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Title

Trees adjust nutrient acquisition strategies across tropical forest secondary succession

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Summary

- Nutrient limitation may constrain the ability of recovering and mature tropical forests to serve as a carbon sink. However, it is unclear to what extent trees can utilize nutrient acquisition strategies – especially root phosphatase enzymes and mycorrhizal symbioses – to overcome low nutrient availability across secondary succession.
- We used a large-scale, full factorial nitrogen and phosphorus fertilization experiment of 76 plots along a secondary successional gradient in lowland wet tropical forests of Panama to test the extent to which root phosphatase enzyme activity and mycorrhizal colonization are flexible, and if investment shifts over succession, reflective of changing nutrient limitation. We also conducted a meta-analysis to test how tropical trees adjust these strategies in response to nutrient additions and across succession.
- We find that tropical trees are dynamic, adjusting investment in strategies – particularly root phosphatase – in response to changing nutrient conditions through succession. These changes reflect a shift from strong nitrogen to weak phosphorus limitation over succession. Our meta-analysis findings were consistent with our field study; we found more predictable responses of root phosphatase than mycorrhizal colonization to nutrient availability.
- We found that nutrient acquisition strategies are responsive to nutrient availability and demand in tropical forests, likely critical for alleviating nutrient limitation.

Plain language summary

Using a large-scale nitrogen by phosphorus fertilization experiment across four stages of lowland wet tropical forest succession, we found that trees adjust nutrient acquisition strategies – particularly root phosphatase activity, reflecting responses to nutrient limitation shifts from strong nitrogen to weak phosphorus limitation during forest recovery. With a meta-analysis, we also find consistent patterns of dynamic nutrient acquisition strategies across the broader tropics.

Key words

Mycorrhizal fungi, nitrogen, nutrient limitation, nutrient acquisition strategies, phosphorus, root phosphatase, secondary succession, tropical forests

Introduction (1277 words)

Tropical forests contain high functional biodiversity and sequester large amounts of carbon in primary and secondary forests (Pan *et al.*, 2011; Brienen *et al.*, 2015; Chazdon, 2016; Friedlingstein *et al.*, 2022). The size of the future carbon sink in tropical forests may depend on soil nutrient availability (Wieder *et al.*, 2015; Fleischer *et al.*, 2019; Terrer *et al.*, 2019). Historically, lowland tropical forests have been considered to be phosphorus-limited because of their highly weathered soils (Vitousek, 1984). More recent evidence suggests that nitrogen may also constrain tropical carbon accumulation (Manu *et al.*, 2022; Vallicrosa *et al.*, 2023), especially following disturbances (Davidson *et al.*, 2004; Batterman *et al.*, 2013a; Nagy *et al.*, 2017; Lu & Hedin, 2019; Wright, 2019). However, it remains unclear to what extent the tropical forest carbon sink may be safeguarded by functional biodiversity (e.g. Turner *et al.*, 2018), allowing trees to utilize different nutrient acquisition strategies to overcome nutrient limitation.

Tropical trees have evolved a variety of strategies to acquire nutrients and support growth. They may invest in roots by allocating carbon towards their root mass fraction, changing root architecture and morphology (e.g. specific root length, the formation of cluster roots) to increase foraging capacity. They may produce root exudates to directly release compounds to either modify the soil environment or increase microbial activity to break down soil organic matter (e.g. Freschet et al., 2021; Reichert et al., 2022). Trees able to invest in symbioses with nitrogen-fixing bacteria may allocate carbon towards symbiotic nitrogen fixation when nitrogen availability is low (Batterman *et al.*, 2013b).

Here, we evaluate two key nutrient acquisition strategies thought to be of critical importance for tropical trees to address limitation by the essential nutrients nitrogen and phosphorus: root phosphatase enzymes and symbiotic associations with mycorrhizal fungi. Root phosphatase enzymes hydrolyze organic phosphorus into a plant-available phosphorus form, and mycorrhizal fungi aid trees in expanding the volume of soil explored, releasing organic acids and

98 capturing nutrients more efficiently (Bolan, 1991; Hodge & Fitter, 2010). Adjustment of
99 phosphatase could occur through the intensity of enzyme activity at the root scale, through the
100 adjustment of fine root biomass at the forest scale, or a combination of the two. These strategies
101 have a carbon and nitrogen cost, so that trees – if they have evolved control of their carbon
102 allocation – could reduce investment in strategies when nutrients are abundant or light becomes
103 limiting (Bloom *et al.*, 1985; Treseder & Vitousek, 2001). Mycorrhizal fungi can cost up to 20%
104 of the net carbon fixed by plants (Jakobsen and Rosendahl 1990, Lynch *et al.* 2005), and root
105 phosphatase enzymes are between 15-20% nitrogen on a mass basis (Wang *et al.*, 2007).
106 Accordingly, as phosphorus becomes limiting, trees would increase investment in root
107 phosphatase and arbuscular mycorrhizal fungi. Furthermore, the alleviation of nitrogen limitation
108 may induce phosphorus limitation (Davidson & Howarth, 2007) and stimulate phosphatase
109 activity (Treseder & Vitousek, 2001). Thus, tropical forests should benefit from flexible
110 strategies – changing allocation of resources for nutrient acquisition – as their nutrient
111 environment changes, either from changing supply from atmospheric deposition, or from
112 changing nutrient demand following disturbances or in response to CO_2 fertilization. Resolving
113 whether and how trees adjust root phosphatases and mycorrhizal symbioses in response to soil
114 nutrient availability will aid our understanding of the role of tropical forest recovery in the global
115 carbon cycle.
116 While flexible nutrient acquisition strategies have been found by dynamic global
117 vegetation models to be a key mechanism for sustaining a carbon sink as atmospheric CO_2
118 increases (Yang *et al.*, 2016; Fleischer *et al.*, 2019; Sulman *et al.*, 2019), empirical evidence is
119 limited. Most studies are at the species scale from greenhouse experiments (Batterman et al.,
120 2013b; Zalamea et al., 2016; Nasto et al., 2019) and a few field observations (Treseder &
121 Vitousek, 2001; Yokoyama *et al.*, 2017; Waring *et al.*, 2019). These empirical studies show that
122 species vary in their ability to downregulate root phosphatase activity in response to phosphorus
123 additions (Zalamea *et al.*, 2016) and mycorrhizal colonization – a proxy for investment in
124 arbuscular mycorrhizal fungi – in response to nutrient availability (Cárate-Tandalla *et al.*, 2018).
125 Some species may have inflexible strategies if they evolved in a constant, resource-poor
126 environment (Bloom *et al.*, 1985), when mycorrhizal fungi are opportunistic (Johnson *et al.*,
127 1997; Whiteside *et al.*, 2019), or when costs of the strategy are low relative to the benefit, such
128 as resorption of nutrients prior to senescence (Allen *et al.*, 2020). Our ability to scale flexibility

129 from species to ecosystems is complicated by the possibility that change in nutrient acquisition
130 strategies at the community level could result from either flexibility within a species or turnover
131 in community composition. Thus, we must resolve the degree to which tropical forest nutrient
132 acquisition strategies are flexible at the ecosystem scale.
133 A secondary succession tropical forest chronosequence is an ideal place to examine
134 flexibility in nutrient acquisition strategies because it captures interacting gradients in light, net
135 nutrient demand and soil nutrient availability. Disturbance causes large nutrient losses, especially
136 nitrogen, contributing to nutrient constraints (Kauffman *et al.*, 1995; McGrath *et al.*, 2001; Neill
137 *et al.*, 2006). Following disturbance, forests have a high net nutrient demand – requiring
138 additional nutrients to support forest biomass accumulation – from the high light availability that
139 stimulates growth (Davidson & Martinelli, 2009; van Breugel *et al.*, 2019). As forests recover
140 and light availability decreases, net biomass accumulation slows (e.g. Poorter *et al.*, 2016), and
141 demand for nutrients decreases (Wright *et al.*, 2018). Soil nitrogen availability also increases due
142 to accumulation of fixed nitrogen (Batterman *et al.*, 2013a). This shift in light, demand and
143 nutrient supply following disturbance likely translates to a switch from strong nitrogen limitation
144 in early succession to limitation by phosphorus (Vitousek, 1984) or another resource such as
145 light, calcium, and phosphorus in late succession (Guariguata & Ostertag, 2001; Herbert *et al.*,
146 2003; Nagy *et al.*, 2017; Cunha *et al.*, 2022). Resolving how nutrient acquisition strategies shift
147 over tropical forest secondary succession remains a critical challenge.
148 We took two approaches to investigate the extent to which tropical forests have flexible
149 nutrient acquisition strategies and adjust them in response to soil nutrient availability and
150 recovery from disturbance. First, we examined nutrient strategies at the community level in the
151 first full factorial nitrogen and phosphorus addition experiment across a complete secondary
152 succession gradient in the lowland wet tropical forests. After four years of fertilizing the
153 youngest three forests (sampled at 4, 14, and 30 years of recovery from abandoned pasture), and
154 22 years of fertilizing the mature forest (>600 years old), we quantified root phosphatase
155 (phosphomonoesterase and phosphodiesterase) activity at both the root and forest scales, and
156 arbuscular mycorrhizal colonization. Second, to contextualize our findings across tropical
157 forests, we conducted a meta-analysis of 38 studies from tropical forests that examined responses
158 of root phosphatase activity and mycorrhizal colonization to nitrogen addition, phosphorus
159 addition, or forest age. We hypothesize that 1) tropical forest nutrient acquisition strategies are

flexible, and 2) tropical forest investment in nutrient acquisition strategies changes over succession, indicative of a shift from nitrogen limitation early in succession to limitation by phosphorus or another resource later in succession. More specifically, we expect that flexibility in nutrient acquisition strategies will include decreases in phosphatase production in response to phosphorus addition in phosphorus-limited forests, increases in phosphatase production in response to nitrogen addition in nitrogen-limited forests, and decreases in arbuscular mycorrhizal colonization decreases in response to addition of either nutrient, indicative of limitation by that nutrient, particularly later in succession when demand for new nutrients decreases (Fig. 1). The degree to which tropical trees utilize flexible nutrient acquisition strategies will inform our ability to better predict how nutrient limitation will constrain the tropical carbon sink in the future.

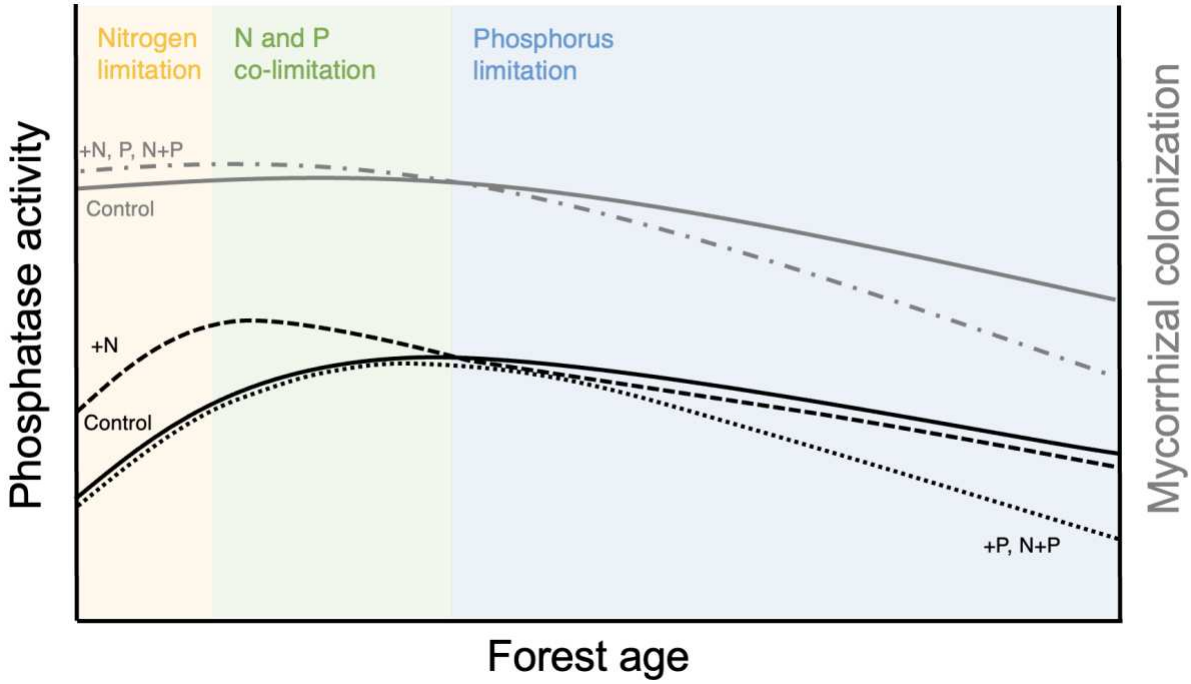

Fig. 1. Hypothesized predictions of how root phosphatase activity (black) and mycorrhizal colonization (gray) change across secondary forest succession in response to nitrogen (N) and phosphorus (P) addition. The different types of lines represent the different treatments (dashed lines) and controls (solid lines). Because nitrogen is more mobile compared to phosphorus and lost relatively quickly after a disturbance event, the ecosystem starts at nitrogen limitation (yellow-shared area). However, nitrogen availability increases over time as nitrogen fixers fix nitrogen, and eventually the ecosystem shifts towards nitrogen and phosphorus co-limitation (green shaded area) and finally to phosphorus limitation (blue shaded area).

Materials and Methods (2016 words)

Field study site

We conducted the study at the Agua Salud Project, a 15 km^2 area near Soberania National Park, and at the Gigante peninsula in the Barro Colorado Nature Monument. Both lowland wet tropical forest areas are located within the Republic of Panama. The study spans nitrogen and phosphorus factorial fertilization for 0.16-ha plots across three forest ages in early secondary succession within the Agua Salud Project and for 0.16-ha plots in mature forest at Gigante, located 13 km away from Agua Salud.

The nitrogen and phosphorus factorial experiment within the Agua Salud Project (9°13'59" N, 79°41'59" W) was conducted on plots in forests recovering from pasture at three different age class groupings: forests that were directly recovering from cleaned pasture (4 years), and forests at approximately 14 and 30 years of recovery (Van Breugel *et al.*, 2013). Forests had been fertilized for four years at the time of sampling (for example, at the time of sampling, the forest that was 0-year-old at the start of the experiment was now 4-year-old forest). Each treatment was replicated five times, for a total of 60 plots. For the mature forest age class (>600 years since human disturbance), the nitrogen and phosphorus factorial fertilization plots (9°06'31" N, 79°50'37"W) were replicated four times, for a total of 16 plots, which had been fertilized for 22 years at the time of sampling. Plots are 0.16 ha (40 x 40 m) plots except for the control plots at Agua Salud, which are 0.1 ha (20 x 50 m). All samples were taken within the inner 0.1 ha area of the plots that were fertilized to allow for a buffer. Across all sites, fertilizer was applied by hand four times a year at rates of 125 kg N ha^{-1} yr^{-1} as urea and 50 kg P ha^{-1} yr^{-1} as triple superphosphate.

The mean annual temperature was 26°C, with a mean annual precipitation of 2700 mm and 2600 mm at Agua Salud and Gigante respectively, with a distinct dry season between January and April (Leigh, 1999; Ogden *et al.*, 2013). Soil types at Agua Salud are Oxisols (Typic Hapludox) and Inceptisols (Typic Dystrudept and Oxic Dystrudept) on pre-Tertiary basalt (Baille and Hall, pers. comm.), while at Gigante, the soils are Oxisols (Typic Kandiudox) derived from a Miocene basalt (Turner *et al.*, 2013b). Across Agua Salud and Gigante, total soil phosphorus averaged 289.6 ± 8.42 (mean ± standard error) and 285 ± 26 mg P kg^{-1} soil in the top 10 cm, respectively (Supporting Information, Fig. S1). Canopy heights ranged from >2 m in the

youngest forests to >35 m in the mature forests (Yavitt *et al.*, 2011). Community composition changes across forest succession as indicated by a change in abundance-based Jaccard dissimilarity indices; however, we controlled for community composition within a forest age by blocking plots with one of each treatment and a control plot located within an area separated by >40 m to minimize fertilizer transfer between plots (van Breugel *et al.*, 2013).

Root collection

We randomly collected five soil cores (0-10 cm depth) within each of the 76 plots during the end of the wet season of 2019. The wet season typically represents periods of higher belowground activity (Turner *et al.*, 2013a; Turner & Wright, 2014). In the laboratory, we sampled the soils for fine roots (0-1 mm) and kept roots in the refrigerator (4°C) prior assaying for no more than three days after collecting the roots in the field. There was no effect the time of storage on root phosphatase activity. The 0-1 mm size class has been found to represent first to third-order roots (Wurzburger & Wright, 2015) that most actively exchange nutrients and have higher phosphatase activity (Cabugao *et al.*, 2021). A second smaller subsample was preserved in 95% ethanol and refrigerated at 4°C for subsequent analysis for arbuscular mycorrhizal colonization. To scale phosphatase activity rates per gram of root to the forest scale, we collected fine root biomass for 64 of the 76 plots (four replicates of each treatment per age class) in the middle of the rainy season (early August to middle September) of 2019 (Tang, 2022). Briefly, five soil cores (6 cm in diameter to 10 cm depth) per plot were sampled and homogenized, and live fine roots (<2 mm) were collected, dried, and weighed. The forest-scale phosphatase activity represents a higher estimate of root phosphatase activity, as 0-1 mm fine roots represent about 65% of 0-2 mm fine roots (Wurzburger & Wright 2015) but typically have higher activity rates per gram of root (Cabugao *et al.*, 2021).

Root phosphatase activity

To quantify root phosphatase activity, we used a method adapted from (Turner et al. 2001), using para-nitrophenyl (pNPP) and bis para-nitrophenyl (bis-pNPP) as analogue substrates for phosphomonoesterase and phosphodiesterase, respectively. Phosphomonoesterase is the dominant root phosphatase enzyme that hydrolyzes simple phosphomonoesters to release a phosphate ion for plant uptake, while phosphodiesterase hydrolyzes nucleic acids and

phospholipids to release a phosphomonoester group (Browman & Tabatabai, 1978; Tabatabai, 1994). Because phosphomonoesters must be hydrolyzed by phosphomonoesterase to produce a phosphate ion for plant uptake, phosphodiesterase represents more plant investment in acquiring phosphorus from less labile sources (Turner, 2008).

For each plot, we collected three subsamples of fine roots for measuring phosphomonoesterase activity, three subsamples for phosphodiesterase activity, and one for measuring color production without substrate added. Briefly, between 200-500 mg of fresh root were added to a glass vial with 9 mL of 50 mM sodium acetate-acetic acid buffer (pH 5.0) and shaken in a water bath at 26°C for five minutes to simulate surface soil temperatures. To initiate the reaction, we added 1.0 mL of 50 mM pNPP or 10 mM bis-pNPP (5.0 mM and 1.0 mM final concentration, respectively). The reaction was terminated by mixing 0.5 mL of buffer/substrate solution with 4.5 mL of 0.11 M NaOH, which was then vortexed and measured for absorbance at 405 nm against paranitrophenol (pNP) standards. Two sodium acetate-acetic acid buffer and substrate blanks were run for every assay. Roots were removed from the vials and dried at 60°C for 3 days to express the activity as μmol pNP g^{-1} dry mass roots h^{-1}.

Arbuscular mycorrhizal colonization

We focused on arbuscular mycorrhizal fungi as 75% of the trees in our plots are identified as arbuscular mycorrhizal (23% of trees did not have a known mycorrhizal association) based on associations derived from (Steidinger *et al.* 2019). We cleared preserved root samples in 5% KOH between 60 and 70°C for up to seven hours, neutralized for 15 minutes in 2% HCl, and stained with 0.05% trypan blue in a 1:1:1 mixture of lactic acid, glycerol, and deionized water for 15 min at 70°C following (Wurzburger & Wright 2015). Roots were destained in a 1:1 lactic acid glycerol solution for a minimum of 12 hours prior to observation. We studied roots under a compound microscope and quantified the number of mycorrhizal structures (arbuscules, vesicles, and hyphae) using a random-intercept method (McGonigle *et al.*, 1990), viewing structures between 40-600x magnification. Colonization quantification was standardized at 100x (Supporting Information, Fig. S2). We evaluated colonization across 10 randomized intersections and a minimum of 10 root segments per plot for an average of 13 roots per plot. Mycorrhizal colonization was calculated as the percentage of fine-root length colonized by either arbuscules, vesicles, or hyphae. If intracellular coils were identified, they were categorized with arbuscules.

Meta-analysis study selection

To search for mycorrhizal colonization rates across secondary forest age, we searched Web of Science using terms "tropic* AND forest AND (secondary OR succession) AND mycorrhiza* AND colonization" which resulted in 49 studies on March 22, 2021. We also searched the terms ((mycorrhiz* OR fine root* OR phosphatase OR fixation) AND forest AND (tropic* OR Borne* OR Amazon* OR Africa* OR Panama OR "Costa Rica" OR Belize OR Brazil OR Peru OR Ecuador OR Colombia OR Venezuela OR "French Guiana" OR Guyana OR Surinam OR Bolivia OR Jamaica OR "Puerto Rico" OR Hawaii OR Cameroon OR Nigeria OR Gabon OR "Central African Republic" OR Malaysia OR Indonesia OR Thailand OR "New Guinea" OR Australia) AND (addition* OR fertiliz*) AND (nutrient OR nitrogen OR phosphorus OR calcium OR potassium)), which resulted in 544 studies on March 22, 2021. To search for responses of root phosphatase and mycorrhizal colonization, we used the terms "forest AND (tropic* OR Borne* OR Amazon* OR Africa* OR Panama OR "Costa Rica" OR Belize OR Brazil OR Peru OR Ecuador OR Colombia OR Venezuela OR "French Guiana" OR Guyana OR Surinam OR Bolivia OR Jamaica OR "Puerto Rico" OR Hawaii OR Cameroon OR Nigeria OR Gabon OR "Central African Republic" OR Malaysia OR Indonesia OR Thailand OR "New Guinea" OR Australia) AND "mycorrhiza colonization" AND (secondary OR succession)) which resulted in 61 studies on March 22, 2021. We based these search terms on Wright (2019).

We focused our meta-analysis on mycorrhizal colonization as a percentage of root length colonized and root phosphomonoesterase activity, the dominant phosphatase enzyme. While mycorrhizal colonization intensity is not a direct measure of plant investment to mycorrhizal fungi, it is the most measured and reported trait. For mycorrhizal colonization, we also recorded dominant tree mycorrhizal type and found that only one study was dominated by ectomycorrhizal tree species. For each case study, we recorded site locations (latitude and longitude), climatic variables (mean annual precipitation [MAP] and temperature [MAT]). If data were not presented, site latitude and longitude were extracted from Google Maps (https://www.google.com/maps) based on the approximate location reported in the publication, and we extracted MAT and MAP from the WorldClim database (https://www.worldclim.org/) (Fick & Hijmans, 2017). When results were presented graphically, we used DataThief to digitize the data (Tummers, 2006).

Statistical analysis

Field study

To test the effects of forest age and nutrient addition on root phosphatase activity and arbuscular mycorrhizal colonization, we used linear mixed effects models (lme4 package) (Bates *et al.*, 2015) with nitrogen, phosphorus and forest age and their interactions as fixed effects and site as a random effect (i.e., landscape effects) for root phosphatase and arbuscular mycorrhizal colonization (R Core Team, 2020). The means of the three subsamples of root phosphatase activity and the percent of individual root colonized were analyzed in our models. Models were selected with stepwise model reduction based on Akaike's Information Criterion (AIC) values, decreases in ΔAIC of > 2 were considered significant. For both root phosphatase and mycorrhizal colonization, the full model with interactions was the best fit model (Supporting Information, Tables S1 and S2). We evaluated the homogeneity of variance of residuals across nutrient treatments and forest age for each model. Phosphomonoesterase and phosphodiesterase did not require data transformation to meet the assumptions of the models, while the ratio of phosphomonoesterase to phosphodiesterase was ln-transformed. All mycorrhizal colonization data required the logit transformation, which is appropriate for continuous proportional data (Warton & Hui, 2011). To analyze treatment effects on each individual forest age class, we ran the contrasts with a Tukey adjustment using the "emmeans" package (Supporting Information, Table S2) (Lenth, 2021). To quantify the variance explained by fixed and random effects in all of our models to report the marginal (variance explained by fixed effects only) and conditional R^2 (variance explained by both fixed and random effects), we used the "MuMIn" package (Bartoń, 2020).

Meta-analysis

To examine how mycorrhizal colonization changes across forest age, we used a linear mixed effect models with forest age, mean annual precipitation and temperature as fixed effects and the study as a random effect. Models were selected with stepwise model reduction based on Akaike's Information Criterion (AIC) values and decreases in ΔAIC of > 2 were considered significant.

To evaluate the effects of fertilization on root phosphatase activity and mycorrhizal colonization, we used the natural log of the response ratio (Hedges *et al.*, 1999).

$$lnRR = ln\left(\frac{X_{N,P,or\ NP}}{X_{control}}\right)$$

The variance (v) of *lnRR* is calculated as:

$$v = \frac{S^2_{N,P,NP}}{N_{N,P,NP} * X^2_{N,P,NP}} + \frac{S^2_{control}}{N_{control} * X^2_{control}}$$

We calculated the percent change of nutrient acquisition strategies in response to nutrient addition, as (e^{R+} -1) × 100, where R^+ is the weighted mean effect size and a random effect for the study. All meta-analyses were performed in the "metafor" package in R (Viechtbauer, 2010).

Results (1094 words)

Nutrient strategy flexibility over gradients in nutrient availability and tropical forest secondary succession

In our field experiment, root phosphatase activity rates at the root scale responded strongly to nutrients and forest age (phosphomonoesterase: marginal $R^2_{GLMM(m)}$=0.48, conditional $R^2_{GLMM(c)}$=0.62; phosphodiesterase: marginal $R^2_{GLMM(m)}$=0.2, conditional $R^2_{G(c)}$=0.46; Fig. 1a; Supporting Information, Fig. S3a; Table S1). Overall, root phosphatase activity was best explained by the interaction of nitrogen and phosphorus with forest age (nitrogen by phosphorus by forest age, phosphomonoeasterase: ΔAIC=-114.4; phosphodiesterase: ΔAIC=-25.7; Supporting Information, Table S1). Nitrogen addition alone increased both phosphomonoesterase and phosphodiesterase activity relative to the control in the 4-year-old forests (phosphomonoesterase: +45%, $p<0.05$; phosphodiesterase: +114%, $p<0.05$) and 14-year-old forests (phosphomonoesterase: +55%, $p<0.001$;), while phosphorus addition had no effect in the 4 and 14-year-old forests ($p>0.05$; Fig.1a; Supporting Information, Fig. S3a, Table S2). In the 30-year-old and mature forests, nitrogen additions had no effect on phosphomonoesterase activity rates, ($p>0.05$), while phosphorus significantly reduced phosphomonoesterase activity in the 30-year-old forests (-25%, $p<0.01$) and in the mature forests (-42%, $p<0.001$). The response of phosphomonoesterase to combined nitrogen and phosphorus addition was similar to the

response to phosphorus addition alone, such that nitrogen and phosphorus did not suppress phosphomonoesterase activity in the young forests ($p>0.05$), but suppressed phosphomonoesterase activity in the 30-year-old forests (26%, $p<0.05$) and mature forests (47%, $p<0.01$).

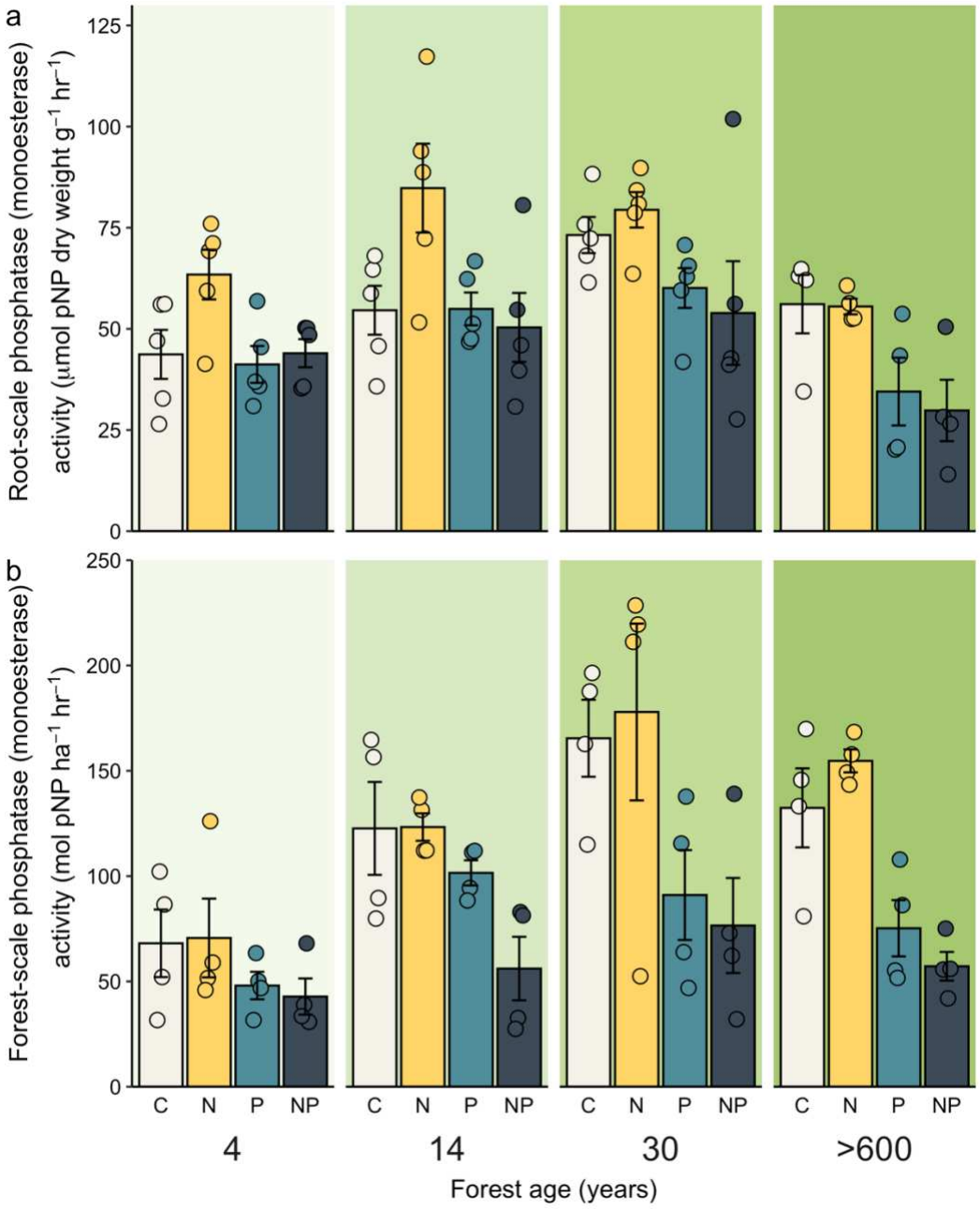

Fig. 2. Root phosphatase activity responds to nitrogen in the younger tropical forests and phosphorus in the older forests at the root scale (a), and root phosphatase decreases in response to phosphorus in the older forests at the forest scale (b). Root phosphatase (phosphomonoesterase) activity per unit root (a) and per unit area (b) in response to four years of nitrogen and phosphorus addition across three ages of forest recovering from pasture abandonment at the Agua Salud project, and in response to 22 years of nutrient addition at a mature lowland tropical forest in the Barro Colorado Nature Monument. All forests are lowland wet tropical forests in Panama. In panel a, the bars represent the mean across plot-level replicates, the standard error bars represent one standard error, and the dots represent the mean of the three replicates with each 0.16 ha plot.

Since changes in phosphatase activity at the forest scale could occur as a result of shifts in fine root biomass (Supporting Information, Fig. S4) as well as at the root level, we scaled phosphomonoesterase activity rates to fine root biomass. Nitrogen and phosphorus had no effect on forest-scale phosphomonoesterase rates in 4-year-old forests (nitrogen by phosphorus by forest age, ΔAIC=-152.5; $R^2_{GLMM(m)}$=0.58, $R^2_{GLMM(c)}$=0.73; Fig 1b; Supporting Information, Table S1); phosphomonoesterase activity rates per root increased in response to nitrogen, while fine root biomass per hectare decreased (Supporting Information, Fig. S4, Table S2). Phosphorus suppressed forest-scale phosphomonoesterase rates in the 14, 30-year-old, and mature forests ($p<0.01$; Fig 1b).

We also found an increase in phosphomonoesterase activity at the root and forest scales over succession. Phosphomonoesterase activity increased with forest age across the control treatments, with an increase of 68% between the 4 and 30-year-old forests ($p<0.05$) and trended (not statistically-significant) towards an increase of 34% between the 14 and 30-year-old forests ($p<0.10$; Fig. 1a; Supporting Information, Table S3). Phosphomonoesterase then trended (not statistically-significant) towards a decrease from the 30-year-old forests to the mature forests by 23% ($p<0.10$). At the forest scale, phosphomonoesterase activity per hectare similarly increased between the 4 and 14-year-old forests by 80% ($p<0.05$; Fig. 1b) and 4 and 30-year-old forests by 143% ($p<0.001$), but did not differ among 14, 30-year-old, or mature forests ($p>0.05$).

Arbuscular mycorrhizal colonization also responded to nutrients, although not as consistently as phosphatase activity. Similar to phosphatase activity, arbuscular mycorrhizal colonization was best explained by the interaction of nitrogen and phosphorus with forest age (nitrogen by phosphorus by forest age, ΔAIC=-16.7; $R^2_{GLMM(m)}$=0.29, $R^2_{GLMM(c)}$=0.42; Supporting Information, Table S4). However, nitrogen and phosphorus had no effect on colonization relative to the control in the 4 or 14-year-old forests (Fig. 2a; Supporting Information, Table S2). Arbuscular mycorrhizal colonization also did not decline across forest age in the control treatments, but nitrogen and phosphorus, when combined, decreased colonization by 22% in the 30-year-old forests ($p<0.05$). Nitrogen and phosphorus additions had a stronger effect in the mature forests, where nitrogen alone decreased colonization by 29% relative to the control ($p<0.05$), while nitrogen and phosphorus together decreased colonization by 40% ($p<0.05$).

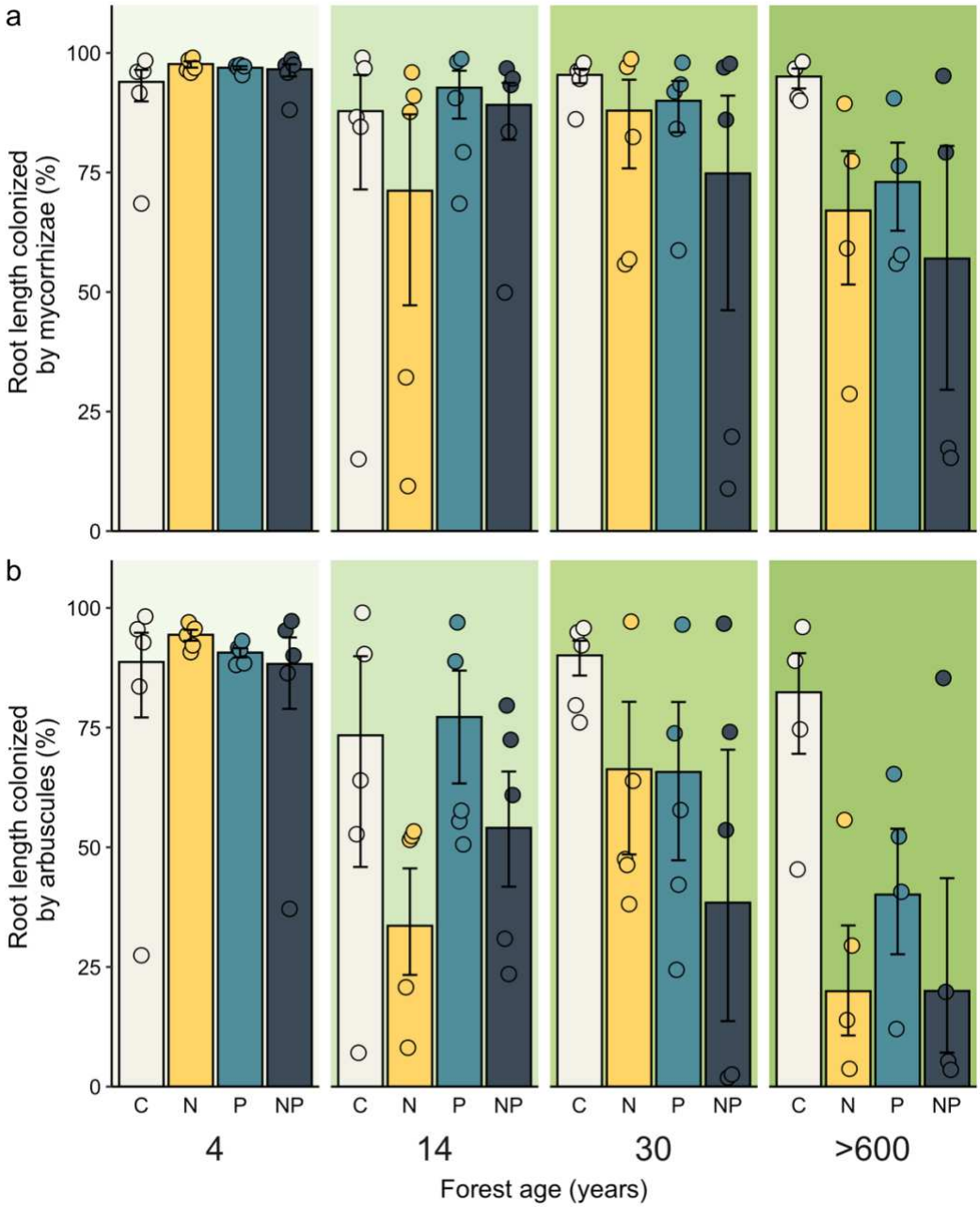

Fig. 3. Mycorrhizal colonization responds to nitrogen and phosphorus in the oldest lowland wet tropical forests in Panama. Arbuscular mycorrhizal colonization (% of root length colonized by mycorrhizal structures) (a) and percent arbuscules (b) in response to four years of nitrogen and phosphorus addition across three ages of forests recovering from pasture abandonment in the Agua Salud project, and 22 years of nutrient addition at a mature lowland tropical forest in the Barro Colorado Nature Monument. The bars represent the back-transformed mean across plot-level replicates, the bars represent a back-transformed standard error, and the dots represent a back-transformed mean of ten roots examined across ten intersections for colonization.

We also examined how the presence of arbuscules – the site of carbon and nutrient exchange between roots and mycorrhizal fungi – responded to fertilization (nitrogen by phosphorus by forest age, ΔAIC=-27.5; $R^2_{GLMM(m)}$=0.38, $R^2_{GLMM(c)}$=0.45; Fig. 2b; Supporting Information, Table S4). We found no strong nutrient effects of nitrogen or phosphorus in the 0-year-old forests ($p>0.05$). Nitrogen additions with and without phosphorus decreased arbuscules by 42% ($p<0.05$) in the 14-year-old forests, and by 33% in the 30-year-old forests ($p<0.10$). In the mature forests, nitrogen (with and without phosphorus) reduced the abundance of arbuscules by 67% ($p<0.05$).

Over the course of secondary succession, we also found evidence that, at the community level, arbuscular mycorrhizal colonization was upregulated in younger secondary forests when nutrient demand was high relative to supply; mycorrhizal colonization did not decline in response to nutrients in the youngest forests and only responded to nutrients in the older forests. While there were no differences in mycorrhizal colonization across forest age in the control treatments ($p > 0.05$), across all treatments, arbuscular mycorrhizal colonization was 20% higher in the 0-year-old forests compared to the mature forests (Fig. 2a; $p<0.05$). These patterns were consistent when we examined arbuscules (Fig. 2b): across all treatments, arbuscule colonization was reduced by 33% and 56%, respectively, from the 4- to the 14-year-old ($p<0.05$) and mature forests ($p<0.005$).

Flexibility of nutrient strategies in tropical forests globally

Across five studies (37 observations) that examined the effect of nutrients on root phosphatase activity, and 25 studies (137 observations) that examined nutrient effects on mycorrhizal colonization, we found evidence that trees use flexible strategies of root phosphatase activity at the seedling stage (23 observations) and in the field at the community level (14 observations), and investment in mycorrhizal colonization at both the seedling stage (64 observations) and in the field at the community level (73 observations; Supporting Information, Fig. S5, Table S5). Root phosphatase activity declined by 44% (CI: 30 to 55%, $p<0.0001$; Fig. 3a) in response to added phosphorus and by 45% (CI: 26 to 59%, $p<0.0001$) in response to phosphorus and nitrogen added together, but did not significantly increase root phosphatase in response to added nitrogen ($p>0.05$). This suggests that trees use a flexible strategy of investment in phosphatase activity based on soil phosphorus availability. Mycorrhizal colonization declined by 13% (CI: -

22 to -3%, $p<0.01$; Fig. 3b) in response nitrogen. However, phosphorus and nitrogen and phosphorus added together ($p>0.05$; Fig. 3b) did not significantly impact mycorrhizal colonization, indicative of either an unpredictable strategy by mycorrhizal fungi or heterogeneity in initial nutrient conditions.

To determine if and how nutrient strategies change at the community level during tropical forest secondary succession, 92 observations from 11 studies across 13 locations indicated that mycorrhizal colonization significantly decreased as forests aged ($p<0.0001$, $F_{1,89.085}=41.402$, $R^2_{GLMM(m)}=0.288$, $R^2_{GLMM(c)}=0.458$; Fig. 3c; Supporting Information, Table S6). This finding is consistent with the idea that trees adjust investment per unit root length in mycorrhizal fungi as net nutrient demand decreases with slowing net biomass accumulation over successional time. Thus, our meta-analysis shows that phosphatase activity was flexible in response to experimental nutrient addition and mycorrhizal colonization was flexible across secondary succession.

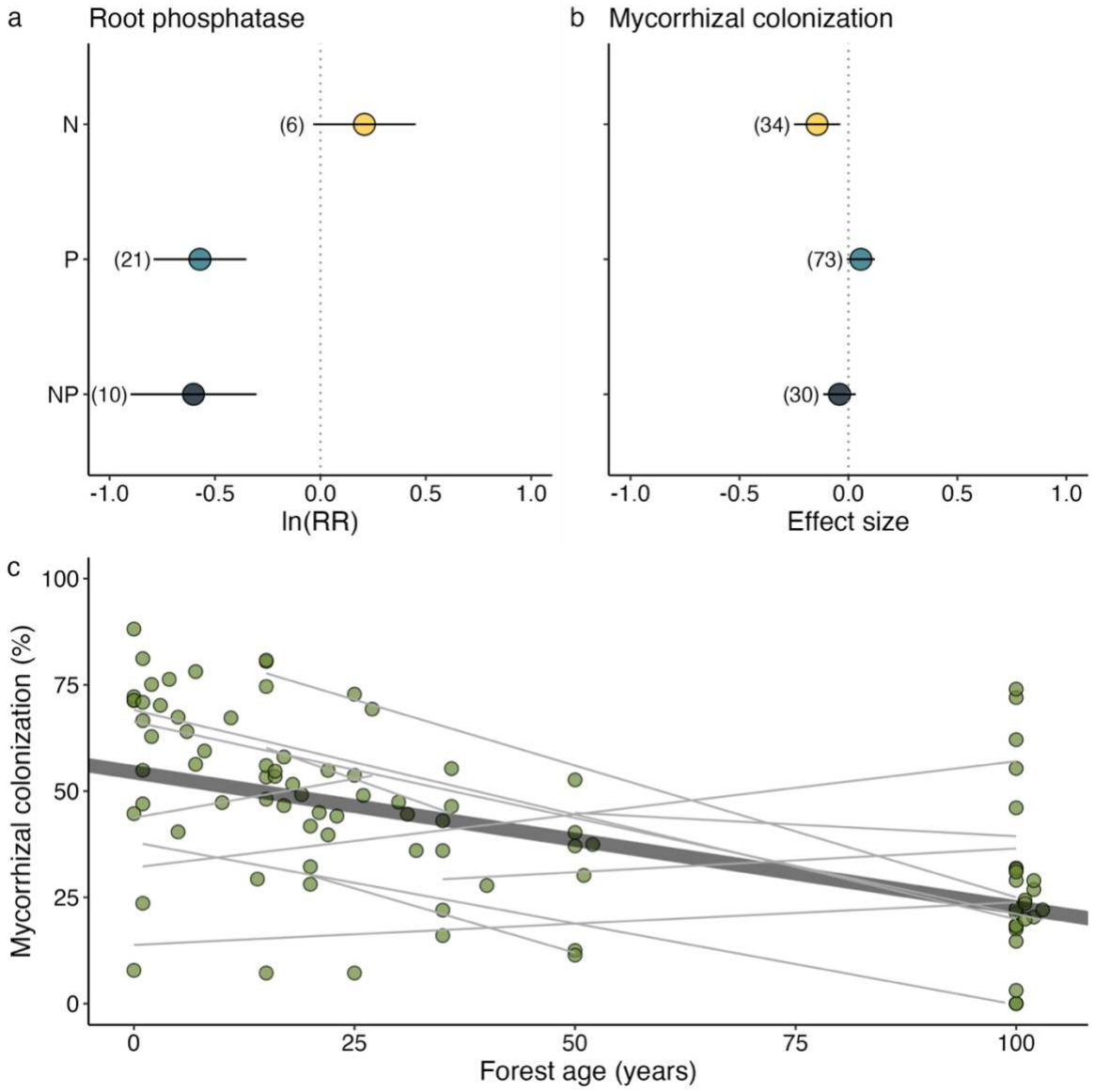

Fig. 4. Root phosphatase responds more to nitrogen and phosphorus than mycorrhizal colonization, and mycorrhizal colonization typically decreases during forest succession from a meta-analysis. The effects (mean effect size of the log response ratio) of nitrogen and phosphorus fertilization on root phosphatase activity (a) and mycorrhizal colonization (b) and (c) mycorrhizal colonization across forest age. For panels (a) and (b), a negative effect size indicates that fertilization decreased root phosphatase activity or mycorrhizal colonization, while a positive effect size indicates a stimulation. Effects were considered significant where the CIs do not overlap zero. The number to the left of each effect size indicates the number of observations from an individual site or species. In panel (c) individual points represent mean values of mycorrhizal colonization (%) from each forest site, mature forested sites without an age classification are indicated as unconfirmed ages and classified here as ~100 years. We repeated this analysis without confirmed forest ages and found consistent results (Supporting Information Fig. S6). Thin grey lines indicate the trend of mycorrhizal colonization within a study site, while the thick black line indicates the overall trend synthesized across studies ($p<0.0001$, $F_{1,89.085}=41.402$, $R^2_{GLMM(m)}=0.288$, $R^2_{GLMM(c)}=0.458$

Discussion (1939 words)

The ability of trees to overcome nutrient limitation and support the tropical forest carbon sink may depend on functional biodiversity (Levy-Varon *et al.*, 2019; Poorter *et al.*, 2021) through a suite of nutrient acquisition strategies, allowing trees to access nutrients and overcome nutrient limitation. But it has remained unclear to what extent tropical forests can use and adjust these strategies – particularly root phosphatase and investment in mycorrhizal fungi – in response to changing soil nutrient availability and tropical forest recovery during succession. Understanding the flexibility of plant nutrient acquisition strategies is particularly critical as nutrient limitation is projected to increase under elevated CO_2 (Wieder *et al.*, 2015; Fleischer *et al.*, 2019; Terrer *et al.*, 2019). Our large-scale, nutrient addition experiment and meta-analysis found that: 1) tropical forests are flexible in their nutrient acquisition strategies; and, 2) tree strategies at the community level change during forest succession, consistent with the expectation of a shift from nitrogen to phosphorus limitation over tropical forest secondary succession.

We found support for our first hypothesis that root phosphatase activity and mycorrhizal colonization are flexible, with stronger and more consistent responses to nutrient availability in root phosphatase activity than mycorrhizal colonization. Phosphatase activity was highly flexible, increasing in response to nitrogen by half, and decreasing in response to phosphorus by half within a forest age class. While the changes in root phosphatase activity could be due to flexibility within tree species or due to changes in tree community composition, we assume that at least within forest age classes where tree species composition does not differ drastically, changes in activity are likely due to species flexibility in response to nutrients. Our meta-analysis – focused on tropical forests – captured predominantly seedling studies and root phosphatase activity in mature forests, and found that root phosphatase activity more consistently responded to phosphorus than nitrogen, which contrasts with patterns at the global level which found strong positive responses to nitrogen (Marklein & Houlton, 2012), potentially indicating weaker nitrogen limitation in mature tropical forests compared to other ecosystems globally. Nonetheless, the flexibility of the phosphatase in response to nitrogen and phosphorus is consistent with responses from previous studies (Treseder & Vitousek, 2001; Marklein & Houlton, 2012).

513 Compared to root phosphatase activity, mycorrhizal colonization responses to nutrients
514 were less consistent and predictable. In our field experiment, the greatest flexibility we observed
515 was in the mature forests in response to nitrogen and phosphorus. Across the experiment, the
516 effect of forest age and nutrients also explained less variation than for phosphatase (12% vs 48%,
517 respectively). While responses from individual studies in the meta-analysis varied widely in
518 mycorrhizal responses to nutrients, there were no consistent responses overall to phosphorus and
519 only a small decrease in response to nitrogen (Fig. 3; Supporting Information, Fig. S7). These
520 results contrast with a global-level meta-analysis which found that mycorrhizal colonization
521 substantially decreased in response to fertilization (Treseder, 2004).
522 The lack of consistent responses of mycorrhizal colonization in tropical forests and
523 species to nutrients could occur for four reasons. First, the flexibility of investment in
524 mycorrhizal fungi may emerge on a longer timescale than root phosphatase activity since the
525 relationship depends on interactions between multiple species (Sheldrake *et al.*, 2017). We found
526 the strongest effects of nutrients on mycorrhizal colonization in the mature forests which had
527 been fertilized for 22 years at the time of sampling, 18 years longer than our youngest forests
528 where we found little effect of nutrients on mycorrhizae. The longer period of fertilization may
529 have allowed long-lived trees to adapt to changing nutrient conditions, decreasing investment in
530 mycorrhizal fungi to minimize carbon costs. Second, mycorrhizal fungi play many roles in
531 resource acquisition and pathogen defense, and therefore may change dynamically with other
532 drivers, such as rainfall, not accounted for in our experiment (Smith & Smith, 2011; Delavaux *et*
533 *al.*, 2017). Indeed, our results in the mature forest were consistent with a previous study after 15
534 years of fertilization from seedlings of the seven most common tree species across the plots
535 (Sheldrake *et al.*, 2018) but not with a study after 14 years of fertilization (Wurzburger &
536 Wright, 2015) at the same experimental site using mixed root samples from randomly collected
537 cores. More specifically, Wurzburger & Wright (2015) found a modest increase of mycorrhizal
538 colonization in response to phosphorus but a decline in response to nitrogen, while Sheldrake *et*
539 *al*. (2018) found a suppression in response to both nitrogen and phosphorus. While the methods
540 Wurzburger & Wright (2015) used were consistent with ours, the modest increase found by their
541 study was attributed to a potential opportunistic response of the mycorrhizal fungi. This response
542 may have declined in the eight years between the Wurzburger & Wright (2015) study and our
543 study after plant and microbial communities have had a longer time to adapt to the altered

544 nutrient conditions. Across time, such as seasonality, and space, the baseline availability of
545 resources may also differ, leading to inconsistent responses to experimental nutrient additions. In
546 contrast, phosphatase is specifically utilized to acquire phosphorus, and therefore would be
547 expected to respond predictably to nitrogen and phosphorus (Treseder & Vitousek, 2001). Third,
548 mycorrhizal fungi involve a complex relationship between two organisms that are highly diverse
549 in tropical forests and the nature of the symbiosis may change as resource availability changes
550 (Whiteside *et al.*, 2019). Fourth, the quantification of mycorrhizal colonization itself may not
551 accurately capture plant investment in the symbiosis (Treseder, 2013), and colonization
552 intensities differ amongst species, which could lead to further variation (Hart & Reader, 2002;
553 Koch *et al.*, 2017) when comparing across forests.
554 We also found support for our second hypothesis, that tropical forest investment in root
555 phosphatase and mycorrhizal colonization changes over secondary succession. Root phosphatase
556 activity increased with forest age in the early years of recovery from disturbance, both at the root
557 and forest scale. Although no study had previously examined root phosphatase activity over
558 tropical forest secondary succession, our meta-analysis showed that, overall, mycorrhizal
559 colonization was highest in young forests and declined in older forests. Our field experiment
560 demonstrated similar patterns: mycorrhizal colonization was upregulated in younger secondary
561 forests when nutrient demand was high relative to supply, stayed high in the older control
562 forests, and only declined in the older forest in response to nutrients, demonstrating the
563 interaction between nutrients and forest age. These findings support the theory that the strength
564 of plant nutrient limitation is greatest in young secondary forests and decreases over succession
565 as nutrients becomes less limiting (Allen & Allen, 1990; Zangaro *et al.*, 2013), or through
566 changes in community composition where early successional tree species have stronger
567 associations with mycorrhizal fungi (Aidar *et al.*, 2004; Guadarrama *et al.*, 2014). However, in
568 some ecosystems, mycorrhizal colonization may increase with succession because of differences
569 in disturbance history (Bachelot *et al.*, 2018) (Fig. 3).
570 Our experimental findings also support the theory and observations that tropical forests
571 shift from nitrogen limitation early in succession to phosphorus limitation later in succession
572 (Davidson *et al.*, 2004, 2007; Nagy *et al.*, 2017). In the 4 and 14-year-old forests, the positive
573 response of phosphatase at the root scale to nitrogen alone suggest that these young forests are
574 nitrogen-limited, and the alleviation of nitrogen limitation stimulated phosphatase to address the

resulting phosphorus limitation. Interestingly, when phosphatase activity was scaled to fine root biomass (Fig. 1) in these young forests to the forest scale, there was no direct nitrogen effect, suggesting that tree roots were more efficient at producing phosphatase per unit of root in response to nitrogen. More broadly, these findings are consistent with nitrogen limitation in the young forests indicated by the increased abundance of nitrogen-fixing trees and root nodule biomass in nearby forests of the same age classes (Batterman *et al.*, 2013b) as well as findings of increased biomass growth in response to nitrogen addition in the 4 and 14-year old forests (Tang, 2022). In contrast, the lack of a response to nitrogen and the strong reduction at the root scale in response to phosphorus in the 30-year-old and mature forests suggests phosphorus limitation, with plants investing more in phosphatase under ambient conditions. Even though we have not seen a growth response to phosphorus in the mature forests at Gigante (Wright *et al.*, 2018), the suppression of root phosphatase in response to phosphorus additions still suggests weak phosphorus limitation. The lack of downregulation at the root scale in response to phosphorus in our youngest forests differed likely because they were at the earliest stages of succession when nitrogen was limiting, or there was co-limitation by nitrogen and phosphorus. The pattern of phosphatase activity over forest age also supports a shift in nitrogen to phosphorus limitation: phosphatase activity at both the root and forest scales increased from the 4 to 14 to 30-year-old forests in control plots without added nutrients. Root phosphatase activity slightly decreased from the 30-year-old forests to the mature forests, suggesting a lower net demand for nutrients in mature forests (Wright *et al.*, 2018). Root phosphatase differed from the bulk soil phosphatase activity, which reflects an increase in microbial phosphorus limitation across succession (Supporting Information, Fig. S8), suggesting a decoupling of plant nutrient limitation and microbial limitation. The lack of an increase in root phosphatase activity in the mature forests was not due to underlying differences in soil phosphorus; total soil phosphorus and available phosphorus were similar in control plots across forest ages (Supporting Information, Fig. S1). Together, our findings of strong flexibility in belowground plant nutrient acquisition strategies across gradients in nutrient availability and forest age contribute to the high functional biodiversity in tropical forests.

In conclusion, we observed changing tropical forest investment in nutrient acquisition strategies across secondary succession, suggesting that trees utilize these strategies to access nutrients and support forest growth rates. Investment in nutrient acquisition strategies in young

606 forests likely contributes to the recuperation of nutrient as well as carbon cycles following
607 disturbance (Davidson *et al.*, 2007; Poorter *et al.*, 2016; Sullivan *et al.*, 2019) by stimulating
608 efficient release of nutrients from decomposing litter. In older forests, the persistence of high
609 investment in nutrient acquisition strategies may explain the lack of an aboveground growth
610 responses to nutrient fertilization (Wright *et al.*, 2018; Wright, 2019). Strong investment in
611 nutrient acquisition in older forests could compensate for the scarcity of soil nutrients by
612 accelerating nutrient cycling and increasing tree access to nutrients such that trees grow similarly
613 across soil nutrient conditions, or in response to future elevated CO_2 as nutrients becoming more
614 limiting as they are bound up in plant tissues under enhanced biomass growth. However, there
615 may be an upper bound on the degree to which these strategies can alleviate nutrient limitation
616 on the future carbon sink because of the associated carbon costs (Vicca *et al.*, 2012; Doughty *et*
617 *al.*, 2018; Allen *et al.*, 2020), the limited availability of soil nutrients they can acquire, and the
618 extent to which investment in strategies equates to overall success in nutrient uptake.
619 Constraining our understanding of the flexibility of nutrient strategies, their associated carbon
620 costs, how root strategies are coordinated, and the underlying drivers of variation of pan-tropical
621 soil nutrient availability will continue to improve our model projections of the tropical forest
622 carbon sink (e.g. Fleischer *et al.*, 2019). Next-generation dynamic global vegetation models
623 should incorporate the emerging, predictable responses of root phosphatase, while the role of
624 mycorrhizal fungi in nutrient uptake needs to be further understood. Ultimately, our findings
625 indicate that nutrient acquisition strategies are flexible in response to changing nutrient
626 conditions during lowland wet tropical forest recovery from disturbance. Nutrient acquisition
627 strategies support rapid growth in young forests, maintain productivity of mature forests, and
628 potentially play a critical role in alleviating nutrient limitation on the tropical carbon sink.
629
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Competing Interests

There are no competing interests.

Author Contributions

M.Y.W. and S.A.B. designed and executed the study. N.W., W.T., J.S.H, and S.J.W. aided with lab analyses, field work, and data analysis. S.A.B., S.J.W., J.S.H., L.O.H., and M.v.B. designed and J.S.H,. S.A.B. and S.J.W. established the original field experiments. K.S. provided additional data. M.Y.W. and S.A.B. drafted the article and all authors provided feedback on the manuscript.

Data availability

Data and code supporting the results of the manuscript have been archived in a public repository (https://doi.org/10.25390/caryinstitute.24088689)

References

Aidar MPM, Carrenho R, Joly CA. **2004**. Aspects of arbuscular mycorrhizal fungi in an atlantic forest chronosequence parque estadual turístico do Alto Ribeira (petar), SP. *Biota Neotropica* **4**: 1–15.

Allen EB, Allen MF. **1990**. *The Mediation of Competition by Mycorrhizae in Successional and Patchy Environments*. Academic Press, Inc.

Allen K, Fisher JB, Phillips RP, Powers JS, Brzostek ER. **2020**. Modeling the Carbon Cost of Plant Nitrogen and Phosphorus Uptake Across Temperate and Tropical Forests. *Frontiers in Forests and Global Change* **3**: 43.

Bachelot B, Uriarte M, Muscarella R, Forero-Montaña J, Thompson J, McGuire K, Zimmerman J, Swenson NG, Clark JS. **2018**. Associations among arbuscular mycorrhizal fungi and seedlings are predicted to change with tree successional status. *Ecology* **99**: 607–620.

Bartoń K. **2020**. MuMIn: Multi-Model Inference. R package, version 1.43.17.

Bates D, Mächler M, Bolker B, Walker S. **2015**. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**.

Batterman SA, Hedin LO, Van Breugel M, Ransijn J, Craven DJ, Hall JS. **2013a**. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **502**: 224–227.

Batterman SA, Hedin LO, Van Breugel M, Ransijn J, Craven DJ, Hall JS. **2013b**. Key role of symbiotic dinitrogen fixation in tropical forest secondary succession. *Nature* **502**: 224–227.

Batterman SA, Wurzburger N, Hedin LO. **2013c**. Nitrogen and phosphorus interact to control tropical symbiotic N_2 fixation: a test in Inga punctata. *Journal of Ecology* **101**: 1400–1408.

Bloom AJ, Chapin FS, Mooney HA. **1985**. Resource Limitation in Plants-An Economic Analogy. *Annual Review of Ecology and Systematics* **16**: 363–392.

Bolan NS. **1991**. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant and Soil* **134**: 189–207.

van Breugel M, Craven D, Lai HR, Baillon M, Turner BL, Hall JS. **2019**. Soil nutrients and dispersal limitation shape compositional variation in secondary tropical forests across multiple scales. *Journal of Ecology* **107**: 566–581.

Van Breugel M, Hall JS, Craven D, Bailon M, Hernandez A, Abbene M, Van Breugel P. **2013**. Succession of ephemeral secondary forests and their limited role for the conservation of floristic diversity in a human-modified tropical landscape. *PLoS ONE* **8**.

van Breugel M, Hall JS, Craven D, Bailon M, Hernandez A, Abbene M, van Breugel P. **2013**. Succession of Ephemeral Secondary Forests and Their Limited Role for the Conservation of Floristic Diversity in a Human-Modified Tropical Landscape (K Bawa, Ed.). *PLoS ONE* **8**: e82433.

Brienen RJW, Phillips OL, Feldpausch TR, Gloor E, Baker TR, Lloyd J, Lopez-Gonzalez G, Monteagudo-Mendoza A, Malhi Y, Lewis SL, *et al.* 2015. Long-term decline of the Amazon carbon sink. *Nature* **519**: 344–348.

Browman MG, Tabatabai MA. **1978**. Phosphodiesterase Activity of Soils. *Soil Science Society of America Journal* **42**: 284–290.

Cabugao KG, Yaffar D, Stenson N, Childs J, Phillips J, Mayes MA, Yang X, Weston DJ, Norby RJ. **2021**. Bringing function to structure: Root–soil interactions shaping phosphatase activity throughout a soil profile in Puerto Rico. *Ecology and Evolution* **11**: 1150–1164.

Cárate-Tandalla D, Camenzind T, Leuschner C, Homeier J. **2018**. Contrasting species responses to continued nitrogen and phosphorus addition in tropical montane forest tree seedlings. *Biotropica* **50**: 234–245.

Chazdon R. **2016**. Carbon sequestration potential of second-growth forest regeneration in the Latin American tropics. *Science Advances* **333**: 988–93.

Cunha HFV, Andersen KM, Lugli LF, Santana FD, Aleixo IF, Moraes AM, Garcia S, Di Ponzio R, Mendoza EO, Brum B, *et al.* 2022. Direct evidence for phosphorus limitation on Amazon forest productivity. *Nature* **608**: 558–562.

Davidson EA, de Carvalho CJR, Figueira AM, Ishida FY, Ometto JPHB, Nardoto GB, Sába RT, Hayashi SN, Leal EC, Vieira ICG, *et al.* 2007. Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature* **447**: 995–998.

Davidson EA, de Carvalho CJR, Vieira ICG, Figueiredo R De, Moutinho P, Ishida FY, Santos MTP, Guerrero JB, Kalif K, Sabá RT. **2004**. Nitrogen and Phosphorus Limitation of Biomass Growth in a Tropical Secondary Forest. *Ecological Applications* **14**: 150–163.

Davidson EA, Howarth RW. **2007**. Nutrients in synergy. *Nature* **449**: 1000–1001.

Davidson EA, Martinelli LA. **2009**. Nutrient limitations to secondary forest regrowth. *Geophysical Monograph Series* **186**: 299–309.

Delavaux CS, Smith-Ramesh LM, Kuebbing SE. **2017**. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology* **98**: 2111–2119.

Doughty CE, Goldsmith GR, Raab N, Girardin CAJ, Farfan-Amezquita F, Huaraca-Huasco W, Silva-Espejo JE, Araujo-Murakami A, da Costa ACL, Rocha W, *et al.* 2018. What controls variation in carbon use efficiency among Amazonian tropical forests? *Biotropica* **50**: 16–25.

Fick SE, Hijmans RJ. **2017**. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology* **37**: 4302–4315.

Fleischer K, Rammig A, De Kauwe MG, Walker AP, Domingues TF, Fuchslueger L, Garcia S, Goll DS, Grandis A, Jiang M, *et al.* 2019. Amazon forest response to CO_2 fertilization dependent on plant phosphorus acquisition. *Nature Geoscience* **12**: 736–741.

Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, Bardgett RD, De Deyn GB, Johnson D, Klimešová J, *et al.* 2021. Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytologist* **232**: 1123–1158.

Friedlingstein P, O'sullivan M, Jones MW, Andrew RM, Gregor L, Hauck J, Le Quéré C, Luijkx IT, Olsen A, Peters GP, *et al.* 2022. Global Carbon Budget 2022. *Earth System Science Data* **14**: 4811–4900.

Guadarrama P, Castillo S, Ramos-Zapata JA, Hernández-Cuevas L V., Camargo-Ricalde SL. **2014**. Arbuscular mycorrhizal fungal communities in changing environments: The effects of seasonality and anthropogenic disturbance in a seasonal dry forest. *Pedobiologia* **57**: 87–95.

Guariguata MR, Ostertag R. **2001**. Neotropical secondary forest succession: Changes in structural and functional characteristics. *Forest Ecology and Management* **148**: 185–206.

Hart MM, Reader RJ. **2002**. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist* **153**: 335–344.

Hedges L V., Gurevitch J, Curtis PS. **1999**. The Meta-Analysis of Response Ratios in Experimental Ecology. *Ecology* **80**: 1150.

Herbert DA, Williams M, Rastetter EB. **2003**. A model analysis of N and P limitation on carbon accumulation in Amazonian secondary forest after alternate land-use abandonment. *Biogeochemistry* **65**: 121–150.

Hodge A, Fitter AH. **2010**. Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. *Proceedings of the National Academy of Sciences of the United States of America* **107**: 13754–13759.

Johnson NC, Graham JH, Smith FA. **1997**. Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* **135**: 575–585.

Kauffman JB, Cummings DL, Ward DE, Babbitt R. **1995**. Fire in the Brazilian Amazon: 1. Biomass, nutrient pools, and losses in slashed primary forests. *Oecologia* **104**: 397–408.

Koch AM, Antunes PM, Maherali H, Hart MM, Klironomos JN. **2017**. Evolutionary asymmetry in the arbuscular mycorrhizal symbiosis: conservatism in fungal morphology does not predict host plant growth. *New Phytologist* **214**: 1330–1337.

Leigh EG. **1999**. *Tropical forest ecology: a view from Barro Colorado Island*. New York, New York, USA.: Oxford University Press.

Lenth R V. 2021. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package, version 1.6.1.

Levy-Varon JH, Batterman SA, Medvigy D, Xu X, Hall JS, van Breugel M, Hedin LO. **2019**. Tropical carbon sink accelerated by symbiotic dinitrogen fixation. *Nature Communications* **10**: 5637.

Lu M, Hedin LO. **2019**. Global plant–symbiont organization and emergence of biogeochemical cycles resolved by evolution-based trait modelling. *Nature Ecology and Evolution* **3**: 239–250.

Manu R, Corre MD, Aleeje A, Mwanjalolo MJG, Babweteera F, Veldkamp E, van Straaten O. **2022**. Responses of tree growth and biomass production to nutrient addition in a semi-deciduous tropical forest in Africa. *Ecology* **103**: 1–15.

Marklein AR, Houlton BZ. **2012**. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist* **193**: 696–704.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. **1990**. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

McGrath DA, Smith CK, Gholz HL, de Assis Oliveira F. **2001**. Effects of Land-Use Change on Soil Nutrient Dynamics in Amazônia. *Ecosystems* **4**: 625–645.

Nagy RC, Rastetter EB, Neill C, Porder S. **2017**. Nutrient limitation in tropical secondary forests following different management practices. *Ecological Applications* **27**: 734–755.

Nasto MK, Winter K, Turner BL, Cleveland CC. **2019**. Nutrient acquisition strategies augment growth in tropical N_2 -fixing trees in nutrient-poor soil and under elevated CO_2. *Ecology* **100**: e02646.

Neill C, Piccolo MC, Cerri CC, Steudler PA, Melillo JM. **2006**. Soil solution nitrogen losses during clearing of lowland Amazon forest for pasture. *Plant and Soil* **281**: 233–245.

Ogden FL, Crouch TD, Stallard RF, Hall JS. **2013**. Effect of land cover and use on dry season river runoff, runoff efficiency, and peak storm runoff in the seasonal tropics of Central Panama. *Water Resources Research* **49**: 8443–8462.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, *et al.* 2011. A large and persistent carbon sink in the world's forests. *Science* **333**: 988–993.

Poorter L, Bongers F, Aide TM, Almeyda Zambrano AM, Balvanera P, Becknell JM, Boukili V, Brancalion PHS, Broadbent EN, Chazdon RL, *et al.* 2016. Biomass resilience of Neotropical secondary forests. *Nature* **530**: 211–214.

Poorter L, Rozendaal DMA, Bongers F, Almeida DJS, Álvarez FS, Andrade JL, Arreola Villa LF, Becknell JM, Bhaskar R, Boukili V, *et al.* 2021. Functional recovery of secondary tropical forests. *Proceedings of the National Academy of Sciences* **118**.

R Core Team. **2020**. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*.

Reichert T, Rammig A, Fuchslueger L, Lugli LF, Quesada CA, Fleischer K. **2022**. Plant phosphorus-use and -acquisition strategies in Amazonia. *New Phytologist* **234**: 1126–1143.

Sheldrake M, Rosenstock NP, Mangan S, Revillini D, Sayer EJ, Olsson PA, Verbruggen E, Tanner EVJ, Turner BL, Wright SJ. **2018**. Responses of arbuscular mycorrhizal fungi to long-term inorganic and organic nutrient addition in a lowland tropical forest. *ISME Journal* **12**: 2433–2445.

Sheldrake M, Rosenstock NP, Revillini D, Olsson PA, Mangan S, Sayer EJ, Wallander H, Turner BL, Tanner EVJ. **2017**. Arbuscular mycorrhizal fungal community composition is

altered by long-term litter removal but not litter addition in a lowland tropical forest. *New Phytologist* **214**: 455–467.

Smith SE, Smith FA. **2011**. Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. *Annual Review of Plant Biology* **62**: 227–250.

Steidinger BS, Crowther TW, Liang J, Van Nuland ME, Werner GDA, Reich PB, Nabuurs G, de-Miguel S, Zhou M, Picard N, *et al.* 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* **569**: 404–408.

Sullivan BW, Nifong RL, Nasto MK, Alvarez-Clare S, Dencker C, Soper FM, Shoemaker KT, Ishida FY, Zaragoza-Castells J, Davidson EA, *et al.* 2019. Biogeochemical recuperation of lowland tropical forest during succession. *Ecology* **100**: 1–14.

Sulman BN, Shevliakova E, Brzostek ER, Kivlin SN, Malyshev S, Menge DNL, Zhang X. **2019**. Diverse Mycorrhizal Associations Enhance Terrestrial C Storage in a Global Model. *Global Biogeochemical Cycles* **33**: 501–523.

Tabatabai MA. **1994**. Soil Enzymes. In: Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. 775–833.

Tang W. **2022**. Will nutrients limit the tropical forest carbon sink?

Terrer C, Jackson RB, Prentice IC, Keenan TF, Kaiser C, Vicca S, Fisher JB, Reich PB, Stocker BD, Hungate BA, *et al.* 2019. Nitrogen and phosphorus constrain the CO_2 fertilization of global plant biomass. *Nature Climate Change* **9**: 684–689.

Treseder KK. **2004**. A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist* **164**: 347–355.

Treseder KK. **2013**. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil* **371**: 1–13.

Treseder KK, Vitousek PM. **2001**. Effects of Soil Nutrient Availability on Investment in Acquistion of N and P in Hawaiian Rain Forests. *Ecology* **82**: 946–954.

Tummers B. **2006**. DataThief III.

Turner BL. **2008**. Resource partitioning for soil phosphorus: A hypothesis. *Journal of Ecology* **96**: 698–702.

Turner BL, Baxter R, Ellwood NTW, Whitton BA. **2001**. Characterization of the phosphatase activities of mosses in relation to their environment. *Plant, Cell and Environment* **24**: 1165–1176.

Turner BL, Wright SJ. **2014**. The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* **117**: 115–130.

Turner BL, Yavitt JB, Harms KE, Garcia MN, Romero TE, Wright SJ. **2013a**. Seasonal Changes and Treatment Effects on Soil Inorganic Nutrients Following a Decade of Fertilizer Addition in a Lowland Tropical Forest. *Soil Science Society of America Journal* **77**: 1357–1369.

Turner BL, Yavitt JB, Harms KE, Garcia MN, Romero TE, Wright SJ. **2013b**. Seasonal Changes and Treatment Effects on Soil Inorganic Nutrients Following a Decade of Fertilizer Addition in a Lowland Tropical Forest. *Soil Science Society of America Journal* **77**: 1357–1369.

Vallicrosa H, Lugli LF, Fuchslueger L, Sardans J, Ramirez-Rojas I, Verbruggen E, Grau O, Bréchet L, Peguero G, Van Langenhove L, *et al.* 2023. Phosphorus scarcity contributes to nitrogen limitation in lowland tropical rainforests. *Ecology*: 1–12.

Vicca S, Luyssaert S, Peñuelas J, Campioli M, Chapin FS, Ciais P, Heinemeyer A, Högberg P, Kutsch WL, Law BE, *et al.* 2012. Fertile forests produce biomass more efficiently. *Ecology Letters* **15**: 520–526.

Viechtbauer W. **2010**. Conducting Meta-Analyses in R with the metafor Package. *Journal of Statistical Software* **36**.

Vitousek PM. **1984**. Litterfall, nutrient cycling, and nutrient fimitation in tropical forests. *Ecology* **65**: 285–298.

Waring BG, Pérez-Aviles D, Murray JG, Powers JS. **2019**. Plant community responses to stand-level nutrient fertilization in a secondary tropical dry forest. *Ecology* **100**: 1–12.

Warton DI, Hui FKC. **2011**. The arcsine is asinine: the analysis of proportions in ecology. *Ecology* **92**: 3–10.

Whiteside MD, Werner GDA, Caldas VEA, van't Padje A, Dupin SE, Elbers B, Bakker M, Wyatt GAK, Klein M, Hink MA, *et al.* 2019. Mycorrhizal Fungi Respond to Resource Inequality by Moving Phosphorus from Rich to Poor Patches across Networks. *Current Biology* **29**: 2043-2050.e8.

Wieder WR, Cleveland CC, Smith WK, Todd-Brown K. **2015**. Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience* **8**: 441–444.

Wright SJ. **2019**. Plant responses to nutrient addition experiments conducted in tropical forests. *Ecological Monographs* **0**: 1–18.

Wright SJ, Turner BL, Yavitt JB, Harms KE, Kaspari M, Tanner EVJ, Bujan J, Griffin EA, Mayor JR, Pasquini SC, *et al.* 2018. Plant responses to fertilization experiments in lowland, species-rich, tropical forests. *Ecology* **99**: 1129–1138.

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

Yang X, Thornton PE, Ricciuto DM, Hoffman FM. **2016**. Phosphorus feedbacks constraining tropical ecosystem responses to changes in atmospheric CO 2 and climate. *Geophysical Research Letters* **43**: 7205–7214.

Yavitt JB, Harms KE, Garcia MN, Mirabello MJ, Wright SJ. **2011**. Soil fertility and fine root dynamics in response to 4 years of nutrient (N, P, K) fertilization in a lowland tropical moist forest, Panama. *Austral Ecology* **36**: 433–445.

Yokoyama D, Imai N, Kitayama K. **2017**. Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant and Soil* **416**: 463–476.

Zalamea PC, Turner BL, Winter K, Jones FA, Sarmiento C, Dalling JW. **2016**. Seedling growth responses to phosphorus reflect adult distribution patterns of tropical trees. *New Phytologist* **212**: 400–408.

Zangaro W, Rostirola LV, de Souza PB, de Almeida Alves R, Lescano LEAM, Rondina ABL, Nogueira MA, Carrenho R. **2013**. Root colonization and spore abundance of arbuscular mycorrhizal fungi in distinct successional stages from an Atlantic rainforest biome in southern Brazil. *Mycorrhiza* **23**: 221–233.

Figure Legends

Fig. 1. Hypothesized predictions of how root phosphatase activity (black) and mycorrhizal colonization (gray) change across secondary forest succession in response to nitrogen (N) and phosphorus (P) addition. The different types of lines represent the different treatments (dashed lines) and controls (solid lines). Because nitrogen is more mobile compared to phosphorus and lost relatively quickly after a disturbance event, the ecosystem starts at nitrogen limitation (yellow-shared area). However, nitrogen availability increases over time as nitrogen fixers fix nitrogen, and eventually the ecosystem shifts towards nitrogen and phosphorus co-limitation (green shaded area) and finally to phosphorus limitation (blue shaded area).

Fig. 2. Root phosphatase activity responds to nitrogen in the younger tropical forests and phosphorus in the older forests at the root scale (a), and root phosphatase decreases in response to phosphorus in the older forests at the forest scale (b). Root phosphatase (phosphomonoesterase) activity per unit root (a) and per unit area (b) in response to four years of nitrogen and phosphorus addition across three ages of forest recovering from pasture abandonment at the Agua Salud project, and in response to 22 years of nutrient addition at a mature lowland tropical forest in the Barro Colorado Nature Monument. All forests are lowland wet tropical forests in Panama. In panel a, the bars represent the mean across plot-level replicates, the standard error bars represent one standard error, and the dots represent the mean of the three replicates with each 0.16 ha plot.

Fig. 3. Mycorrhizal colonization responds to nitrogen and phosphorus in the oldest lowland wet tropical forests in Panama. Arbuscular mycorrhizal colonization (% of root length colonized by mycorrhizal structures) (a) and percent arbuscules (b) in response to four years of nitrogen and phosphorus addition across three ages of forests recovering from pasture abandonment in the Agua Salud project, and 22 years of nutrient addition at a mature lowland tropical forest in the Barro Colorado Nature Monument. The bars represent the back-transformed mean across plot-level replicates, the bars represent a back-transformed standard error, and the dots represent a back-transformed mean of ten roots examined across ten intersections for colonization.

Fig. 4. Root phosphatase responds more to nitrogen and phosphorus than mycorrhizal colonization, and mycorrhizal colonization typically decreases during forest succession from a meta-analysis. The effects (mean effect size of the log response ratio) of nitrogen and phosphorus fertilization on root phosphatase activity (a) and mycorrhizal colonization (b) and (c) mycorrhizal colonization across forest age. For panels (a) and (b), a negative effect size indicates that fertilization decreased root phosphatase activity or mycorrhizal colonization, while a positive effect size indicates a stimulation. Effects were considered significant where the CIs do not overlap zero. The number to the left of each effect size indicates the number of observations from an individual site or species. In panel (c) individual points represent mean values of mycorrhizal colonization (%) from each forest site, mature forested sites without an age classification are indicated as unconfirmed ages and classified here as ~100 years. We repeated this analysis without confirmed forest ages and found consistent results (Supporting Information Fig. S10). Thin grey lines indicate the trend of mycorrhizal colonization within a study site, while the thick black line indicates the overall trend synthesized across studies ($p<0.0001$, $F_{1,89.085}=41.402$, $R^2_{GLMM(m)}=0.288$, $R^2_{GLMM(c)}=0.458$