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Plant responses to fertilization experiments in lowland, species-rich, tropical forests

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Running head: Nutrient limitation in tropical forests

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Abstract – We present a meta-analysis of plant responses to fertilization experiments conducted in lowland, species-rich, tropical forests. We also update a key result and present the first species-level analyses of tree growth rates for a 15-year factorial nitrogen (N), phosphorus (P) and potassium (K) experiment conducted in central Panama. The update concerns community-level tree growth rates, which responded significantly to the addition of N and K together after 10 years of fertilization (Wright et al. 2011) but not after 15 years (this study). Our experimental

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soils are infertile for the region, and species whose regional distributions are strongly associated with low soil P availability dominate the local tree flora. Under these circumstances, we expect muted responses to fertilization, and we predicted species associated with low-P soils would respond most slowly. The data did not support this prediction – species-level tree growth responses to P addition were unrelated to species-level soil P associations. The meta-analysis demonstrated that nutrient limitation is widespread in lowland tropical forests and evaluated two directional hypotheses concerning plant responses to N addition and to P addition. The meta-analysis supported the hypothesis that tree (or biomass) growth rate responses to fertilization are weaker in old growth forests and stronger in secondary forests, where rapid biomass accumulation provides a nutrient sink. The meta-analysis found no support for the long-standing hypothesis that plant responses are stronger for P addition and weaker for N addition. We do not advocate discarding the latter hypothesis. There are only 14 fertilization experiments from lowland, species-rich, tropical forests, 13 of the 14 experiments added nutrients for five or fewer years, and responses vary widely among experiments. Potential fertilization responses should be muted when the species present are well adapted to nutrient-poor soils, as is the case in our experiment, and when pest pressure increases with fertilization, as it does in our experiment. The statistical power and especially the duration of fertilization experiments conducted in old growth, tropical forests might be insufficient to detect the slow, modest growth responses that are to be expected.

Key words: Barro Colorado Nature Monument, fertilization, fine litter production, foliar nutrient concentrations, meta-analysis, nitrogen, old growth forest, phosphorus, potassium, secondary forest, tree growth rates

1 **Introduction**

2 Tropical forests cover just 7% of the Earth's land surface but store 25% of terrestrial
3 carbon and account for 33% of terrestrial net primary productivity (Bonan 2008). The vast
4 majority of these forests spread across the humid lowlands of tropical Africa, Asia and the
5 Americas and support tremendous numbers of species. How these lowland, species-rich, tropical
6 forests respond to atmospheric and climate change will have profound implications for future

7 global carbon and hydrological cycles (Bonan 2008), with the potential for nutrient supplies to
8 limit future carbon sequestration being a crucial uncertainty (Wieder et al. 2015).

9 Comparative nutrient cycling studies generated the hypothesis that phosphorus (P) is
10 limiting in many lowland tropical forests while nitrogen (N) is not. Briefly, leaf N:P ratios
11 increase dramatically before abscission in many lowland tropical forests, suggesting more
12 efficient reabsorption of P than N (McGroddy et al. 2004). Partly as a result, P tends to cycle
13 more efficiently than N in fine litter in tropical lowland forests relative to temperate, boreal and
14 tropical montane forests (Vitousek 1984, Vitousek and Sanford 1986). In addition, gaseous and
15 hydrological losses of N tend to be much larger in tropical lowland forests than in temperate and
16 boreal forests, suggesting N supplies exceed plant demand in tropical lowland forests (Houlton et
17 al. 2006, Hedin et al. 2009). Finally, foliar P concentrations correlate strongly with total soil P
18 stocks (Cleveland et al. 2011), and fine litter production increases with litter P but not litter N
19 concentration in lowland tropical forests (Vitousek 1984). These comparative studies are
20 consistent with the hypothesis that P is limiting in many lowland tropical forests while N is not.

21 At least two mechanisms contribute to the nutrient cycling differences observed between
22 tropical lowland forests versus temperate, boreal and tropical montane forests. Bedrock and
23 biological fixation are the primary sources of P and N, respectively, and Walker and Syers
24 (1976) showed that P availability declines as bedrock decomposes and P leaches away during
25 pedogenesis and N availability increases as biological fixation develops. Rapid erosion keeps
26 many montane soils in early stages of pedogenesis (Porder et al. 2007), and Quaternary
27 glaciation exposed fresh bedrock and spread dust and till from ground bedrock over a large
28 portion of temperate and boreal but not tropical latitudes (Vitousek 1984). In addition, warm,
29 moist conditions enhance decomposition rates in the lowland tropics, preventing accumulation of
30 unavailable organic N in an organic horizon. These regional differences in soil age and
31 decomposition rates and the consistent results of comparative nutrient cycling studies motivate
32 the hypothesis that lowland tropical forests growing on old, highly weathered Ultisols and
33 Oxisols tend to be limited by P (or another rock derived nutrient) and not by N (Vitousek 1984,
34 Vitousek and Sanford 1986, Vitousek et al. 2010). This hypothesis is now widely accepted (Elser
35 et al. 2007), but experimental tests are few.

36 Operational definitions of nutrient limitation of biological processes include positive
37 responses to experimental fertilization (Vitousek and Howarth 1991, Vitousek et al. 2010).

38 Classic fertilization experiments confirmed the hypothesis that N and P limit Hawaiian forests
39 growing on young versus old soils, respectively (Harrington et al. 2001). Strong, positive
40 responses to N and P fertilization indicate N and P both limit plants in most terrestrial biomes
41 (Elser et al. 2007, LeBauer and Treseder 2008). These meta-analyses report reasonable numbers
42 of experiments for tropical forests, but these mainly concern montane forests, Hawaiian forests
43 dominated by a single tree species, species-poor mangrove and planted forests, and even potted
44 seedlings (see *Discussion: Experimental evidence for nutrient limitation*). Just 14 fertilization
45 experiments have been conducted in lowland, species-rich, tropical forests (LSRTEF), with more
46 than half published in the 10 years since the two meta-analyses (Appendix S1: Tables S1 and
47 S2). In a third global meta-analysis, temperate and boreal forest plants respond strongly to
48 potassium (K) fertilization; however, the single tropical study concerned potted seedlings
49 (Tripler et al. 2006). Experimental evidence of nutrient limitation of plants in LSRTEF is scant
50 and a systematic review is lacking.

51 Here, we update a key result from our own factorial NPK fertilization experiment
52 conducted in central Panama and perform a meta-analysis of the 14 fertilization experiments
53 conducted in LSRTEF. In our experiment, each added nutrient increased plant tissue
54 concentrations of that nutrient; K addition (henceforth +K) decreased fine root biomass and
55 increased rates of seedling growth, fine root turnover and decomposition; +P increased fine litter
56 production and rates of photosynthesis, stomatal conductance and decomposition; combined N
57 and P addition (henceforth +NP) increased seedling growth rates; +PK further increased stomatal
58 conductance; and +NK ameliorated declining tree growth rates (Kaspari et al. 2008, Wright et al.
59 2011, Yavitt et al. 2011, Pasquini and Santiago 2012, Santiago et al. 2012, Mayor et al. 2014,
60 Turner et al. 2014, Pasquini et al. 2015). The key result that has changed as the fertilization
61 treatments continue concerns tree growth rates, which are no longer responsive to +NK.

62 We also use newly available information on species-level responses to a natural gradient
63 of P availability (Condit et al. 2013) to inform the first species-level analysis of tree growth rates
64 for our experiment. We test the hypothesis that species whose regional distributions are
65 associated with P-rich soils respond more strongly to P addition than do species associated with
66 P-poor soils.

67 Our meta-analysis focuses on two hypotheses. The first hypothesis is that P limitation is
68 stronger and N limitation is weaker in LSRTEF. The second hypothesis is that nutrient limitation

69 is stronger in secondary forests (and in high light tree-fall gaps) and weaker in old growth
70 forests. The second hypothesis is motivated by the large nutrient sink imposed by rapid biomass
71 accumulation in secondary forests and tree-fall gaps. The meta-analysis is limited to N and P
72 because just two fertilization experiments have considered any other nutrient for LSRTF. The
73 meta-analysis is also limited to four types of responses – foliar N and P concentrations, fine litter
74 N and P concentrations, fine litter production, and tree or biomass growth rates – because too
75 few experiments have considered other types of responses. The meta-analysis indicates that N
76 and P are equally likely to be limiting in LSRTF and are more likely to limit tree/biomass growth
77 rates in secondary forests than in old growth forests.

78

79 **Methods**

80 *Meta-analysis*

81 We compiled 18 published articles from 14 fertilization experiments conducted in
82 LSRTF (Appendix S1). For each experiment, we extracted a soil description, forest type
83 (secondary or old growth), forest age for secondary forests, fertilizer type, number of years of
84 fertilization, plot area and number, and any special circumstances. We tallied the number of
85 significant responses to any type of fertilizer for four types of responses – foliar nutrient
86 concentrations, fine litter nutrient concentrations, fine litter production and tree or biomass
87 growth rates. We are limited to these four response types because too few studies documented
88 any other response.

89 We also conducted a formal meta-analysis for the subset of experiments that compared
90 control versus +N and/or +P treatments (or in one case –N and –P treatments versus a complete
91 fertilizer). We extracted community-level and/or species-level treatment means, standard
92 deviations and sample sizes for each response. We treated analyses for different species and for
93 different tree size categories as separate tests. We excluded community-level analyses if analyses
94 were also partitioned by species or size. Several studies did not report standard deviations for
95 particular responses and these responses were excluded (tree growth, Mirmanto et al. 1999;
96 species-level growth, Gehring et al. 1999; all responses, Newbery et al. 2002; foliar nutrient
97 concentrations, Davidson et al. 2004). Appendix S1, Metadata S1 and Data S1 present the
98 articles and extracted data included in the meta-analysis.

99 We conducted the meta-analysis with the ‘metafor’ package (version 2.0-0, Viechtbauer
100 2010) in R version 3.3.2. We used the `escalc()` function to calculate Hedge’s g and random effect
101 models to estimate 95% confidence intervals for each response. Plant performance improved
102 significantly with fertilization when lower 95% confidence intervals were positive. We added
103 moderators to random effect models to test two directional hypotheses. Fertilization responses
104 are (1) stronger for P addition than for N addition and (2) stronger for secondary forests than for
105 old growth forests (see *Introduction* for rationale). Just two experiments conducted in secondary
106 forests reported litter production and litter nutrient concentrations (Appendix S1: Table S2), so
107 we could not isolate forest type for litter responses. We evaluated the first hypothesis with
108 secondary and old growth forests pooled for all four types of response and for each forest type
109 separately for foliar nutrient concentrations and tree/biomass growth. We also evaluated the
110 second hypothesis for foliar nutrient concentrations and tree/biomass growth. We report Wald
111 chi-square and one-tailed p -values for directional hypotheses. We followed the recommendations
112 of Jennions et al. (2013) to evaluate potential publication bias.

113 *Study site*

114 Our experiment is located on the mainland in the Barro Colorado Nature Monument in
115 central Panama (9° 06' 31" N, 79° 50' 37" W). Tree species composition and stature (canopy
116 heights up to 43 m) are characteristic of old growth (>200 years) forest. Aerial photographs
117 confirm the presence of tall forest in 1927 (SJW, personal observation). The soils developed on
118 Miocene basalt and transition from Oxisols (Typic Hapludox in Soil Taxonomy; Soil Survey
119 Staff 1999) in the upper northeast corner of the 38.4-ha experimental plot to poorly drained
120 Inceptisols (Aeric Epiaquepts) in the low lying, southwest corner (B. L. Turner, *unpublished*
121 *data*). Although our experimental forest is fertile relative to many Amazonian forests (Wright et
122 al. 2011), it is infertile for central Panama, with very low concentrations of exchangeable
123 phosphate (< 1 mg P kg⁻¹ by resin extraction) and moderately low exchangeable K (Yavitt et al.
124 2009, Condit et al. 2013, Mirabello et al. 2013, Turner et al. 2013, 2015).

125 *Experimental design*

126 We replicated the eight treatments of a 2x2x2 factorial NPK experiment four times.
127 Within each replicate, we blocked the N, P, K and NPK treatments versus the NP, NK, PK and
128 control treatments (see App. Fig. 1 in Wright et al. 2011). This balanced, incomplete-block
129 design minimizes uncontrolled error associated with spatial variation, enables evaluation of main

130 effects and two-way interactions, but limits power to evaluate the three-way interaction (Winer
131 1971). The 32 experimental plots are each 40 by 40 m and are separated by a minimum distance
132 of 40 m, with the exception of two plots separated by 20 m and a 2-m deep streambed.

133 Beginning in 1998, we added fertilizer by hand in four equal doses each wet season with
134 6–8 weeks between applications (approximate dates May 15–30, July 1–15, September 1–15 and
135 October 15–30). Nitrogen was added as coated urea ((NH₂)₂CO), P as triple superphosphate
136 (Ca(H₂PO₄)₂·H₂O), and K as potassium chloride (KCl). Annual doses were 125 kg N ha⁻¹ yr⁻¹,
137 50 kg P ha⁻¹ yr⁻¹ and 50 kg K ha⁻¹ yr⁻¹, which equals 69%, 470% and 88% of annual inputs from
138 fine litter at a site 3-km to the north, respectively (Yavitt et al. 2004). Similar large additions of P
139 relative to annual litter inputs are standard practice in tropical nutrient addition experiments (see
140 studies in Appendix S1: Tables S1 and S2) because tropical soils, including the soils at our site,
141 tend to sequester large amounts of added P in forms believed to be inaccessible to plants
142 (Mirabello et al. 2013).

143 We identified trees and measured diameter at breast height (DBH defined as 1.3 m) in
144 1997, 1998, 1999, 2000, 2001 (DBH ≥ 100 mm only), 2003, 2008 and 2013, using the methods
145 of Condit (1998). We recorded measurement height if buttresses or deformities prevented
146 measurement at 1.3 m. We censused all trees with DBH ≥ 100 mm and, for a central 20-by-30 m
147 subset of each plot, all trees with DBH ≥ 10 mm. We used the 1998, 2003, 2008 and 2013
148 censuses to calculate relative growth rates (RGR) for three 5-yr census intervals as:

$$149 \quad \text{RGR} = (\ln(\text{DBH}_f) - \ln(\text{DBH}_i)) / (\text{DOC}_f - \text{DOC}_i) / 365.25,$$

150 where DOC refers to day of century of the corresponding DBH measurement and the subscripts *f*
151 and *i* refer to final and initial values, respectively. We excluded palms because diameter growth
152 is absent or limited and dicots with broken main trunks, changes in measurement height, or
153 multiple stems at the measurement height.

154 *Analyses of community-level growth rates*

155 We used repeated measures ANOVA and a linear mixed effects analysis to analyze RGR.
156 Repeated measures ANOVA is the appropriate analysis for our designed experiment. The
157 response variable was the average RGR value for each plot-census interval combination,
158 repeated measures were on census interval and main effects were nutrient treatments, their two-
159 way interactions, blocks within replicates and all interactions with census interval. RGR tends to
160 decline with DBH ($r = -0.11$, $n = 17,824$ in our data). To minimize this source of variation, we

161 performed repeated measures ANOVAs for five relatively narrow size classes as follows: shrubs
162 and saplings ($10 \text{ mm} \leq \text{DBH}_i < 25 \text{ mm}$), small poles ($25 \text{ mm} \leq \text{DBH}_i < 50 \text{ mm}$), large poles (50
163 $\text{mm} \leq \text{DBH}_i < 100 \text{ mm}$), small trees ($100 \text{ mm} \leq \text{DBH}_i < 250 \text{ mm}$) and large trees ($\text{DBH}_i \geq 250$
164 mm). We performed repeated measures ANOVAs with the ‘aov’ command in R 3.3.2. We
165 repeated each analysis in SYSTAT© 11 (Richmond, CA) to evaluate the compound symmetry
166 assumption, which was satisfied for all five size classes (Huynh-Feldt Epsilon close to 1).

167 The repeated measures ANOVAs partitioned by tree size suggested a possible
168 fertilization-tree size interaction (see *Results: Community-level growth rates*). We used a linear
169 mixed effects analysis to evaluate this possibility, incorporating initial size (DBH_i) as a
170 covariate. The response variable was the RGR value for each individual-census interval
171 combination. Fixed effects were the N, P and K treatments, their two-way interactions and their
172 interactions with DBH_i . Random effects were census interval, individual within species, and plot
173 within block and replicate. We included species with 20 or more individuals. We compared AIC
174 values for models that included all random effects and all possible combinations of fixed effects.

175 *Analyses of species-level growth rates*

176 We used the P effect sizes of Condit et al. (2013) to characterize species-level P affinities
177 for the regional species pool and the experimental forest. Condit et al. (2013) evaluated
178 relationships between occurrence and soil fertility for 550 tree species, using 72 tree and soil
179 surveys conducted within 50 km of our site. Strong effect sizes, with absolute values > 0.5 ,
180 characterized relationships between occurrence probability and soil resin P availability for 57.6%
181 of the 550 species (Condit et al. 2013). To describe P affinity for the regional species pool, we
182 examined the distribution of the 550 central Panama species among P effect sizes. To describe P
183 affinity for the experimental forest, we examined the distribution of individual trees among P
184 effect sizes.

185 We used a linear mixed effects analysis to evaluate relationships between species-level
186 RGR, P addition and P affinity. We treated P effect sizes as a covariate to represent species-level
187 P affinity, P addition as a fixed effect, and census interval, individual within species and plot
188 within block and replicate as random effects. We did not consider the N and K treatments
189 because they were insignificant in the previous analysis (see *Results: Community-level growth*
190 *rates*) and N and K effect sizes were consistently small in the analysis of Condit et al. (2013).
191 We included species with four or more individuals in each P treatment. We compared AIC

192 values for models that included all random effects and all combinations of P addition, P affinity
193 and the interaction between P addition and P affinity. To avoid the compounding number of
194 interactions associated with a second covariate, we performed this analysis for the four smaller
195 size classes described previously (see *Methods: Analyses of community-level growth rates*). We
196 excluded the largest size class because too few species had four or more large individuals in each
197 P treatment.

198 *Power analysis*

199 We conducted two simulations of our repeated measures ANOVA to evaluate statistical
200 power. We used simulations because, to the best of our knowledge, a standard power analysis is
201 not available for our incomplete block design (see *Methods: Experimental Design*). The first
202 simulation used a common overall plot mean RGR value and its standard deviation (SD). The
203 second simulation used plot mean RGR and SD values observed for each block and census
204 interval. To simulate positive responses to P addition, we increased means observed for no-P
205 plots by 1%, 3%, 5%, ... and 41% for +P plots. We then drew random values from normal
206 distributions with the appropriate means and SDs for all 32 plots and performed the repeated
207 measures ANOVA. We repeated these steps 1,000 times for each percentage increase in +P
208 means and tallied the number of times the main effect of P was significant.

209 **Results**

210 *Community-level growth rates*

211 The number of RGR values ranged from 330 to 6,633 for the five tree size classes
212 (Appendix S2: Table S1). The main effects of N, P and K addition and their two-way interactions
213 were insignificant for all five size classes in the repeated measures ANOVAs, although the main
214 effect of K addition was marginally insignificant ($p = 0.057$) for the largest trees (Appendix S2:
215 Table S2). RGR varied significantly among census intervals for shrubs and saplings, with lower
216 RGR in the second census interval (2003 to 2008), but did not vary significantly among census
217 intervals for the four larger size classes (Appendix S2: Table S2). The repeated measures
218 ANOVAs provide little to no statistical evidence that fertilizers affected RGR.

219 The relative growth rates of shrubs, saplings and small poles (< 50 mm DBH) tended to
220 be larger in the control treatment than in any of the nutrient addition treatments (Fig. 1). This
221 insignificant tendency was absent for large poles and tended to be reversed for small and large
222

223 trees (Fig. 1). This suggested a possible interaction between nutrient treatments and tree size. To
224 evaluate this possibility, we performed a linear mixed effects analysis of RGR that included trees
225 of all sizes and treated initial size (DBH_i) as a covariate. This analysis included species
226 represented by 20 or more individuals, 13,688 RGR measurements and 5,510 individual trees.
227 The number of RGR values ranging from 73 to 208 (mean = 143) among plot-census interval
228 combinations. The model that included DBH_i as a covariate minimized AIC, with $\Delta AIC = 12$ for
229 the next best model and $\Delta AIC = 183$ for the null model that included just random effects
230 (Appendix S2: Table S3). The linear mixed effects analysis provides no statistical evidence that
231 fertilizers affected RGR.

232 *Species-level growth rates*

233 The 550 species for which Condit et al. (2013) determined P effect sizes include 93.1%
234 of the species and 98.3% of the individuals in our experimental forest. Species with strong
235 positive P effect sizes (>0.5) are associated with P-rich soils (high-P affinity) and comprised
236 20% of the species in the regional species pool (Fig. 2A), but just 6% of the individuals in the
237 experimental forest (Fig. 2B). Species associated with P-rich soils are underrepresented in the
238 experimental forest relative to the regional species pool.

239 The number of RGR values ranged from 747 to 5,851 for the four tree size classes for the
240 linear mixed effects analysis that included P affinity (Appendix S2: Table S4). The main effects
241 of P addition, P affinity and their interaction were insignificant for all size classes (Fig. 2C,
242 Appendix S2: Table S5). As in the repeated measures ANOVAs, RGR varied significantly
243 among census intervals for shrubs and saplings, with lower RGR in the second census interval
244 (2003 to 2008), but not for the three larger size classes (Appendix S2: Table S5).

245 *Power analysis*

246 Our repeated measures ANOVA had a 46% chance of detecting a 20% increase in RGR
247 (Appendix S2: Fig. S1). Relaxing control of spatial and temporal variation associated with
248 blocks and census intervals had little effect on power (Appendix S2: Fig. S1). This is consistent
249 with the uniformly insignificant effects of blocks and replicates in the repeated measures
250 ANOVAs (Appendix S2: Table S2).

251

252

253 *Meta-analysis*

254 Seven and nine of the 14 fertilization experiments enable isolation of N and/or P
255 responses, respectively (Appendix S1: Tables S1 and S2). Sample sizes can be larger when
256 single studies reported responses for multiple species or tree size classes. Sample sizes can also
257 be smaller when only a subset of studies documented a particular response. There was no
258 evidence for publication bias after accounting for heterogeneity between secondary and old
259 growth forests (Appendix S1: Table S3).

260 All mean effect sizes were positive, indicating plants tend to be limited by N and by P in
261 LSRTF (Fig. 3). Ten of the 16 mean effect sizes were individually significant ($p < 0.05$). Four of
262 the six insignificant effect sizes concerned tree/biomass growth rates in old growth forests (Fig.
263 3F) and litter element concentrations (Fig. 3B).

264 We evaluated the interaction between forest type (secondary versus old growth) and
265 fertilizer type (+N versus +P) and the directional hypothesis that responses are stronger in
266 secondary forests and weaker in old growth forests for foliar nutrient concentrations and
267 tree/biomass growth rates. Forest*fertilizer interactions were insignificant for foliar nutrient
268 concentrations (Fig. 3E; $QM_1 = 0.287$, $p = 0.296$) and for tree/biomass growth rates (Fig. 3F;
269 $QM_1 = 0.255$, $p = 0.307$). For foliar nutrient concentrations, the null hypothesis that fertilization
270 responses were similar in secondary and old growth forests was accepted (Fig. 3E; $QM_1 = 1.64$,
271 $p = 0.100$ for pooled responses; $QM_1 = 1.44$, $p = 0.116$ for N responses to +N; $QM_1 = 0.327$, $p = 0.284$
272 for P responses to +P). For tree/biomass growth rates, the null hypothesis that fertilization
273 responses were similar in secondary and old growth forests was rejected (Fig. 3F; $QM_1 = 8.41$,
274 $p = 0.0019$ for responses to pooled fertilizers; $QM_1 = 5.59$, $p = 0.0091$ for responses to +N;
275 $QM_1 = 2.97$, $p = 0.0423$ for responses to +P). Tree/biomass growth responses were significantly
276 stronger in secondary forests and weaker in old growth forests (Fig. 3F).

277 We evaluated the directional hypothesis that responses are stronger for P addition and
278 weaker for N addition for all four responses with secondary and old growth forests pooled and
279 for foliar nutrient concentrations and tree/biomass growth rates for each forest type. The null
280 hypothesis that responses are similar for +P and +N could never be rejected. Figures 3A through
281 3D present tests with secondary and old growth forests pooled (Fig. 3A, $QM_1 = 2.66$, $p = 0.051$ for
282 foliar nutrient concentrations; Fig. 3B, $QM_1 = 0.153$, $p = 0.348$ for fine litter nutrient
283 concentrations; Fig. 3C, $QM_1 = 0.111$, $p = 0.370$ for fine litter production; Fig. 3D, $QM_1 = 0.430$,
284 $p = 0.256$ for tree/biomass growth rates). Figure 3E presents foliar nutrient concentrations for each

285 forest type ($QM_1=0.0957$, $p=0.378$ for secondary and $QM_1=2.35$, $p=0.063$ for old growth
286 forests). Figure 3F presents tree/biomass growth rates for each forest type ($QM_1=0.0253$,
287 $p=0.437$ for secondary and $QM_1=0.758$, $p=0.192$ for old growth forests). The meta-analysis
288 provides no evidence that plant responses differ for +P versus +N treatments.

289

290 **Discussion**

291 When just two census intervals were available, our experiment included significant
292 N*K*census interval interactions for growth for the three smaller tree size classes (Wright et al.
293 2011). Growth rates were larger in the first census interval, and +NK ameliorated the decline to
294 the second census interval (see Fig. 1 in Wright et al. 2011). Now, with three census intervals
295 available, all N*K*interval interactions are insignificant and the second census interval has
296 significantly slower growth rates for the smallest size class only (Appendix S2: Table S2). Our
297 new analysis includes 50% more RGR values and years of fertilization and must replace the
298 earlier analysis. After 15 years of chronic nutrient additions, our experiment provides virtually
299 no evidence that nutrients limit tree growth (Fig. 1, Appendix S2: Tables S2 and S3).

300 Our treatments have significantly increased soil nutrient availability and many measures
301 of plant performance. Phosphorus addition increased soil phosphate availability by 2800%; +K
302 increased K availability by 91%; and +N increased nitrate availability by 120% (Yavitt et al.
303 2011, Mirabello et al. 2013, Turner et al. 2013). Species- and community-level plant responses,
304 ranging from increases in metabolism (photosynthesis, stomatal conductance) to increases in
305 production (fine litter), standing biomass and tissue turnover rates (fine roots) were reviewed
306 earlier (see *Introduction*). We now consider why these increases in soil nutrient availability and
307 plant performance do not lead to increased tree growth. We begin with a meta-analysis of 14
308 fertilization experiments conducted in lowland, species-rich tropical forests (LSRTF).

309 *Experimental evidence for nutrient limitation*

310 All 14 fertilization experiments address the hypothesis that some combination of
311 nutrients limit plants in LSRTF, and a tally of statistically significant effects indicates that
312 nutrient limitation is widespread (Appendix S1: Tables S1 and S2). Foliar and fine litter
313 concentrations of at least one nutrient increased significantly in seven of eight and five of five
314 experiments, respectively. Fine litter production increased significantly in five of seven
315 experiments. Tree/biomass growth responses varied with forest environment. Tree/biomass

316 growth rates increased significantly in seven of eight experiments conducted in secondary forests
317 or with saplings in high light microsites in old growth forests. In contrast, six experiments
318 conducted in old growth forests documented tree growth responses without finding significant
319 responses for trees larger than 100-mm DBH and with a significant increase for smaller trees in
320 just one study. The contrast between forest environments is significant (Fisher Exact Test,
321 $p=0.0256$ for trees < 100 -mm DBH and $p=0.00466$ for trees > 100 -mm DBH). To summarize,
322 fertilization is often associated with significant increases in tissue nutrient concentrations and
323 fine litter production and, in secondary forests and tree-fall gaps, with significant increases in
324 tree/biomass growth rates. There is, however, no experimental evidence for nutrient limitation of
325 growth rates for trees larger than 100 mm DBH in old growth LSRTF.

326 Our formal meta-analysis of the subset of these experiments with +N or +P treatments
327 produced broadly similar results. All 16 mean effect sizes were positive and 10 were strong
328 (mean Hedge's $g > 0.5$), indicating widespread limitation by N and by P (Fig. 3). The directional
329 hypothesis that N and P limitation of tree/biomass growth rates is stronger in secondary forests
330 and weaker in old growth forests was also supported (Fig. 3F), and once again there was no
331 evidence for N or P limitation of tree growth rates in old growth forests (open circles in Fig. 3F).

332 Our meta-analysis produced two related surprises. Nitrogen limits foliar N concentrations
333 and fine litter production in LSRTF (Figs. 3A and 3C), and there is no evidence that P limitation
334 is stronger than N limitation in LSRTF (all tests in Fig. 3). This is consistent with a meta-
335 analysis of terrestrial N addition experiments in which "The degree of N limitation in the
336 remainder of the tropical forest studies [when Hawaiian forests on recent lava flows were
337 excluded]... was comparable to that of temperate forests..." (LeBauer and Treseder 2008). This
338 conclusion of LeBauer and Treseder (2008) and our own meta-analysis are inconsistent with a
339 second meta-analysis in which "... most fertilization experiments in forests were conducted in
340 tropical latitudes, and this habitat type had a stronger response to added P than added N,
341 suggesting support for the long-held belief that tropical ecosystems on old soils are
342 predominantly P limited (Walker and Syers 1976)." (Elser et al. 2007). To reconcile these
343 contrasting conclusions concerning N limitation, we examined the tropical forest studies in both
344 earlier meta-analyses.

345 Both meta-analyses include experiments conducted in a wide range of tropical forest
346 environments. Elser et al. (2007) include mangrove forests (3 studies), montane forests (8), a

347 monospecific *Eucalyptus* stand (1) and seedlings planted into pots (2), abandoned land (2) and
348 forest understory (1). Several of these studies are of questionable relevance to their conclusion
349 concerning P limitation on old soils because the experimental soils are artificial (pot
350 experiments) or relatively young (many montane forests, Porder et al. 2007). These 17 studies
351 are also irrelevant to our interest in LSRTF. LeBauer and Treseder (2008) also include seven
352 studies conducted in montane forests. Just six and three experiments conducted in LSRTF remain
353 in the compilations of Elser et al. (2007) and LeBauer and Treseder (2008), respectively. Our
354 meta-analysis included these experiments plus eight additional experiments conducted in
355 LSRTF, and we believe the conclusions of our meta-analysis stand for LSRTF.

356 To summarize those conclusions, both N and P addition are associated with strong
357 increases in foliar nutrient concentrations (Figs. 3A and 3E), fine litter production (Fig. 3C) and
358 fine litter nutrient concentrations (Fig. 3B) in LSRTF. The increases in fine litter nutrient
359 concentrations are highly variable, however, suggesting variation in concentrations and/or
360 resorption among tissues and studies (Fig. 3B, Schreeg et al. 2014, Alvarez-Clare et al. 2015).
361 Both N and P addition are also associated with strong increases in tree/biomass growth in
362 secondary forests, where rapid biomass accumulation ensures a nutrient sink (filled circles in
363 Fig. 3F). There is, however, no evidence for the long-standing hypothesis that P limitation is
364 stronger and N limitation is weaker in lowland tropical forests (Figs. 3A through 3F) nor for
365 nutrient limitation of tree growth rates in old growth forests (open circles in Fig. 3F).

366 *Why is tree growth unresponsive to fertilization in old growth forests?*

367 At least four mutually compatible mechanisms might contribute to the absence of tree
368 growth responses to fertilization in old growth LSRTF (open circles in Fig. 3F). The first
369 concerns local species composition and potential growth responses. Species adapted to low
370 resource levels tend to have limited potential to increase growth rates in response to increased
371 resource levels (Coley et al. 1985). This could limit fertilization responses until species adapted
372 to high nutrient soils arrive changing species composition (Chapin et al. 1986). At our
373 experimental forest, species whose regional distributions are strongly associated with P-poor and
374 P-rich soils comprise 47% and just 6% of the individual trees, respectively (Fig. 2B). We should
375 expect modest and slow responses to P addition when species associated with P-poor soils
376 dominate local species composition (Chapin et al. 1986, Kitayama 2005, Dalling et al. 2016).

377 A second possible mechanism for muted growth responses to fertilization concerns plant
378 enemies. Fertilization often increases tissue nutrient concentrations (Figs. 3A, 3B and 3E),
379 making fertilized plants more attractive to herbivores and possibly other pests. Two fertilization
380 experiments conducted in LSRTF considered herbivory. Herbivory increased with +P in 10-year
381 old forests in Mexico (Campo and Dirzo 2003) and with +K and +P in our experiment (Santiago
382 et al. 2012). Potassium addition also reduced net adverse effects of foliar bacteria in our
383 experiment (Griffin et al. 2016). Spatial scale becomes important if fertilized plants attract pests.
384 Fertilizers applied to individual plants or small plots might create nutrient hotspots that recruit
385 nearby pests. Most fertilization experiments are conducted at spatial scales of 10 to 50 m in
386 LSRTF with measurements limited to a central core area (see plot sizes in Appendix S1: Tables
387 S1 and S2). This might limit problems posed by immigration; however, enemy populations
388 might still increase in large fertilized plots if their local demography changes. As an example,
389 forest floor arthropod abundance increased with +K and +P in the central area of our 1,600-m²
390 experimental plots (Kaspari et al. 2017, also see Bujan et al. 2016). If fertilization increases pest
391 pressure, those pests might consume increased primary production, limiting potential tree growth
392 responses (Anderson et al. 2010).

393 As an aside, pest pressure might also contribute to a striking difference between growth
394 responses to +P in our experiment versus a growing house experiment conducted with a subset of
395 our species. In the growing house experiment, species-specific growth responses to +P increase
396 steadily with the strength of species-level associations with P-rich soils (Zalamea et al. 2017). In
397 our forest experiment, sapling growth responses were unrelated to these same species-level
398 associations with P-rich soils (Fig. 2C). Species adapted to high resource levels tend to be poorly
399 defended against herbivores and other pests (Coley et al. 1985). Pests that are absent from the
400 growing house experiment might prevent species associated with P-rich soils from achieving
401 their potential growth responses in the forest experiment. Of course, with so few individuals of
402 species associated with P-rich soils present (Fig. 2B), our statistical power to evaluate their
403 responses is also limited (Fig. 2C).

404 A third possible mechanism for muted growth responses to fertilization concerns time.
405 Thirteen of the 14 fertilization experiments added nutrients for five or fewer years (Appendix S1:
406 Tables S1 and S2) while tropical trees can live for centuries (Chambers 1998, Worbes and Junk
407 1999). There is evidence for size-dependent responses to fertilization. Three studies fertilized *in*

408 *situ* seedlings, and seedling growth rates increased in all three studies (Hättenschwiler 2002,
409 Yavitt et al. 2008, Santiago et al. 2012). Five studies fertilized saplings only (Villagra et al. 2013,
410 Chou et al. 2017) or partitioned growth analyses by tree size (Fisher et al. 2013, Alvarez-Clare et
411 al. 2013, this study), and growth rates of saplings or the smallest tree size class increased in three
412 of the five studies (Alvarez-Clare et al. 2013, Villagra et al. 2013, Chou et al. 2017). In contrast,
413 N and P fertilization had no effect on the growth rates of trees larger than 100 mm DBH in the
414 six fertilization experiments that evaluated larger trees in old growth LSRTF (Appendix S1:
415 Table S1). Large trees can accumulate large reserves of nutrients and carbohydrates, and many
416 years might be required to capture their growth responses to nutrient addition.

417 The final reason for insignificant growth responses concerns statistical power. Our
418 experiment has a reasonable chance of detecting a 20% increase in RGR (Appendix S2: Fig. S1).
419 Sample sizes (see numbers of plots in Appendix S1: Tables S1 and S2) suggest power is likely to
420 be similar or lower for 12 of the 13 remaining experiments conducted in LSRTF unless plot-to-
421 plot variation is unexpectedly low. To summarize, the potential fertilization response of tropical
422 forest trees will be limited if the species present are well adapted to nutrient-poor soils, as in our
423 experiment (Fig. 2B), and if pest pressure increases with fertilization, as in our experiment
424 (Santiago et al. 2012). The statistical power and especially the duration of fertilization
425 experiments conducted in old growth, tropical forests might also be insufficient to detect the
426 slow, modest growth responses that are to be expected.

427 *Conclusions*

428 Our review of 14 fertilization experiments conducted in LSRTF indicates that nutrient
429 limitation is widespread (Appendix S1: Tables S1 and S2). Nutrient availability is already likely
430 to be limiting the ability of these forests to sequester carbon despite rising atmospheric CO₂
431 concentrations (Wieder et al. 2015). Our formal meta-analysis of the subset of these experiments
432 that include +N and/or +P treatments supports the hypothesis that nutrient limitation is stronger
433 in secondary forest and weaker in old growth forest, but does not support the hypothesis that P
434 limitation is stronger and N limitation is weaker. As an aside, evidence for P limitation is also
435 suspect because every experiment that included a +P treatment used simple or triple super
436 phosphate fertilizer. Super phosphate fertilizers supply calcium (Ca) and P at a 1:2 ratio of Ca to
437 P. Bedrock is the primary source for Ca as well as P, and Ca like P might limit tropical forests
438 growing on highly weathered soils (Vitousek 1984, Sanford and Vitousek 1986, Baillie et al.

439 1987, Cuevas and Medina 1988). Nonetheless, we believe it would be premature to discard the
440 hypothesis that P limitation is stronger than N limitation in lowland tropical forests for two
441 reasons.

442 First, the number of fertilization experiments conducted in LSRTF remains small
443 (Appendix S1: Tables S1 and S2) and most of the experiments share modest sample sizes and
444 short durations (see *Discussion: Why is tree growth unresponsive to fertilization in old growth*
445 *forests?*). Effect sizes tend to be larger for plant responses to P addition than to N addition;
446 however, the difference is small and insignificant (Fig. 3). In contrast, a recent meta-analysis of
447 microbial responses to fertilization experiments conducted in tropical forests found strong
448 evidence for P limitation over all tropical forests and evidence for N limitation in montane but
449 not lowland tropical forests (Camezind et al., in press). The contrasting generation times and
450 fertilization responses of microbes and plants suggest that the responses of long-lived plants
451 might strengthen as the duration of fertilization experiments increases.

452 The second reason we believe it would be premature to discard the hypothesis that plant
453 limitation by P is stronger than limitation by N in lowland tropical forests concerns evidence
454 from our own experiment after 15 years of chronic nutrient additions. Soil Ca availability is
455 extraordinarily high in our control plots (averaging 1,690 mg kg⁻¹, Yavitt et al. 2009), and the Ca
456 added with the triple super phosphate fertilizer is not an issue. In contrast to the results of our
457 meta-analysis, our own experiment provides much more evidence for P (and K) limitation than
458 for N limitation. The evidence includes a wide range of bacterial, fungal, arthropod and plant
459 responses (summarized in Table S2 in Kaspari et al. 2017; additional responses in Schreeg et al.
460 2014; Pasquini et al. 2015; Wurzburger and Wright 2015; Griffin et al. 2016, 2017; Bujan et al.
461 2016; Sheldrake et al. 2017). Statistically significant plant responses are roughly equally divided
462 between the +P and +K treatments, with just one significant response to the +N treatment (an
463 increase in tissue N concentrations). We conclude that N rarely limits plant function at our site,
464 and N addition is unlikely to affect tree growth in the future. We predict that the many significant
465 plant responses to +P and +K will, with time, lead to significant increases in tree growth and net
466 primary production. After 15 years, a marginally insignificant trend ($p = 0.057$) suggests that
467 growth responses might be developing first in response to K addition among trees larger than
468 249 mm DBH (Fig. 1, Appendix S2: Table S2). Lloyd et al. (2015) recently hypothesized that K
469 availability plays a key role determining tropical forest structure. We are now in the 20th year of

470 our chronic nutrient addition treatments, and we plan to continue indefinitely to test these and
471 other predictions.

472

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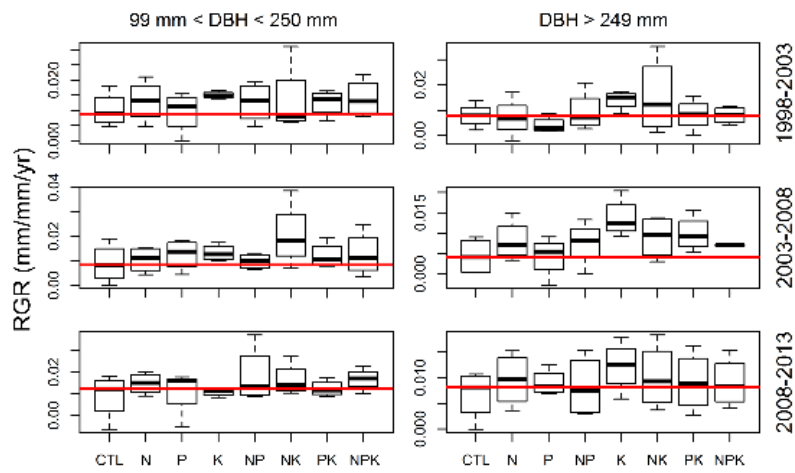
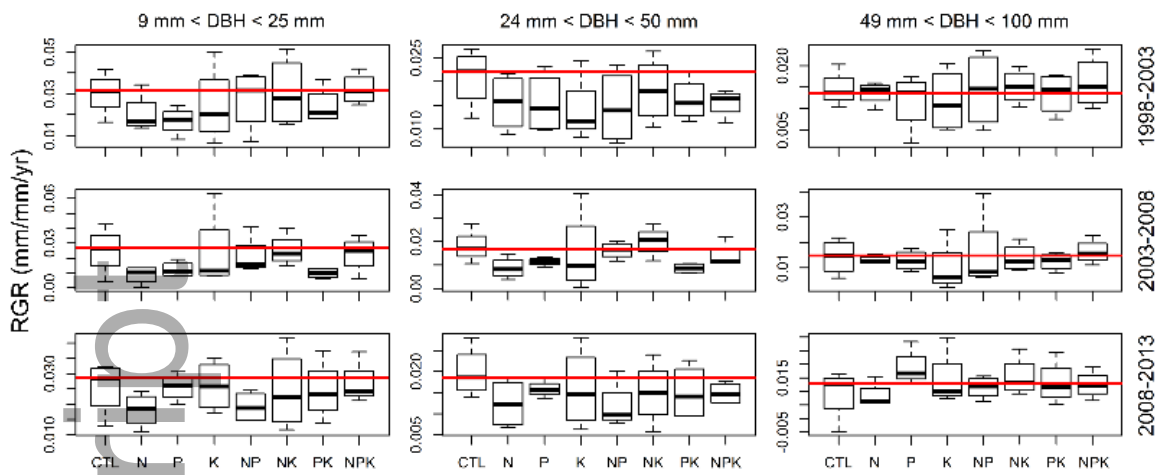
Figure Captions

Figure 1. Bar-and-whisker plots of mean relative growth rates (RGR) for five size classes and three census intervals. The solid red lines represent median RGR for the control treatment for each size class and census interval. The three census intervals are 1998-2003, 2003-2008, and 2008-2013. The five size classes are shrubs and saplings ($10 \text{ mm} \leq \text{DBH}_i < 25 \text{ mm}$), small poles ($25 \text{ mm} \leq \text{DBH}_i < 50 \text{ mm}$), large poles ($50 \text{ mm} \leq \text{DBH}_i < 100 \text{ mm}$), small trees ($100 \text{ mm} \leq \text{DBH}_i < 250 \text{ mm}$) and large trees ($\text{DBH}_i \geq 250 \text{ mm}$). In the first four size classes, treatments order is control, one nutrient (+N, +P and +K), two nutrients (+NP, +NK and +PK) and three nutrients (+NPK). In the final size class, treatment order groups -K (control, +N, +P and +NP) versus +K (+K, +NK, +PK and +NPK) treatments to illustrate the marginally significant effect of K addition ($p = 0.057$). Thick horizontal lines represent medians, boxes represent the interquartile range (25% to 75%) and whiskers represent extreme values.

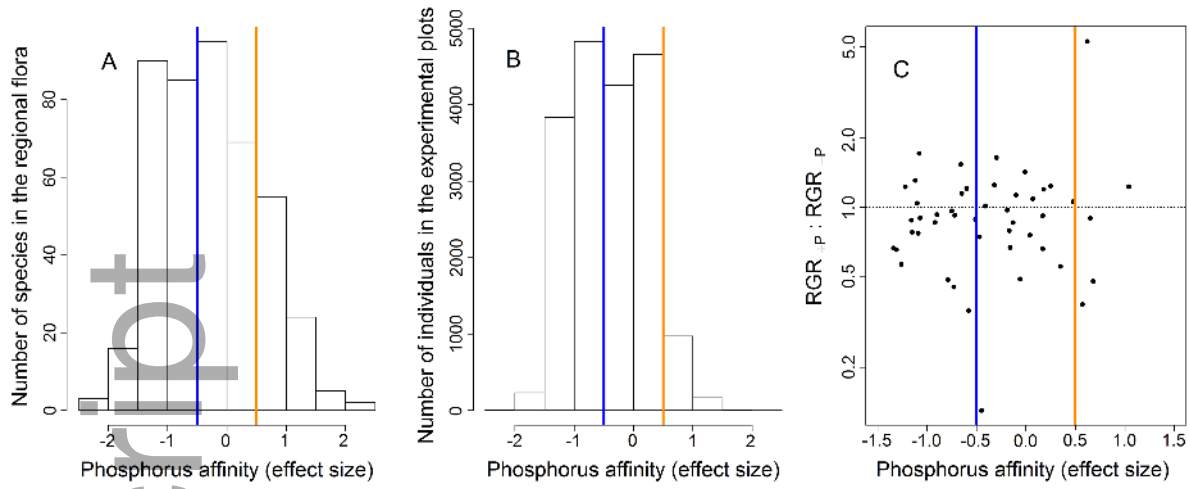
Figure 2. Histograms of the distributions of species in the regional species pool (panel A) and individuals in the 38.4-ha experimental plot (panel B) with respect to species-level phosphorus affinity and the relationship between species-level phosphorus affinity and the ratio of mean relative growth rates (RGR) of conspecifics with versus without added phosphorus ($\text{RGR}_{+P} : \text{RGR}_{-P}$) for shrubs and saplings ($10 \text{ mm} \leq \text{DBH} < 25 \text{ mm}$) (panel C). In panel C, each symbol represents a species with four or more individuals in each phosphorus treatment, and the

horizontal dashed line represents equal RGR values in both phosphorus treatments. The orange and blue vertical lines represent strong positive and negative phosphorus affinity thresholds, respectively.

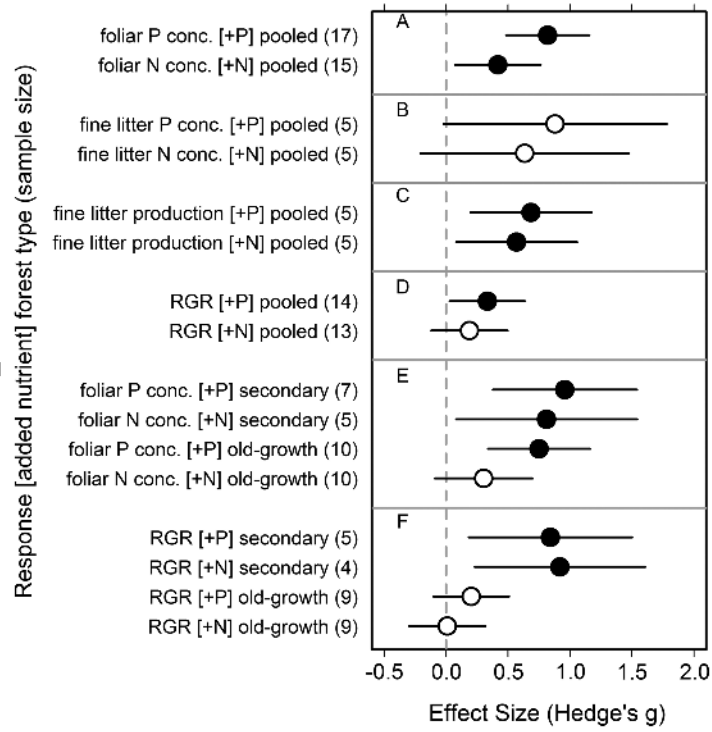
Figure 3. Meta-analysis of nitrogen (N) and phosphorus (P) responses for fertilization experiments conducted in lowland, species-rich, tropical forests (see Data S1). Points are mean effect sizes, with 95% confidence intervals. Filled and empty points identify significant (lower 95% CI > 0) and insignificant effect sizes, respectively. The vertical axis label identifies the type of response (foliar or fine litter nutrient concentrations, fine litter production, or tree/biomass growth (RGR)), the added nutrient in square brackets (+N or +P), the type of forest (secondary, old growth or pooled), and sample size in parentheses. The first three gray horizontal lines separate four tests of the directional hypothesis that responses are stronger for P addition and weaker for N addition, with secondary and old growth forests pooled (sections A, B, C and D). The final gray horizontal line separates tests for interactions between fertilizer type (+N or +P) and forest type (secondary or old growth) and for the directional hypothesis that responses are stronger for secondary forests and weaker for old growth forests for foliar nutrient concentrations (E) and tree/biomass growth rates (F).



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