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ARTICLE

Phosphorus scarcity contributes to nitrogen limitation in lowland tropical rainforests

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Abstract

There is increasing evidence to suggest that soil nutrient availability can limit the carbon sink capacity of forests, a particularly relevant issue considering today's changing climate. This question is especially important in the tropics, where most part of the Earth's plant biomass is stored. To assess whether tropical forest growth is limited by soil nutrients and to explore N and P limitations, we analyzed stem growth and foliar elemental composition of the five stem widest trees per plot at two sites in French Guiana after 3 years of nitrogen (N), phosphorus (P), and N + P addition. We also compared the results between potential N-fixer and non-N-fixer species. We found a positive effect of N fertilization on stem growth and foliar N, as well as a positive effect of P fertilization on stem growth, foliar N, and foliar P. Potential N-fixing species had greater stem growth, greater foliar N, and greater foliar P concentrations than non-N-fixers. In terms of growth, there was a negative interaction between N-fixer status, N + P, and P fertilization, but no interaction with N fertilization. Because N-fixing plants do not show to be completely N saturated, we do not anticipate N providing from N-fixing plants would supply non-N-fixers. Although the soil-age hypothesis only anticipates P limitation in highly weathered systems, our results for stem growth and foliar elemental composition indicate the existence of considerable N and P co-limitation, which is alleviated in N-fixing plants. The evidence suggests that certain mechanisms invest in N to obtain the scarce P through soil phosphatases, which potentially contributes to the N limitation detected by this study.

KEYWORDS

nitrogen, nutrient limitation, phosphorus, tropical ecology

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INTRODUCTION

Nitrogen (N) and phosphorus (P) are two essential macronutrients that are fundamental in molecules and in the process underlying CO₂ fixation. Thus, they are key in the most basic plant functions such as growth and reproduction (Ellsworth et al., 2022; Schlesinger, 1997; Sterner & Elser, 2002). Accordingly, N and P are essential for plant growth and as such exercise a vital control of the carbon (C) sink capacity of vegetation. Therefore, limitations on their availability could constrain the capacity of plants to sequester C (Fernández-Martínez et al., 2014; Hungate et al., 2003; Terrer et al., 2019; Vicca et al., 2012; Wang et al., 2017; Wright, 2019). The foliar elemental composition provides important information about the nutrient status of plants (Sterner & Elser, 2002) and, along with plant growth capacity, is widely used to determine nutrient limitation before and after fertilization treatments (Elser et al., 2010; Ostertag & DiManno, 2016). Some estimates based on foliar nutrient resorption suggest that, globally, nearly 18% of land plants are already limited by N, 43% are limited by P, and 39% could be co-limited by both these elements (Du et al., 2020). As well, the decrease in foliar N and P concentrations in European forests during the past 30 years has been highlighted as a warning about the potential nutrient limitations in European forests (Penuelas et al., 2020).

Of all biomes, tropical, and subtropical forests have the greatest amounts of biomass C, estimated at around 550 Gt of C (Trummer et al., 2009). Yet, the greatest uncertainties in the ecosystem models forecasting increases in CO₂ occur with these forest types (Fleischer et al., 2019; Koch et al., 2021; Pan et al., 2011), most probably due to their great diversity and the scarcity of empirical data. Most tropical forests grow on highly weathered acid soils that have developed on old parent material, under warm temperatures, and with high rainfall, which make them very prone to nutrient depletion (Morley, 2000; Quesada et al., 2011; Vitousek et al., 2010). This can jeopardize their potential as C sinks in light of the increase in atmospheric CO₂ (Fleischer et al., 2019; Wieder et al., 2015).

Generally, most P initial supply originates from the weathering of parent material, which is only replenished at a geological scale, yet in Guiana Shield there is also P deposition originating from Saharan dust (Prospero et al., 2020). Conversely, N is continuously supplied via atmospheric deposition and biological fixation in soils by N-fixing bacteria and is known to accumulate in the biomass (Houlton et al., 2018; Vitousek, 1984; Walker & Syers, 1976). For example, in French Guiana, N-fixation processes can result in the addition of around 18 kgN⁻¹ ha⁻¹ year⁻¹ on unmanaged land (Reis et al., 2020), of which

13 kgN⁻¹ ha⁻¹ year⁻¹ are calculated to originate from N deposition (Van Langenhove, Verryckt, et al., 2020). Given the fact that many tropical forests grow on very old soils, N is expected to accumulate as P is depleted during soil development (Walker & Syers, 1976). Thus, tropical forests are assumed to be P-, rather than N limited (Du et al., 2020; Vallicrosa et al., 2022). Although some nutrient manipulation experiments in tropical forests support the P limitation theory (Lugli et al., 2021; Mao et al., 2021), others are inconclusive about N and P limitation levels (Alvarez-Clare & Mack, 2015; Mayor et al., 2014; Tanner et al., 1990), or even demonstrate that both N and P limitations exist in tropical forests (Wright, 2019; Wright et al., 2018), most probably due to the variability across tropical forests. In general, P limitation is commonly observed as a factor in the tropics, while the role of N limitation and its interaction with P limitation still needs to be clarified.

Due to the influence of high temperatures and precipitation, tropical evergreen forests are estimated to have the highest levels of N-fixing on the Earth's surface (Cleveland et al., 1999). Aside from the free-living soil N-fixers, species from the Fabaceae, Rosaceae, Cucurbitaceae, and Fagaceae families are known to be associated with root N-fixing microorganisms (Tedersoo et al., 2018) that also contribute to the N-fixing process. The species associated with N-fixing microorganisms are capable of directly fixing N in the plant roots, which is thought to facilitate N acquisition and plant development in N-limiting environments (Baribault et al., 2012; Poorter, 1993; Tedersoo et al., 2018). Evidence from lowland tropical nutrient-poor forests suggest that N-fixer-associated species had higher growth rates as seedlings than non-N-fixing species (Nasto et al., 2019). Nonetheless, there is still a poor understanding about how far this N supply extends; whether this N fixation is providing only to the symbiont organisms or if they can reach other species in the ecosystem such as the non-N-fixers (Lai et al., 2018).

Acid P-depleted soils are consequently orthophosphate depleted, which is the most common P form to be absorbed by plants (Raven, 2015; Treseder & Vitousek, 2001). Consequently, the mineralization of organic P may be the most important source of P in these tropical environments (Vitousek & Sanford, 1986), together with the resorption of P by leaves and litter before it is leached from soils (Grau et al., 2017; Urbina et al., 2021). Phosphatases are enzymes that are responsible for P mineralization and phosphatase activity plays a crucial role in P nutrient dynamics and availability in P-depleted soils. Conversely, the creation of these enzymes is extremely N costly, and is directly linked to N availability (Margalef et al., 2017), which thus encourages nutrient trading of N to P in soil phosphatases (Chen et al., 2020; Reichert et al., 2022). Nevertheless, the N cost in tropical

ecosystems for alleviating P limitation remains undetermined.

The benchmarks for this study were as follows:

Hypothesis 1. We assumed our sites to be P limited, as anticipated by the soil-age hypothesis, due to the progressive P depletion of the acid parental material. As well, we expected that the high levels of N fixation in tropical forests would provide enough N in the ecosystem, reaching N-fixers and non-N-fixer species, precluding any N limitations.

Hypothesis 2. We assumed that N-fixers would not have any differential responses to fertilization because trees are not expected to be N limited or P limited.

We assessed the effects of 3 years of fertilization (N, P, and N + P) on tropical tree stem growth and foliar nutrient composition using data from biennial censuses of tree growth collected in 2015–2020 and in green leaves collected in 2015 to 2019, comparing between N-fixer and non-N-fixers species.

MATERIALS AND METHODS

Study site

The study was conducted in two forest sites in French Guiana, Paracou, and Nouragues, both dominated by primary lowland rainforest. Paracou is located 15 km from the coast (5°16' N, 52°55' W), while Nouragues is located further inland (4°05' N, 52°41' W). The French Guianese climate is characterized by a wet and a dry season. This region receives heavy rains from December to July but also has a dry season, typically characterized by less than 100 mm rainfall per month, from August to November (Aguilos et al., 2018). The soils at both sites are classified as Oxisols according to the USDA soil classification (Anjos et al., 2015), with pH values between 3.7 and 4.5. Its Precambrian geological substrate is particularly low in total P content (Van Langenhove et al., 2021) compared with the generally younger, nutrient-rich soils of western Amazonia (Grau et al., 2017). Both study sites receive similar annual rainfall: mean annual rainfall is 3100 mm in Paracou (Aguilos et al., 2019; 2004–2015 period) and 2990 mm in Nouragues (Bongers et al., 2001). The mean annual air temperature is near 26°C for both sites (Bongers et al., 2001; Gourlet-Fleury et al., 2004). Soils at Paracou range from loamy sand to sandy loam, while soils at Nouragues contain more clay and span the range

of sandy loam to silty clay (Van Langenhove et al., 2021). At both sites, tree diversity is ~200 species ha⁻¹, the most common families were the Lecythidaceae, Fabaceae, Sapotaceae, and Chrysobalanaceae. Trees in Paracou are slightly smaller than in Nouragues, with an average canopy height of 30–35 m and emergent trees up to 45 m high (Gourlet-Fleury et al., 2004), while in Nouragues the average canopy height is 40–45 m and emergent trees are up to 60 m high (Ho Tong Minh et al., 2016).

Field experiment

Twelve 50 × 50 m plots were established in 2015, grouped in three blocks of four plots along a toposequence at both sites, Paracou and Nouragues, giving a total of 24 plots. The toposequence included plots at the top of the hill (Top plots) on the slope of the hill (Slope plots), and at the bottom of the hill (Bottom plots) (Figure 1). The valley bottoms and hilltops differ by 20–50 m in elevation over horizontal distances of 200–400 m (Van Langenhove et al., 2019), with maximum altitudes of 70 and 120 m for Paracou and Nouragues, respectively (Courtois et al., 2018). The nutrient addition experiment started in October 2016. In each group of four plots, one remained unfertilized as a control, and the three other plots were N, P, or N + P enriched. The fertilizer was applied twice per year by hand using a commercial urea ((NH₂)₂CO) and/or triple superphosphate (Ca (H₂PO₄)₂) at a rate of 125 kg N ha⁻¹ year⁻¹ (N treatment), 50 kg P ha⁻¹ year⁻¹ (P treatment), or both amounts together (N + P treatment). These amounts were fixed in light of the ongoing experiments in Barro Colorado Nature Monument in Panama, begun in 1998 (Wright et al., 2011), and the Amazon Fertilization Experiment (AFEX) near Manaus in Brazil, initiated in 2017 (Lugli et al., 2021), to enable future comparison of our results.

Leaf sampling

Leaves were collected twice after fertilization in 2019, during the rainy season from May to the end of June, and during the dry season from the beginning of October until late November. To avoid border effects of the nutrient addition, sampling was done within the central 20 × 20 m area, within the larger 50 × 50 m plots. In each plot, the five biggest trees in diameter were selected, for canopy leaf collection. Green leaves were collected (between ~3–15, depending on the leaf size) both from the top sunlit canopy and the low shaded canopy. In total, we sampled 60 trees per site, which gave a total of ~480 samples (i.e., two sites, two seasons, and two canopy levels). Five trees could not be sampled due to their death

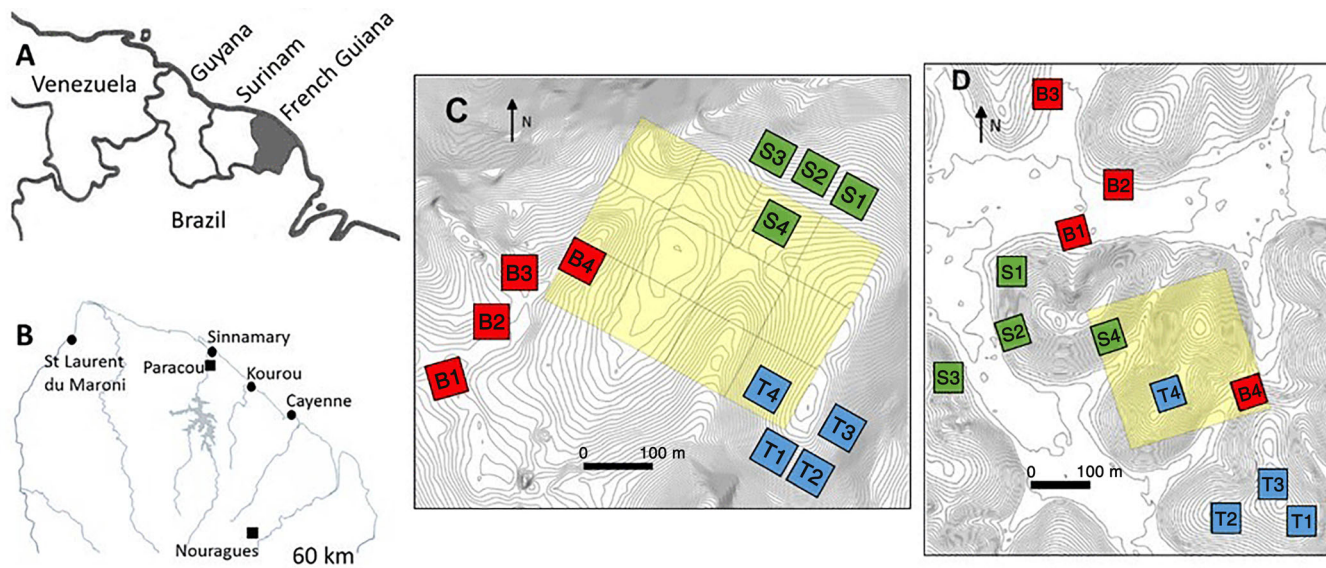


FIGURE 1 Adapted from Verryckt et al. (2022). Situational map: (A) northeastern part of South America; (B) northern French Guiana with its main cities (circles), and the experimental sites of Paracou and Nouragues (squares); (C) the twelve 50 × 50 m plots of this study at Nouragues-Inselberg, and (D) the twelve 50 × 50 m plots of this study at Paracou. Plots are marked by a letter describing the topography (B = bottom, S = slope, T = top) and a number describing the nutrient addition treatment (1 = +N; 2 = +NP; 3 = +P; 4 = control). Yellow boxes represent the long-term undisturbed permanent plots in place before our experiment began and used in our control plots.

during the experiment or leaf senescence during the sampling campaign. The leaves were stored in paper envelopes and immediately frozen in liquid N in the field, and subsequently lyophilized in the laboratory (Christ Freeze Dryer ALPHA 1-2 LDplus, Osterode am Harz, Germany). In total, 65 different tree species were identified during the sampling.

Growth measurement

Manual band dendrometers EMS DB20 and manual dendrometers D1 were installed at Nouragues and Paracou, respectively, on all trunks over 5 cm in diameter at breast height (dbh) in each plot in October 2015. Periodic readings were performed every 2–3 months in Paracou and every 6 months in Nouragues. The final reading at Paracou was taken in June 2020 and at Nouragues in September 2019.

Chemical analysis

The leaf samples were ground with a ball mill (Retsch, model MM400, Restch GmbH) and weighed with an AB204 Mettler Toledo (Mettler Toledo) balance. Total leaf C and N concentrations (%) were determined by gas chromatography using an elemental analyzer interfaced with an isotope ratio mass spectrometer (PDZ Europa

ANCA-GSL and PDZ Europa 20–20; Sercon Ltd.) at the Stable Isotope Facility of UC Davis. We used 4.5 mg of pulverized dry sample for total C and N (%) analyses, calibrated with reference materials. Total leaf P concentrations were determined by inductively coupled plasma (ICP) mass spectrometry (ICP-MS Agilent 7500 CE) using 250 mg of leaf material digested in 5 mL of concentrated HNO_3 (Milestone Ultrawave digester; Sorisole, BG, Italy) following the NIST 1573 (tomato leaves) protocol.

Calculations

To determine whether N, P, or N + P fertilization affected stem growth over time, we calculated the mean annual dbh as:

$$\text{Growth}_{\text{stem}} = (\text{DBH}_{t2} - \text{DBH}_{t1}) / t2 - t1,$$

where DBH_{t2} and DBH_{t1} were the values measured during the final (2020 for Paracou and 2019 for Nouragues) and first (2015) dendrometer readings, respectively. 8.75% of the data were slightly negative values, which were reclassified and considered to be 0. Three outliers were excluded due to high values.

Tree species were classified as either N-fixers or non-N-fixers following the classification provided by Tedersoo et al. (2018). In the Nouragues and Paracou tree growth database, 181 individuals of 26 tree species were

categorized as potentially N-fixers, and 333 individuals of 128 species were categorized as non-N-fixers. In the foliar elemental composition analysis, 36 individuals from eight tree species were considered to belong to N-fixing species and 79 individuals from 57 tree species to non-N-fixing species.

Statistics analyses

Data analyses were conducted using *glmmTMB* R (Brooks et al., 2017), *lme4* (Bates et al., 2015) and *lmerTest* (Kuznetsova et al., 2017) packages in R, with *ggplot* (Wickham, 2016) and *ggpubr* used for visualization (Kassambara, 2020).

We evaluated the effects of fertilization on tree growth using generalized linear mixed models (“*glmmTMB*” function) to model $\text{Growth}_{\text{stem}}$ as a function of treatment (N, P, N + P, or control), N-fixer status, and their interaction as fixed effects, with topography (Top, Slope or Bottom), and site (Paracou, Nouragues) as crossed random effects (i.e., “(1|Site)”). The Tweedie family linked to log distribution was used for null-inflated exponential-shaped datasets.

We assessed whether fertilization affected the foliar elemental composition in comparison with control plots (2019 data) using mixed effect models (“*lmer*” function).

We modeled each foliar elemental composition (in percentage of leaf dry weight) as a function of treatment (N, P, or N + P, or control), N-fixer status, and their interaction as fixed effects, with season (Wet, Dry), site, and topography as random effects. We reported significant results at $p < 0.05$ and log-transformed each element concentration prior to model implementation to fulfill normality requirements.

Given the high tree species diversity of the area most species are not consistent in all plots, which could alter the results of the analysis. To control the effect of species diversity we are including an additional analysis considering the species as a random factor in addition to site and topography, which does not significantly vary the results of previous analysis or its conclusions (Vallicrosa, 2023; TS1.xlsx).

RESULTS

Growth

Fertilization with N, P, or N + P significantly stimulated stem growth, 3.05, 3.88 and 4.01-fold, respectively, in comparison with control plots (Figure 2A; Vallicrosa, 2023: TS2.xlsx). In all treatments, potential N-fixers had greater growth than species not regarded as N-fixers

Stem growth

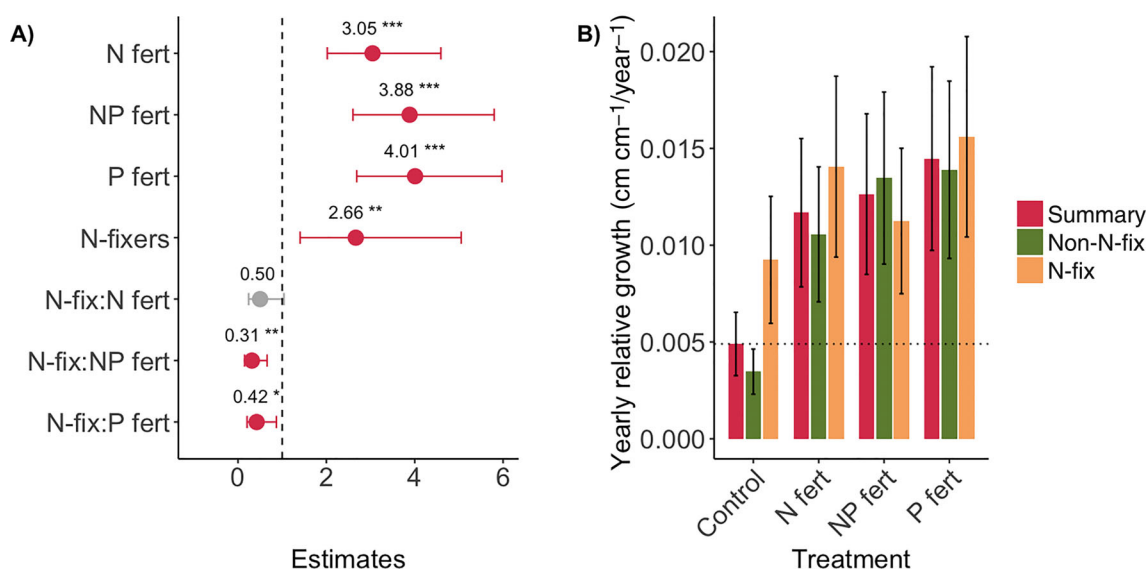


FIGURE 2 (A) Model estimates explaining stem growth between treatments. Red color stands for significant relationships and gray color stands for a nonsignificant relationship. The vertical dashed line indicates no effect. Estimates below 1 indicate negative effects. The numbers represent the estimated values and asterisks their significance. (B) Predicted stem growth values predicted for each group in dbh relative growth. The summary represents the weighted means of each treatment; the dashed horizontal line represents the control summary value.

(Figure 2B). However, we noted a significant negative interaction between N-fixers and P (0.42) and N + P (0.31) fertilization (Figure 2A, Vallicrosa, 2023: TS2.xlsx) and a close link between N-fixers and N fertilization ($p = 0.062$; Figure 2A, Vallicrosa, 2023: TS2.xlsx).

Control plots had a yearly relative growth of 0.0049 cm, while N-fertilized plots, N + P fertilized plots, and P fertilized plots grew 0.0117, 0.0126, and 0.0145 cm, respectively (Figure 2B, https://github.com/helenavallicrosa/FG_leaves/TS3.xlsx). In control and N-fertilized plots, N-fixers grew significantly more than non-N-fixers (0.0035 vs. 0.0092 cm and 0.0106 vs. 0.0141 cm, respectively). Nevertheless, as anticipated by the significant negative interaction between N-fixers and N + P, and N-fixers and the P fertilization treatment, N-fixers grew less in N + P fertilized plots and P fertilized plots (0.0135 vs. 0.0113 cm, and 0.0139 and 0.0156 cm, respectively: Figure 2, Vallicrosa, 2023: TS3.xlsx).

Leaf elemental composition

N and N + P addition plots showed significantly higher leaf N content than control plots, with 1.44% in control plots and 1.77% and 1.70% in N and N + P fertilized plots, respectively. N-fixing species had also higher foliar N in control plots than non-N-fixers, reaching 1.69% compared to 1.34% in nonfixing species. Neither fertilization with P

nor the interactions between the treatments and the N-fixers category significantly affected the amount of foliar N (Figure 3, Vallicrosa, 2023: TS4.xlsx).

Leaf P content increased from 0.052% in controls to 0.056% in N, 0.071% in N + P, and 0.061% in P fertilized plots. Interestingly, N-fixers had also had higher P content in their leaves, reaching levels of 0.066% compared to X% in nonfixing species. The interaction between N-fixers and fertilization treatments did not show any significant relationships in the models describing leaf P content (Figure 4, Vallicrosa, 2023: TS4.xlsx).

The foliar N:P ratio was positively affected by N fertilization, which increased from 27.73 in controls, to 31.47 in N-fertilized plots, but was negatively affected by N + P and P fertilization (23.86 and 23.42, respectively). In this case, neither N-fixers nor their interactions with the different N, P, and N + P treatments had any significant effects on the foliar N:P ratio (Figure 5, Vallicrosa, 2023: TS4.xlsx).

DISCUSSION

Our results for stem growth and foliar elemental composition suggest a strong N and P co-limitation in the two studied forests in French Guiana. This strong nutrient limitation affected both N-fixing and non-N-fixing trees. Nutrient limitation in terrestrial ecosystems refers to a constraint on primary production or other ecosystem

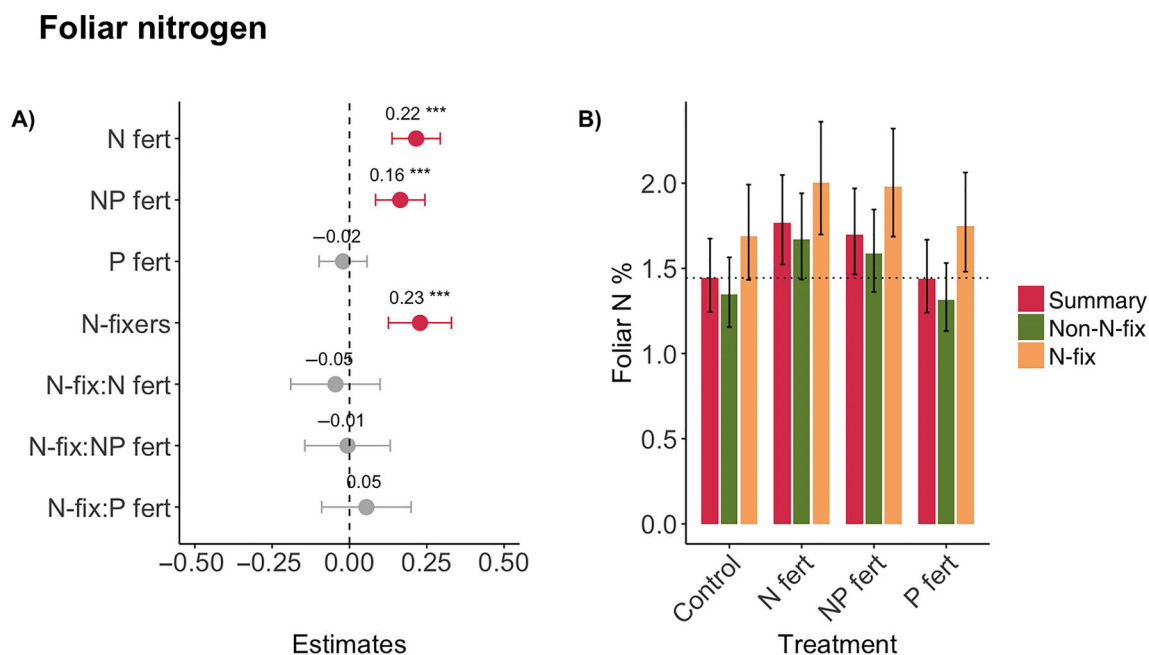


FIGURE 3 (A) Model estimates explaining foliar nitrogen between treatments. Red color stands for significant relationships and gray color stands for nonsignificant relationships. The vertical dashed line indicates no effect. Estimates below 0 indicate negative effects. The numbers represent the estimated values and the asterisks their significance. (B) Foliar N % values are predicted for each group. The summary represents for the weighted means of each treatment; the dashed horizontal line represents the control summary value.

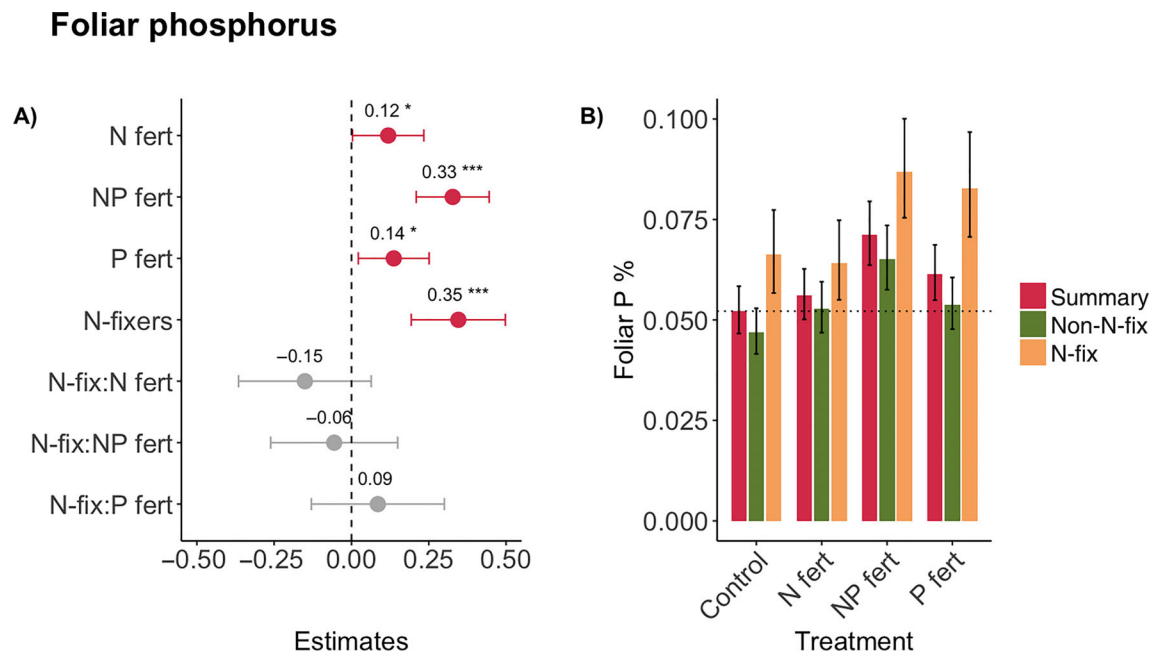


FIGURE 4 (A) Model estimates explaining foliar phosphorus between treatments. Red color stands for significant relationships and gray color stands for nonsignificant relationships. The vertical dashed line indicates no effect. Estimates below 0 indicate negative effects. The numbers represent the estimated values and the asterisks their significance. (B) Foliar P % values are predicted for each group. The summary represents the weighted means of each treatment; the dashed horizontal line represents the control summary value.

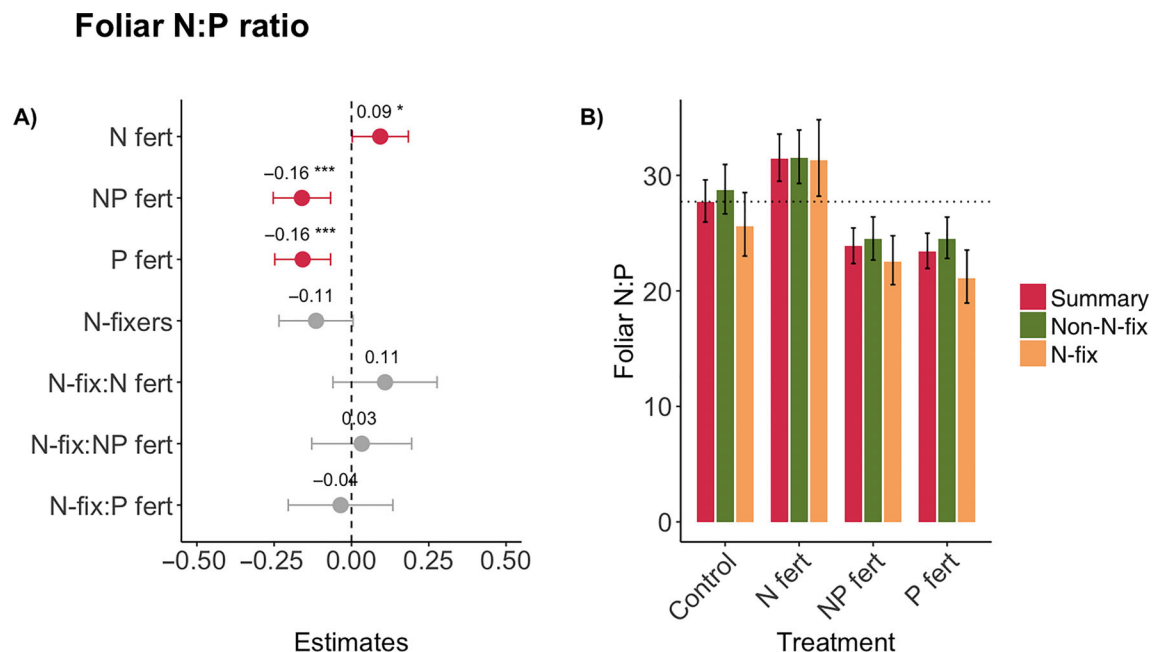


FIGURE 5 (A) Model estimates explaining foliar nitrogen-to-phosphorus ratio between treatments. Red color stands for significant relationships and gray stands for nonsignificant relationships. The vertical dashed line indicates no effect. Estimates below 0 indicate negative effects. The numbers represent the estimated values and the asterisks their significance. (B) Foliar N:P values are predicted for each group. The summary represents the weighted means of each treatment; the dashed horizontal line represents the control summary value.

processes due to low rates of nutrient supply (Chapin et al., 1986; Vitousek & Farrington, 1997) and can often be quantified by an increase in growth (Eviner

et al., 2000; Sullivan et al., 2014) or by foliar nutrients (Elser et al., 2010; Ostertag & DiManno, 2016) after experimental nutrient additions. In our study, we

observed an increase in stem growth and foliar N when N was added, as well as an increase in stem growth and foliar P when P was added. Aside from the nutrient additions, the N-fixing tree species had higher levels of foliar N and stem growth (Figures 2 and 3), which can be associated with an increase in N acquisition due to its plant-bacteria symbiosis (Nasto et al., 2019). On the other hand, we found no significant interactions between N-fixers and N fertilization in the N leaf content (Figure 3), P (Figure 4), N:P (Figure 5) and stem growth (Figure 2), which suggests that N fertilization equally affected N-fixers and non-N-fixers equally. Yet, the interaction between N-fixers and stem growth was only slightly significant ($p = 0.06$), so N limitation could be slightly alleviated. We therefore conclude that, although N-fixing plants do potentially have more access to N, they may not reach the N saturation remaining N-limited, and not be able to provide N to other non-N-fixing species in our experimental sites in French Guiana.

This co-limitation of N and P on tree growth in tropical rainforests has also been detected by a long-term fertilization experiment carried out in Panama, which applied the same loads of N and P as our experiments in French Guiana (Wright et al., 2011, 2018). As well, an AFEX experiment carried out in the lowland tropical forests of Manaus, Brazil that was fertilized with the same levels of N and P showed strong limitations on leaf and root productivity with P-only fertilization after 2 years of fertilization (Cunha et al., 2022). A meta-analysis of 48 tropical lowland and montane forests located in the Neotropics, Hawaii, and Southeast Asia highlights the existence of generalized N and P limitations (Wright, 2019). Conversely, a fertilizing experiment conducted in Africa showed N to be more relevant than P on stem growth (Manu et al., 2022). The same pattern of co-limitation has also often been commonly documented in terrestrial, freshwater, and marine environments (Elser et al., 2007).

The evidence of both N and P limitation in most of the above-mentioned studies challenges the common assumption of N saturation in the tropics (Houlton et al., 2018; Vitousek, 1984; Walker & Syers, 1976). The soil-age hypothesis suggests that the constant fixation of N in the tropics results in N accumulation in soils and biomass, making N available to be absorbed or recycled by plants (Walker & Syers, 1976). Another theory proposing the presence of intense leaching of N from the system has often been used to argue for N sufficiency in the tropics. This latter theory assumes that high N losses due to N leaching indicate N saturation given that greater N leaching is thought to occur in the tropics than in homologous systems at other latitudes (Brookshire et al., 2012; Perakis & Hedin, 2002). In addition, the N:P ratio theory

also suggests a lack of N limitation in the tropics, where leaf N:P levels of more than 20 are considered to correspond to P-limited environments (Greenwood et al., 2008; Güsewell, 2004). Our control plots had an N:P ratio of 27.76 and so we suggest that there is a mismatch between the assumptions made in previous studies regarding N limitation and the results of several fertilization experiments (including our study) conducted in certain tropical locations.

Interestingly, our study found greater leaf P in N-fertilized plots (Figure 4) with no addition of P. Similarly, species associated with N-fixing microorganisms also showed higher foliar P than non-N-fixing species (Figure 4). In addition, in N + P plots, foliar N:P decreased, thereby suggesting that foliar P increased even more than N. Therefore, our results suggest that the studied species possess a mechanism for investing part of the provided N that enables them to increase their P acquisition. In turn, this investment of N alleviates P limitation and prevents species from becoming N saturated.

Soil phosphatases N-rich enzymes released by both plants and soil microorganisms mineralize P pools into available forms (e.g., orthophosphates) (Margalef et al., 2017; Vance et al., 2003). Due to its high N requirements, soil phosphatase production can be N limited and is therefore enhanced by N fertilization (Chen et al., 2020). A rise in phosphatase activity could increase plant P availability and P acquisition, thereby alleviating limitations in nutrient-poor environments (Nasto et al., 2019). Studies testing whether N alleviates P limitation due to soil phosphatases offer inconclusive results (Lugli et al., 2021), whereas studies embracing multiple tree species were warranted (Batterman et al., 2013, 2018). In our study, which included 65 different tree species in the foliar elemental composition analysis, we found evidence supporting the trading of N for P at the plot and community levels. Accordingly, the trading of N for P seems to be equally effective as well when N fixed by N-fixers is used, deriving higher foliar P levels (Figure 4). Furthermore, these results, as well as those showing an increase in stem growth and foliar N, fit the theory that N-fixers may have competitive advantages in nutrient-limited environments (Nasto et al., 2017, 2019).

Tropical forests have some of the highest N-fixing rates of all terrestrial ecosystem types (Cleveland et al., 1999) and they have one of the highest abundances of N-fixer trees in terms of basal area (Steidinger et al., 2019). After fixation, N becomes available for acquisition by biomass and when this biomass decomposes it is likely to be leached out, which ensures that the tropics have one of the highest rates of N leaching as well (Brookshire et al., 2012). Van Langenhove, Janssens, et al. (2020) compared the removal of N and P in the same French

Guyana experiment following the addition of nutrients in control soil versus rhizosphere-free soil. They observed a clear rhizosphere effect for P, but not for N. The fact that the rhizospheres rapidly took up P, but not N, supports the assumption that trees growing in these study sites are primarily P limited. Soil P availability estimates (Bray-P), determined before the start of the nutrient addition experiment, suggest very low P availability, typically ranging between <1 and 3 ppm (Verryckt et al., 2022). Reactive N (nitrate + ammonium) availability in these soils exhibited values of 5–25 ppm (Verryckt et al., 2022).

The hypothesized trading of N to P seems to imply an investment by N in phosphatase production that otherwise could be used directly by the plants. Given the strong P limitation widely detected in the tropics (Cunha et al., 2022; Du et al., 2020; Hou et al., 2020), plants may in fact invest part of their available N in obtaining P as opposed to satisfying their N needs. Although the intake of N is constant due to N fixation and N deposition, P is constantly depleted from the soil, which makes its acquisition through mineralization so crucial. Following this reasoning, it thus seems possible that plants in the tropics could also be N limited due to their efforts to negate P limitation.

In contradiction with our initial hypothesis, we found evidence of N and P limitation in a lowland tropical forest based on observed changes in stem growth and foliar elemental composition after a fertilization experiment. We documented advantages in growth and in foliar elemental composition in N-fixer tree species but, despite the potential advantage of N-fixing, these species are still N and P limited. We provide evidence supporting a trading mechanism for obtaining P through extra N availability in the system (e.g., phosphatase exudation). This extra N investment could help explain the N limitation found in the tropics, paradoxically, one of the Earth's ecosystems with the greatest number of N-fixing and N-fixer plants.

AUTHOR CONTRIBUTIONS

Helena Vallicrosa designed the research; Helena Vallicrosa, Laynara F. Lugli, Lucia Fuchslueger, Pere Roc-Fernández, Laura Márquez, Joan Llusà and Romà Ogaya performed the field and laboratory work; Helena Vallicrosa analyzed the data; Helena Vallicrosa, Laynara F. Lugli, Lucia Fuchslueger, Jordi Sardans, Laëticia Bréchet and César Terrer interpreted the data and participated in the discussions; Helena Vallicrosa wrote the manuscript; Ivan Janssens and Josep Peñuelas obtained the funding, and all coauthors supervised the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supplementary tables, data, and code (Vallicrosa, 2023) are available in Zenodo at <https://doi.org/10.5281/zenodo.7781944>.

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REFERENCES

- Aguilos, M., B. Hérault, B. Burban, F. Wagner, and D. Bonal. 2018. "What Drives Long-Term Variations in Carbon Flux and Balance in a Tropical Rainforest in French Guiana?" *Agricultural and Forest Meteorology* 253–254: 114–23. <https://doi.org/10.1016/j.agrformet.2018.02.009>.
- Aguilos, M., C. Stahl, B. Burban, B. Hérault, E. Courtois, S. Coste, F. Wagner, C. Ziegler, K. Takagi, and D. Bonal. 2019. "Interannual and Seasonal Variations in Ecosystem Transpiration and Water Use Efficiency in a Tropical Rainforest." *Forests* 10(1): 14.
- Alvarez-Clare, S., and M. C. Mack. 2015. "Do Foliar, Litter, and Root Nitrogen and Phosphorus Concentrations Reflect Nutrient Limitation in a Lowland Tropical Wet Forest?" *PLoS One* 10(4): e0123796.
- Anjos, L., C. Gaistardo, J. Deckers, S. Dondeyne, E. Eberhardt, M. Gerasimova, B. Harms, et al. 2015. "World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps." In *World Soil Resources Reports No. 106*, edited by P. Schad, C. van Huyssteen, and E. Micheli. Rome, Italy: FAO. <https://doi.org/10.1017/S0014479706394902>.
- Baribault, T. W., R. K. Kobe, and A. O. Finley. 2012. "Tropical Tree Growth is Correlated with Soil Phosphorus, Potassium, and Calcium, though Not for Legumes." *Ecological Monographs* 82: 189–203. <https://doi.org/10.1890/11-1013.1>.
- Bates, D., M. Maechler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67(1): 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Batterman, S. A., J. S. Hall, B. L. Turner, L. O. Hedin, J. K. LaHaela Walter, P. Sheldon, and M. van Breugel. 2018. "Phosphatase Activity and Nitrogen Fixation Reflect Species Differences, Not Nutrient Trading or Nutrient Balance, across Tropical Rainforest Trees." *Ecology Letters* 21: 1486–95. <https://doi.org/10.1111/ele.13129>.
- Batterman, S. A., N. Wurzbarger, and L. O. Hedin. 2013. "Nitrogen and Phosphorus Interact to Control Tropical Symbiotic N₂

- Fixation: A Test in *Inga punctata*." *Journal of Ecology* 101: 1400–8. <https://doi.org/10.1111/1365-2745.12138>.
- Bongers, F., P. Charles-Dominique, P. Forget, and M. Th  ry. 2001. *Nouragues Dynamics and Plant-Animal Interactions in a Neotropical Rainforest*. Kluwer Academic Publishers. <https://doi.org/10.1007/978-94-015-9821-7>.
- Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. "glmmTMB Balances Speed and Flexibility among Packages for Zero-inflated Generalized Linear Mixed Modeling." *The R Journal* 9(2): 378–400. <https://doi.org/10.32614/RJ-2017-066>.
- Brookshire, E. N. J., S. Gerber, D. N. L. Menge, and L. O. Hedin. 2012. "Large Losses of Inorganic Nitrogen from Tropical Rainforests Suggest a Lack of Nitrogen Limitation." *Ecology Letters* 15: 9–16. <https://doi.org/10.1111/j.1461-0248.2011.01701.x>.
- Chapin, F. S., P. M. Vitousek, and K. Van Cleve. 1986. "The Nature of Nutrient Limitation in Plant Communities." *The American Naturalist* 127(1): 48–58. <http://www.jstor.org/stable/2461646>.
- Chen, J., K. J. van Groenigen, B. A. Hungate, C. Terrer, J. W. Groenigen, F. T. Maestre, S. C. Ying, et al. 2020. "Long-Term Nitrogen Loading Alleviates Phosphorus Limitation in Terrestrial Ecosystems." *Global Change Biology* 26: 5077–86. <https://doi.org/10.1111/gcb.15218>.
- Cleveland, C. C., A. R. Townsend, D. S. Schimel, H. Fisher, R. W. Howarth, L. O. Hedin, S. S. Perakis, et al. 1999. "Global Patterns of Terrestrial Biological Nitrogen (N₂) Fixation in Natural Ecosystems." *Global Biogeochemical Cycles* 13: 623–45.
- Courtois, E. A., C. Stahl, J. Van den Berge, L. Br  chet, L. Van Langenhove, A. Richter, I. Urbina, J. L. Soong, J. Pe  uelas, and I. Janssens. 2018. "Spatial Variation of Soil CO₂, CH₄ and N₂O Fluxes across Topographical Positions in Tropical Forests of the Guiana Shield." *Ecosystems* 21: 1445–58. <https://doi.org/10.1007/s10021-018-0232-6>.
- Cunha, H. F. V., K. M. Andersen, L. F. Lugli, F. D. Santana, I. F. Aleixo, A. M. Moraes, S. Garcia, et al. 2022. "Direct Evidence for Phosphorus Limitation on Amazon Forest Productivity." *Nature* 608: 558–62. <https://doi.org/10.1038/s41586-022-05085-2>.
- Du, E., C. Terrer, A. F. A. Pellegrini, A. Ahlstr  m, C. J. van Lissa, X. Zhao, N. Xia, X. Wu, and R. B. Jackson. 2020. "Global Patterns of Terrestrial Nitrogen and Phosphorus Limitation." *Nature Geoscience* 13: 221–6. <https://doi.org/10.1038/s41561-019-0530-4>.
- Ellsworth, D. S., K. Y. Crous, M. G. de Kauwe, L. T. Verryckt, D. Goll, S. Zaehle, K. J. Bloomfield, et al. 2022. "Convergence in Phosphorus Constraints to Photosynthesis in Forests around the World." *Nature Communications* 13: 5005. <https://doi.org/10.1038/s41467-022-32545-0>.
- Elser, J. J., M. E. S. Bracken, D. S. Gruner, W. S. Harpole, N. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. "Global Analysis of Nitrogen and Phosphorus Limitation of Primary Producers in Freshwater, Marine and Terrestrial Ecosystems." *Ecology Letters* 10: 1135–42.
- Elser, J. J., W. F. Fagan, A. J. Kerkhoff, N. G. Swenson, and B. J. Enquist. 2010. "Biological Stoichiometry of Plant Production: Metabolism, Scaling and Ecological Response to Global Change." *The New Phytologist* 186(3): 593–608.
- Eviner, V. T., F. S. Chapin, III, and C. E. Vaughn. 2000. "Nutrient Manipulations in Terrestrial Ecosystems." In *Methods in Ecosystem Science*, edited by O. E. Sala, R. B. Jackson, H. A. Mooney, and R. W. Howarth, 291–307. New York: Springer-Verlag.
- Fern  ndez-Mart  nez, M., S. Vicca, I. A. Janssens, J. Sardans, S. Luyssaert, M. Campioli, F. S. Chapin, III, et al. 2014. "Nutrient Availability as the Key Regulator of Global Forest Carbon Balance." *Nature Climate Change* 4: 471–6.
- Fleischer, K., A. Rammig, M. G. De Kauwe, A. P. Walker, T. F. Domingues, L. Fuchslueger, S. Garcia, et al. 2019. "Amazon Forest Response to CO₂ Fertilization Dependent on Plant Phosphorus Acquisition." *Nature Geoscience* 12: 736–41. <https://doi.org/10.1038/s41561-019-0404-9>.
- Gourlet-Fleury, S., J. M. Guehl, and O. Laroussinie. 2004. "Ecology and Management of a Neotropical Rainforest." In *Lessons Drawn from Paracou, a Long-Term Experimental Research Site in French Guiana*. Paris, France: Elsevier.
- Grau, O., J. Pe  uelas, B. Ferry, V. Freycon, L. Blanc, M. Desprez, C. Baraloto, et al. 2017. "Nutrient-Cycling Mechanisms Other than the Direct Absorption from Soil May Control Forest Structure and Dynamics in Poor Amazonian Soils." *Scientific Reports* 7: 45017. <https://doi.org/10.1038/srep45017>.
- Greenwood, D. J., T. V. Karpinets, K. Zhang, A. Bosh-Serra, A. Boldrini, and L. Karawulova. 2008. "A Unifying Concept for the Dependence of Whole-Crop N: P Ratio on Biomass: Theory and Experiment." *Annals of Botany* 102(6): 967–77. <https://doi.org/10.1093/aob/mcn188>.
- G  sewell, S. 2004. "N:P Ratios in Terrestrial Plants: Variation and Functional Significance." *New Phytologist* 164: 243–66. <https://doi.org/10.1111/j.1469-8137.2004.01192.x>.
- Ho Tong Minh, D., T. Le Toan, F. Rocca, S. Tebaldini, L. Villard, M. R  jou-M  chain, O. L. Phillips, et al. 2016. "SAR Tomography for the Retrieval of Forest Biomass and Height: Cross-Validation at Two Tropical Forest Sites in French Guiana." *Remote Sensing of Environment* 175: 138–47. <https://doi.org/10.1016/j.rse.2015.12.037>.
- Hou, E., Y. Luo, Y. Kuang, C. Chen, X. Lu, L. Jiang, X. Luo, and D. Wen. 2020. "Global Meta-Analysis Shows Pervasive Phosphorus Limitation of Aboveground Plant Production in Natural Terrestrial Ecosystems." *Nature Communications* 11: 637. <https://doi.org/10.1038/s41467-020-14492-w>.
- Houlton, B. Z., S. L. Morford, and R. A. Dahlgren. 2018. "Convergent Evidence for Widespread Rock Nitrogen Sources in Earth's Surface Environment." *Science* 360: 58–62.
- Hungate, B. A., J. S. Dukes, M. R. Shaw, Y. Luo, and C. B. Field. 2003. "Nitrogen and Climate Change." *Science* 302: 1512–3.
- Kassambara, A. 2020. "ggpubr: 'ggplot2' Based Publication Ready Plots." R Package Version 0.4.0. <https://CRAN.R-project.org/package=ggpubr>.
- Koch, A., W. Hubau, and S. L. Lewis. 2021. "Earth System Models Are Not Capturing Present-Day Tropical Forest Carbon Dynamics." *Earth's Future* 9: 1–19.
- Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. "lmerTest Package: Tests in Linear Mixed Effects Models." *Journal of Statistical Software* 82(13): 1–26. <https://doi.org/10.18637/jss.v082.i13>.
- Lai, H. R., J. S. Hall, S. A. Batterman, B. L. Turner, and M. van Breugel. 2018. "Nitrogen Fixer Abundance has No Effect on

- Biomass Recovery During Tropical Secondary Forest Succession." *Journal of Ecology* 106: 1415–27. <https://doi.org/10.1111/1365-2745.12979>.
- Lugli, L. F., J. S. Rosa, K. M. Andersen, R. Di Ponzio, R. V. Almeida, M. Pires, A. L. Cordeiro, et al. 2021. "Rapid Responses of Root Traits and Productivity to Phosphorus and Cation Additions in a Tropical Lowland Forest in Amazonia." *The New Phytologist* 230: 116–28. <https://doi.org/10.1111/nph.17154>.
- Manu, R., M. D. Corre, A. Aleje, M. J. Mwanjalolo, F. Babweteera, E. Veldkamp, and O. van Straaten. 2022. "Responses of Tree Growth and Biomass Production to Nutrient Addition in a Semi-Deciduous Tropical Forest in Africa." *Ecology* 103(6): e3659.
- Mao, Q., H. Chen, C. Wang, Z. Pang, J. Mo, and X. Lu. 2021. "Effect of Long-Term Nitrogen and Phosphorus Additions on Understory Plant Nutrients in a Primary Tropical Forest." *Forests* 12: 803. <https://doi.org/10.3390/f12060803>.
- Margalef, O., J. Sardans, M. Fernández-Martínez, R. Molowny-Horas, I. A. Janssens, P. Ciais, D. Goll, et al. 2017. "Global Patterns of Phosphatase Activity in Natural Soils." *Scientific Reports* 7: 1337. <https://doi.org/10.1038/s41598-017-01418-8>.
- Mayor, J. R., S. J. Wright, and B. L. Turner. 2014. "Species-Specific Responses of Foliar Nutrients to Long-Term Nitrogen and Phosphorus Additions in a Lowland Tropical Forest." *Journal of Ecology* 102(1): 36–44.
- Morley, R. J. 2000. *Origin and Evolution of Tropical Rain Forests*. New York, NY: Wiley.
- Nasto, M. K., B. B. Osborne, Y. Lekberg, G. P. Asner, C. S. Balzotti, S. Porder, P. G. Taylor, A. R. Townsend, and C. C. Cleveland. 2017. "Nutrient Acquisition, Soil Phosphorus Partitioning, and Competition among Trees in a Lowland Tropical Rain Forest." *New Phytologist* 3: 4243–55.
- Nasto, M. K., K. Winter, B. L. Turner, and C. C. Cleveland. 2019. "Nutrient Acquisition Strategies Augment Growth in Tropical N₂-Fixing Trees in Nutrient-Poor Soil and under Elevated CO₂." *Ecology* 100(4): e02646. <https://doi.org/10.1002/ecy.2646>.
- Ostertag, R., and N. M. DiManno. 2016. "Detecting Terrestrial Nutrient Limitation: A Global Meta-Analysis of Foliar Nutrient Concentrations after Fertilization." *Frontiers in Earth Science* 4: 2296–6463.
- Pan, Y., R. A. Birdsey, J. Fang, R. Houghton, P. E. Kauppi, W. A. Kurz, O. L. Phillips, et al. 2011. "A Large and Persistent Carbon Sink in the world's Forests." *Science* 333: 988–93.
- Penuelas, J., M. Fernández-Martínez, H. Vallicrosa, J. Maspons, P. Zuccarini, J. Carnicer, T. G. M. Sanders, et al. 2020. "Increasing Atmospheric CO₂ Concentrations Correlate with Declining Nutritional Status of European Forests." *Commun Biol* 3: 125. <https://doi.org/10.1038/s42003-020-0839-y>.
- Perakis, S., and L. Hedin. 2002. "Nitrogen Loss from Unpolluted South American Forests Mainly Via Dissolved Organic Compounds." *Nature* 415: 416–9.
- Poorter, H. 1993. "Interspecific Variation in the Growth Response of Plants to an Elevated Ambient CO₂ Concentration." In *CO₂ and Biosphere. Advances in Vegetation Science*, Vol 14, edited by J. Rozema, H. Lambers, S. C. van de Geijn, and M. L. Cambridge. Dordrecht: Springer. https://doi.org/10.1007/978-94-011-1797-5_6.
- Prospero, J. M., A. E. Barkley, C. J. Gaston, A. Gatineau, A. Campos y Sansano, and K. Panechou. 2020. "Characterizing and Quantifying African Dust Transport and Deposition to South America: Implications for the Phosphorus Budget in the Amazon Basin." *Global Biogeochemical Cycles* 34: e2020GB006536. <https://doi.org/10.1029/2020GB006536>.
- Quesada, C. A., J. Lloyd, L. O. Anderson, N. M. Fyllas, M. Schwarz, and C. Czimczik. 2011. "Soils of Amazonia with Particular Reference to the RAINFOR Sites." *Biogeosciences* 8: 1415–40. <https://doi.org/10.5194/bg-8-1415-2011>.
- Raven, J. A. 2015. "Interactions between Nitrogen and Phosphorus Metabolism." *Annual Plant Reviews* 48: 187–214. <https://doi.org/10.1002/9781118958841.ch7>.
- Reichert, T., A. Rammig, L. Fuchslueger, L. F. Lugli, C. A. Quesada, and K. Fleischer. 2022. "Plant Phosphorus-Use and -Acquisition Strategies in Amazonia." *The New Phytologist* 234: 1126–43. <https://doi.org/10.1111/nph.17985>.
- Reis, C. R., F. S. Pacheco, S. C. Reed, G. Tejada, G. B. Nardoto, M. C. Forti, and J. P. Ometto. 2020. "Biological Nitrogen Fixation across Major Biomes in Latin America: Patterns and Global Change Effects." *Science of the Total Environment* 746: 140998. <https://doi.org/10.1016/j.scitotenv.2020.140998>.
- Schlesinger, W. H. 1997. *Biogeochemistry: An Analysis of Global Change*. San Diego, Calif: Academic Press.
- Steidinger, B. S., T. W. Crowther, J. Liang, M. E. Van Nuland, G. D. Werner, P. B. Reich, G. J. Nabuurs, et al. 2019. "Climatic Controls of Decomposition Drive the Global Biogeography of Forest-Tree Symbioses." *Nature* 569: 404–8. <https://doi.org/10.1038/s41586-019-1128-0>.
- Sterner, R. W., and J. J. Elser. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Woodstock, UK: Princeton University Press. <http://www.jstor.org/stable/j.ctt1jktrp3>.
- Sullivan, B. W., S. Alvarez-Clare, S. C. Castle, S. Porder, S. C. Reed, L. Schreag, A. R. Townsend, and C. C. Cleveland. 2014. "Assessing Nutrient Limitation in Complex Forested Ecosystems: Alternatives to Large-Scale Fertilization Experiments." *Ecology* 95: 668–81. <https://doi.org/10.1890/13-0825.1>.
- Tanner, E., V. Kapos, S. Freskos, J. Healey, and A. Theobald. 1990. "Nitrogen and Phosphorus Fertilization of Jamaican Montane Forest Trees." *Journal of Tropical Ecology* 6(2): 231–8. <https://doi.org/10.1017/S0266467400004375>.
- Tedersoo, L., L. Laanisto, S. Rahimlou, A. Toussaint, T. Hallikma, and M. Pärtel. 2018. "Global Database of Plants with Root-Symbiotic Nitrogen Fixation: NodDB." *Journal of Vegetation Science* 29: 560–8. <https://doi.org/10.1111/jvs.12627>.
- Terrer, C., R. B. Jackson, I. C. Prentice, T. F. Keenan, C. Kaiser, S. Vicca, J. B. Fisher, et al. 2019. "Nitrogen and Phosphorus Constrain the CO₂ Fertilization of Global Plant Biomass." *Nature Climate Change* 9: 684–9. <https://doi.org/10.1038/s41558-019-0545-2>.
- Treseder, K. K., and P. M. Vitousek. 2001. "Effects of Soil Nutrient Availability on Investment in Acquisition of N and P in Hawaiian Rain Forests." *Ecology* 82(4): 946–54.
- Trummer, K., M. Bertzky, B. Dickson, G. van der Heijden, M. Jenkins, and P. Manning. 2009. *The Natural Fix? The Role of Ecosystems in Climate Mitigation*. A UNEP Rapid Response Assessment. Cambridge, UK: United Nations Environment Programme, UNEP WCMC.
- Urbina, I., O. Grau, J. Sardans, O. Margalef, G. Peguero, D. Asensio, J. LLusià, et al. 2021. "High Foliar K and P Resorption

- Efficiencies in Old-Growth Tropical Forests Growing on Nutrient-Poor Soils." *Ecology and Evolution* 11: 8969–82. <https://doi.org/10.1002/ece3.7734>.
- Vallicrosa, H. 2023. "helenavallicrosa/FG_leaves: v1.0.0 (FG_N+P_fertilization_leaves+growth.v1)." Zenodo. <https://doi.org/10.5281/zenodo.7781944>.
- Vallicrosa, H., J. Sardans, J. Maspons, and J. Peñuelas. 2022. "Global distribution and drivers of forest biome foliar nitrogen to phosphorus ratios (N:P)." *Global Ecology and Biogeography* 31: 861–71.
- Van Langenhove, L., T. Depaepe, L. T. Verryckt, H. Vallicrosa, L. Fuchslueger, L. F. Lugli, L. Brechet, et al. 2021. "Impact of Nutrient Additions on Free-Living Nitrogen Fixation in Litter and Soil of Two French-Guianese Lowland Tropical Forests." *Journal of Geophysical Research Biogeosciences* 126: e2020JG006023. <https://doi.org/10.1029/2020JG006023>.
- Van Langenhove, L., T. Depaepe, S. Vicca, J. van den Berge, C. Stahl, E. Courtois, J. Weedon, et al. 2019. "Regulation of Nitrogen Fixation from Free-Living Organisms in Soil and Leaf Litter of Two Tropical Forests of the Guiana Shield." *Plant and Soil* 450: 93–110. <https://doi.org/10.1007/s11104-019-04012-1>.
- Van Langenhove, L., I. A. Janssens, L. Verryckt, L. Brechet, I. P. Hartley, C. Stahl, E. Courtois, et al. 2020. "Rapid root assimilation of added phosphorus in a lowland tropical rainforest of French Guiana." *Soil Biology and Biochemistry* 140: 107646.
- Van Langenhove, L., L. T. Verryckt, L. Bréchet, E. A. Courtois, C. Stahl, F. Hofhansl, M. Bauters, et al. 2020. "Atmospheric Deposition of Elements and its Relevance for Nutrient Budgets of Tropical Forests." *Biogeochemistry* 149: 175–93. <https://doi.org/10.1007/s10533-020-00673-8>.
- Vance, C. P., C. Uhde-Stone, and D. L. Allan. 2003. "Phosphorus Acquisition and Use: Critical Adaptations by Plants for Securing a Nonrenewable Resource." *New Phytologist* 157: 423–47. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>.
- Verryckt, L. T., S. Vicca, L. van Langenhove, C. Stahl, D. Asensio, I. Urbina, R. Ogaya, et al. 2022. "Vertical Profiles of Leaf Photosynthesis and Leaf Traits and Soil Nutrients in Two Tropical Rainforests in French Guiana before and after a 3-Year Nitrogen and Phosphorus Addition Experiment." *Earth System Science Data* 14(1): 5–18. <https://doi.org/10.5194/essd-14-5-2022>.
- Vicca, S., S. Luyssaert, J. Peñuelas, M. Campioli, F. S. Chapin, III, P. Ciais, A. Heinemeyer, et al. 2012. "Fertile Forests Produce Biomass more Efficiently." *Ecology Letters* 15: 520–6.
- Vitousek, P. M. 1984. "Litterfall, Nutrient Cycling, and Nutrient Limitation in Tropical Forests." *Ecology* 65: 285–98. <https://doi.org/10.2307/1939481>.
- Vitousek, P. M., and H. Farrington. 1997. "Nutrient Limitation and Soil Development: Experimental Test of a Biogeochemical Theory." *Biogeochemistry* 37: 63–75. <https://doi.org/10.1023/A:1005757218475>.
- Vitousek, P. M., S. Porder, B. Z. Houlton, and O. A. Chadwick. 2010. "Terrestrial Phosphorus Limitation: Mechanisms, Implications and Nitrogen–Phosphorus Interactions." *Ecological Applications* 20: 5–15.
- Vitousek, P. M., and R. L. Sanford, Jr. 1986. "Nutrient Cycling in Moist Tropical Forest." *Annual Review of Ecology and Systematics* 17(1): 137–67.
- Walker, T. W., and J. K. Syers. 1976. "Fate of Phosphorus during Pedogenesis." *Geoderma* 15: 1–19.
- Wang, R., D. Goll, Y. Balkanski, D. Hauglustaine, O. Boucher, P. Ciais, I. Janssens, et al. 2017. "Global Forest Carbon Uptake Due to Nitrogen and Phosphorus Deposition from 1850 to 2100." *Global Change Biology* 23: 4854–72.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. New York: Springer-Verlag.
- Wieder, W. R., C. C. Cleveland, W. K. Smith, and K. Todd-Brown. 2015. "Future Productivity and Carbon Storage Limited by Terrestrial Nutrient Availability." *Nature Geoscience* 8: 441–4. <https://doi.org/10.1038/ngeo2413>.
- Wright, S. J. 2019. "Plant Responses to Nutrient Addition Experiments Conducted in Tropical Forests." *Ecological Monographs* 89(4): e01382. <https://doi.org/10.1002/ecm.1382>.
- Wright, S. J., B. L. Turner, J. B. Yavitt, K. E. Harms, M. Kaspari, E. V. J. Tanner, J. Bujan, et al. 2018. "Plant Responses to Fertilization Experiments in Lowland, Species-Rich, Tropical Forests." *Ecology* 99: 1129–38. <https://doi.org/10.1002/ecy.2193>.
- Wright, S. J., J. B. Yavitt, N. Wurzbarger, B. L. Turner, E. V. J. Tanner, E. J. Sayer, L. S. Santiago, et al. 2011. "Potassium, Phosphorus, or Nitrogen Limit Root Allocation, Tree Growth, or Litter Production in a Lowland Tropical Forest." *Ecology* 92: 1616–25. <https://doi.org/10.1890/10-1558.1>.

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