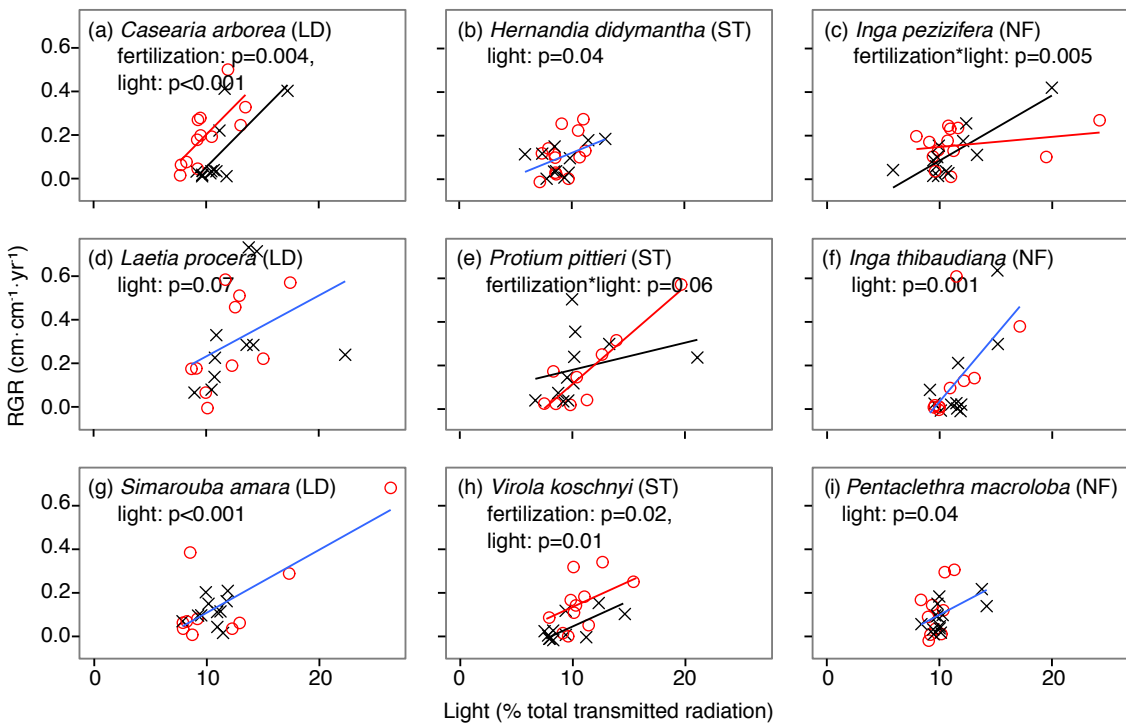
Fig. 5. Relationship between height RGR and light availability by species and fertilization treatment (red circles = fertilized, black crosses = unfertilized). Significance values and lines represent the minimal adequate model for each species (red = fertilized, black = unfertilized, blue = no significant fertilization treatment). Species are arranged by functional group columns (LD = light-demanding, ST = shade-tolerant, NF = N_2-fixing).

871 **SUPPORTING INFORMATION**

872 Additional supporting information may be found in the online version of this article:

873

874 **Appendix S1.** Repeated measures regression analysis.

875 **Table S1.** Diameter repeated measures growth rate regression table.

876 **Table S2.** Height repeated measures growth rate regression table.

877

878

879

880

881

882

883

884

885

886

887

888

889

890 As a service to our authors and readers, this journal provides supporting information supplied by

891 the authors. Such materials may be re-organized for online delivery, but are not copy-edited or

892 typeset. Technical support issues arising from supporting information (other than missing files)

893 should be addressed to the authors.

1   **Appendix S1. Repeated measures regression analysis.**

2   For a repeated measures analysis of sapling response to nutrients and light, we first

3   calculated individual sapling growth rates (mm/yr for diameter and cm/yr for height) through a

4   repeated measures regression of sapling size from each of the six censuses, after correcting for

5   initial size. This allowed us to include individuals that could only be used for part of the study,

6   although we excluded those with overall negative height growth due to stem breakage.

7   As expected, we found a significant and substantial positive relationship between both

8   diameter and height and the fixed effect of time for all saplings (parameter estimates = 1.98

9   mm/yr and 23.74 cm/yr, respectively; marginal R^2 = 0.26 and 0.21, respectively; p<0.001 for

10   both). Additionally, we found that a large portion of the total variance was explained by the

11   combination of the fixed effect of time in conjunction with the variance of each sapling modelled

12   as a random effect (conditional R^2 = 0.93 and 0.88, respectively; Nakagawa & Schielzeth 2013).

13   Therefore, by summing the mean parameter estimate for time with the random effect of each

14   sapling to calculate the growth rates of each individual sapling, we captured a high proportion of

15   the total variance in the data.

16   We then used these calculated individual sapling growth rates as the response variable to

17   determine the minimal adequate model for each functional group and each species and whether

18   that model included light availability, fertilization, or their interaction (Tables S1 and S2). To do

19   this, we used stepwise linear regression as described in the Methods section. We used the "lme4"

20   (Bates et al. 2015), "lmerTest" (Kuznetsova et al. 2016), and "MuMIn" (Barton 2013) packages

21   in R for mixed effects models.

22   Despite the overall similarity of the repeated measures results to the main text linear

23   regression analysis results, we did find two qualitative differences for the diameter data. First,

24   the light-demanding functional group did show a significant positive growth response to a

25   fertilization × light interaction in the repeated measures analysis (Table S1), which was not seen

26   in the main analysis (Fig. 2a). This is indicative of a weaker response to fertilization in this

27   functional group compared to the responses to fertilization seen in the other two functional

28   groups (Figs 2b and c), which were clear even without the amplification provided by the repeated

29   measures analysis. The second qualitative difference was that *Hernandia didymantha* did show a

30   positive growth response to light availability in the repeated measures analysis (Table S1), which

31   was not seen in the main analysis (Fig. 3b). Once again, this is indicative of a weaker response to

32   light availability in this species compared to the responses to light seen in the other species (Fig.

33   3), which were clear in the main regression analysis.

34

35

36

37

38   **References**

39   Barton, K. (2013) MuMIn: Multi-model inference. R package version 1.9.13. Available at

40   https://CRAN.R-project.org/package=MuMIn.

41   Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models

42   using lme4. *Journal of Statistical Software*, **67**, 1–48, doi:10.18637/jss.v067.i01.

43   Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. (2016). Tests in Linear Mixed Effects

44   Models. Available at https://CRAN.R-project.org/package=lmerTest.

45   Nakagawa, S. & Schielzeth, H. (2013) A general and simple method for obtaining R^2 from

46   generalized linear mixed-effects models. *Methods in Ecology and Evolution*, **4**, 133–142.

47   **Table S1.** Diameter repeated measures growth rate (mm/yr) regression table: sample size (n),

48   regression parameter estimates, adjusted multiple R^2, and whole model p-value for the minimal

49   adequate model of each functional group and species. Species are arranged by functional groups

50   (LD = light-demanding, ST = shade-tolerant, NF = N_2-fixing). NA indicates that the factor or

51   interaction was not included in the minimal adequate model.

52    $\cdot$ *P < 0.1; * P < 0.05, ** P<0.01; *** P <0.001*

Functional group	n	Intercept	Light	Fertilization	Light × Fertilization	Adjusted R^2	Model p-value
Light-demanding	76	-3.96	0.57***	-4.55*	0.48**	0.60	<0.001
Shade-tolerant	77	-2.92	0.47***	-3.66**	0.41**	0.64	<0.001
Nitrogen-fixing	77	-2.46	0.31***	1.98*	-0.16*	0.35	<0.001
Species							
C. arborea (LD)	26	-0.86	0.18	-4.66	0.60*	0.40	0.0025
L. procera (LD)	25	-2.92	0.56**	NA	NA	0.24	0.0074
S. amara (LD)	25	-10.38	1.18***	NA	NA	0.84	<0.001
H. didymantha (ST)	25	-1.75	0.39**	NA	NA	0.25	0.0060
P. pittieri (ST)	26	-3.70	0.53***	-3.65$\cdot$	0.38*	0.79	<0.001
V. koschnyi (ST)	26	-3.32	0.51*	-9.17**	0.94**	0.65	<0.001
I. pezizifera (NF)	27	-2.62	0.34***	2.84**	-0.26**	0.56	<0.001
I. thibaudiana (NF)	24	-3.32	0.39**	NA	NA	0.36	0.0012
P. macroloba (NF)	26	-0.89	0.18*	NA	NA	0.15	0.027

53

54

55

56

57

58

59 **Table S2.** Height repeated measures growth rate (cm/yr) regression table: sample size (n),

60 regression parameter estimates, adjusted multiple R^2, and whole model p-value for the minimal

61 adequate model of each functional group and species. Species are arranged by functional groups

62 (LD = light-demanding, ST = shade-tolerant, NF = N$_2$-fixing). NA indicates that the factor or

63 interaction was not included in the minimal adequate model.

64 $^\cdot$ *P < 0.1; * P < 0.05, ** P<0.01; *** P <0.001*

Functional group	n	Intercept	Light	Fertilization	Light × Fertilization	Adjusted R^2	Model p-value
Light-demanding	68	-83.94	10.85***	NA	NA	0.58	<0.001
Shade-tolerant	72	-45.27	6.97***	NA	NA	0.42	<0.001
Nitrogen-fixing	72	-55.40	6.92***	46.77**	-4.29**	0.40	<0.001
Species							
C. arborea (LD)	24	-91.14	10.79***	19.34$^\cdot$	NA	0.47	<0.001
L. procera (LD)	21	-9.09	5.43*	NA	NA	0.17	0.035
S. amara (LD)	23	-125.28	13.96***	NA	NA	0.85	<0.001
H. didymantha (ST)	25	19.07	NA	NA	NA	NA	NA
P. pittieri (ST)	24	-56.38	7.77***	NA	NA	0.60	<0.001
V. koschnyi (ST)	23	-9.36	2.84	-99.93*	10.72*	0.48	0.0013
I. pezizifera (NF)	26	-50.23	6.49***	51.28*	-4.72**	0.50	<0.001
I. thibaudiana (NF)	20	-81.98	9.07***	NA	NA	0.50	<0.001
P. macroloba (NF)	26	-15.68	3.30*	NA	NA	0.12	0.047

65

66

67

68