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Research article

Contrasting responses of fine root biomass and traits to large-scale nitrogen and phosphorus addition in tropical forests in the Guiana shield

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Fine roots mediate plant nutrient acquisition and growth. Depending on soil nutrient availability, plants can regulate fine root biomass and morphological traits to optimise nutrient acquisition. Little is known, however, about the importance of these parameters influencing forest functioning. In this study, we measured root responses to nutrient additions to gain a mechanistic understanding of plant adaptations to nutrient limitation in two tropical forests in French Guiana, differing twofold in their soil nutrient statuses. We analysed the responses of root biomass, mean root diameter (RD), specific root length (SRL), specific root area (SRA), root tissue density (RTD) and carbon (C), nitrogen (N) and phosphorus (P) concentrations in roots down to 15 cm soil depth after three years of N and P additions. At the lower-fertility site Paracou, no changes in root biomass or morphological traits were detected with either N or P addition, although P concentrations in roots increased with P addition. In the higher fertility site, Nouragues, root biomass and P concentrations in roots increased with P addition, with no changes in morphological traits. In contrast, N addition shifted root traits from acquisitive to more conservative by increasing RTD. A significant interaction between N and P in Nouragues pointed to stronger responses to P addition in the absence of N. Our results suggest that the magnitude and direction of root biomass and trait expression were regulated by soil fertility, corroborated by the response to N or P additions. At low fertility sites, we found lower plasticity in root trait expression compared to more fertile conditions, where N and P additions caused stronger and

antagonistic responses. Identifying the exact role of mechanisms affecting root nutrient uptake in Amazon forests growing in different soils will be crucial to foresee if and how rapid global changes can affect their carbon allocation.

Keywords: Amazon rainforest, experimental nutrient manipulation, fine root biomass stocks, nutrient limitation, root morphological traits, soil fertility

Introduction

Tropical forests contribute more than one-third to global terrestrial gross primary productivity (Beer et al. 2010), playing a crucial role in global carbon (C) cycling and storage. Despite the significance of Amazon forests for the global C cycle (Le Quéré et al. 2018), their actual role as a future sink or source of C facing increased atmospheric CO₂ concentrations (Terrer et al. 2019, Fleischer and Terrer 2022) or increased atmospheric nutrient deposition (Galloway et al. 2004, Wang et al. 2017) is still under debate. We need, however, a better understanding of factors limiting tropical forest growth, driving plant biomass allocation and consequently tropical forest C cycling and long-term C storage (Walker et al. 2021), in particular belowground (Obersteiner and Klein 2022). Belowground, fine roots (< 2 mm diameter) are important mediators of water, nutrient and C cycling (Cleveland et al. 2011, Cusack et al. 2021). Tropical forests display high fine root biomass stocks, root productivity and turnover (Finér et al. 2011, Wang et al. 2018), with fine roots accounting for 5–49% of total net primary productivity (NPP) across Pantropical lowland forests (Huaraca Huasco et al. 2021). Still, most knowledge about links and feedbacks between above and belowground forest functioning and how they change in a future climate stem from temperate forests (Wang et al. 2021).

Global patterns of soil nutrient availability predict that tropical forest NPP is rather limited by P than by nitrogen (N) (Wang et al. 2010, Du et al. 2020). While N derives from atmospheric deposition and biological N₂ fixation and accumulates with soil development, P is almost exclusively derived from parent rock material and declines over geological time scales due to continuous soil weathering (Walker and Syers 1976). Hence, in old and highly weathered tropical soils, such as the many soils across the Amazon basin, low P availability might play a crucial role in affecting forests' C allocation pattern and modulate their responses to elevated CO₂ and other global changes (Ainsworth and Long 2005, Norby et al. 2016, Peñuelas et al. 2017). Understanding how, in a hyper-diverse ecosystem such as tropical rainforests, plants can acquire and use those nutrients is critical to predicting tropical forest responses to environmental change and their actual role in a future climate (Goll et al. 2012). Nonetheless, our knowledge of how environmental properties affect fine root trait expression and plasticity remains limited.

To acquire nutrients, plants invest not only in root biomass but can also, to a certain extent, adjust their trait expression at a certain C cost (Aerts and Chapin 1999, McCormack and Iversen 2019). Such root traits (e.g. morphology,

architecture, exudation of compounds) and symbiotic associations with fungi and bacteria tend to reflect a plant's environment aiming to balance C investments and nutrient uptake efficiency (Freschet et al. 2021). For instance, in low fertility soils, plants tend to allocate more C belowground and invest in root biomass (Huaraca Huasco et al. 2021) and display more conservative traits, i.e. greater diameter (RD) and tissue density (RTD) but lower specific root length (SRL) and area (SRA), which results in lower autotrophic respiration rates and increased root longevity (Ushio et al. 2015, Weemstra et al. 2016, Reichert et al. 2022). On the other end of the spectrum, in higher fertility soils, root biomass tends to be lower and fine roots display more acquisitive traits, being thinner and longer, with higher nutrient tissue concentrations, which in turn results in higher respiration costs, higher turnover and short lifespan (McCormack et al. 2015, Cusack et al. 2021). The highly discussed 'root economics' spectrum' (Kong et al. 2016, Kramer-Walter et al. 2016, Weemstra et al. 2016, Bergmann et al. 2020) points out, however, that root traits do not necessarily converge along a single axis from conservative to acquisitive traits along soil resource availability (Dallstream et al. 2022). The ecological significance of soil gradient studies might be constrained as they provide limited insights into root trait plasticity, as other environmental properties such as soil physical properties and tree species composition that evolved with time also impact forest dynamics (Soong et al. 2020), and root biomass allocation (Huaraca Huasco et al. 2021).

Despite the complex interplay between environmental properties and root trait expression, recent studies in tropical forests have found evidence for root trait plasticity controlled by soil nutrient availability (Wurzburger and Wright 2015, Wright et al. 2018, Lugli et al. 2021, Cunha et al. 2022). Large-scale manipulation experiments testing nutrient limitations in mature tropical forests showed that both N and P (Alvarez-Clare et al. 2013, Wright et al. 2018), but also potassium (K) (Wright et al. 2011, Sardans and Peñuelas 2015, Wurzburger and Wright 2015, Manu et al. 2022) and other cations (Lugli et al. 2021, Bauters et al. 2022) affected different aspects of tropical forest functioning. Fine root responses to nutrient manipulation have, however, yielded mixed responses so far (Yavitt et al. 2011, Alvarez-Clare and Mack 2015, Wurzburger and Wright 2015, Wright et al. 2018, Lugli et al. 2021). For instance, the addition of N+P+K decreased root biomass, and RTD and SRL from standing stock roots in forests in Panama (Wright et al. 2011, Yavitt et al. 2011, Wurzburger and Wright 2015). In another fertilisation experiment in central Amazon, no responses to N addition were detected, but P addition stimulated fine root productivity and increased fine root diameter using roots from

ingrowth cores (Lugli et al. 2021). Since N and P are part of a wide range of plant physiological and metabolic processes, soil and plant nutrient stoichiometry and the antagonistic or synergistic interactions between N and P (Marklein and Houlton 2012, Nasto et al. 2019, Toro et al. 2022) could determine the potential for alleviation or exacerbation of nutrient limitation in forests growing in soils of different fertility.

Here, we aimed to understand the consequences of nutrient limitation affecting root nutrient acquisition mechanisms by conducting a large-scale nutrient manipulation experiment in two tropical forests in French Guiana varying two-fold in their soil nutrient statuses (Van Langenhove et al. 2020a) and consequently in tree species richness and diversity (Peguero et al. 2023). After three years of experiment, we specifically tested the potential effects of alleviating soil N and P limitations on various root properties, including root biomass, morphological traits and nutrient contents in root tissues. We hypothesised that if N or P were limiting nutrients in these soils, root traits would change in response to the respective nutrient addition. More specifically, we expected that P additions would prompt stronger responses than N additions, but the magnitude and direction of the response would depend on forest nutrient status. At the lower-fertility site, fine root traits would shift towards more acquisitive traits, with decreased root biomass, RD, RTD and increasing SRL and SRA as well as nutrient content of root tissues. Since N is generally not limiting in tropical forest soils, we predicted that N addition would rather exacerbate P limitation, resulting in the diametrical trait responses as hypothesised for P additions, with fine roots shifting towards more conservative traits. Taken together, our results would enhance our understanding of how soil nutrient limitation affects fine root traits, which can be decisive for Amazonian forests responses to elevated atmospheric CO₂.

Material and methods

Site description

The study was conducted at two primary forest sites in French Guiana, in the research stations of Paracou and Nouragues (Bongers et al. 2001, Gourlet-Fleury et al. 2004). Paracou is situated 15 km from the coast, while Nouragues is located inland, with similar annual rainfall and temperature in both sites (Table 1) (Bongers et al. 2001, Gourlet-Fleury et al. 2004). Table 1 details site location, climate variables, tree species abundance and richness and soil properties. The French Guianese climate is characterised by a wet and a dry season due to the north/south movement of the Inter-Tropical Convergence Zone, with a dry period, typically characterised by less than 100 mm rainfall month⁻¹, from August to November. Soils at Paracou range from loamy sand to sandy loam (sand content = 71.63 ± 1.06%; clay content = 12.13 ± 0.69%), while soils at Nouragues contain more clay (sand content = 46.40 ± 2.50%; clay content = 29.03 ± 1.44%) and span the range of sandy loam to

silty clay (Van Langenhove et al. 2020a). In addition to soil physical properties differences, total contents of soil C, N and P are around twice as high in Nouragues than in Paracou (Table 1). Total species richness (Table 1) and diversity are higher in Nouragues than in Paracou, with different above-ground (Peguero et al. 2023) and belowground functional trait expression between sites (Table 1).

Nutrient addition experimental set-up

The nutrient addition experiment in both forest sites was initiated in October 2016. At each forest site, three blocks of four 50 × 50 m plots were selected (Supporting information; see Courtois et al. (2018) for more details). Each of the three blocks corresponded to different topographical levels, being classified as plateaus, slope and bottom with three plots each (total of 12 plots in Paracou and 12 plots in Nouragues; Courtois et al. 2018, Verryckt et al. 2022). In each block, one plot served as a control plot (no nutrients added), the remaining three plots received one of three nutrient addition treatments: +N, +P, or +NP. Fertiliser was applied twice per year, being half between mid-October and mid-November at the peak of the dry season and the other half in March, during a slightly dry period within the wet season. Application was done by hand-broadcasting commercial urea ((NH₂)₂CO) at a rate of 125 kg N ha⁻¹ year⁻¹ (+N treatment) and triple superphosphate (Ca(H₂PO₄)₂) at a rate of 50 kg P ha⁻¹ year⁻¹ (+P treatment), or both 125 kg N ha⁻¹ year⁻¹ and 50 kg P ha⁻¹ year⁻¹ together (+NP treatment). These amounts were chosen to enable comparison of our results with other tropical fertilisation experiments (Wright et al. 2011). Although our main goal was to determine the effects of the addition of N and P on fine root traits, triple superphosphate also contains about 15% of calcium (Ca) in its composition.

Soil sampling and chemical analyses

Five soil samples were collected per plot in the central 20 × 20 m area of each plot (12 plots per site) with a 15 cm long and 8 cm diameter auger. The five cores down to 15 cm depth were sampled in both the wet (May and June) and dry season (September and October) of 2019 and pooled per plot. Sieved soils were dried at 60°C to constant mass. Total C and N concentrations were determined by dry combustion and analysed by IRMS (EA1110, CE Instruments, coupled to Thermo Fisher Scientific). Total P concentrations were determined by acid digestion followed by ICP-MS analysis (7500ce model, Agilent Technologies). A complete description of soil chemical analysis can be found in Verryckt et al. (2022).

Fine root biomass stocks

Root sampling was conducted in the central 20 × 20 m area of each plot (12 plots per site). Inside this central area, five samples were collected with an 8 cm diameter soil core at two depths, 0–5 and 5–15 cm. Roots were sampled in both the wet and dry season of 2019. The roots were carefully removed,

using sieves and brushes, by wetting and placing running tap water to wash the soil, stones and organic matter. Clean roots from all soil layers were separated by diameter classes (< 2 ; > 2 mm). Only roots < 2 mm diameter (fine roots) from the 0–5 and 5–15 cm soil layer are discussed in this study. Fine roots were first scanned fresh as described below, and later dried at 70°C for 72 h. Fine root stocks were calculated in Mg ha^{-1} as the sum of the 0–15 cm soil layers per plot.

Carbon and nutrient concentrations and stocks in roots

Dried fine roots from the 0–5 and 5–15 cm soil layer were ground to pass a 0.2 mm sieve in an ultra-centrifugal mill (Model ZM 200, Retsch GmbH). Total C and N concentrations (%) were determined by dry combustion, based on the Dumas method using an elemental analyser (model FLASH 2000 NC Soil Analyser, Interscience). P concentration in roots was determined by acid digestion in an ultraWAVE digester, followed by ICP-MS (7500ce model, Agilent Technologies) analysis. Root C, N and P contents are reported as weighted average values for the 0–15 cm soil layer based on fine root biomass of each soil layer. Based on element concentration and fine root biomass stocks, we calculated C (Mg ha^{-1}), N (kg ha^{-1}) and P (kg ha^{-1}) stocks in roots for the 0–15 cm layer.

Root morphology

All fresh fine roots from the 0–5 cm and 5–15 cm soil layers were scanned at 600 dpi and images analysed using WinRHIZO (WinRHIZO Regular 2015, Regent Instruments) to provide root mean diameter, total length, area and volume. With these morphological parameters together with fine root dry weight we were able to determine specific root length (SRL), specific root area (SRA), root tissue density (RTD) and mean root diameter (RD) (Metcalfe et al. 2008). SRL (m g^{-1}) was calculated as root length per unit root dry mass, SRA ($\text{cm}^2 \text{g}^{-1}$) was calculated as root superficial area per unit dry mass and RTD (g cm^{-3}) was calculated as root dry mass per unit root volume. Root morphological traits are reported as weighted average values for the 0–15 cm soil layer based on fine root biomass of each soil layer.

Statistical analyses

To determine the effect of nutrient additions and their interactions in the factorial design $\text{N} \times \text{P}$, we used linear mixed models using the lmer function in R packages 'lme4' (www.r-project.org, Bates et al. 2014) and 'lmerTest' (Kuznetsova et al. 2017). The addition of N and P was used as a fixed factor, and topography (Bottom, Slope, Plateau) as well as seasons (Dry and Wet) as random factors (Vallicrosa et al. 2023) following the structure: $\text{lmer}(\text{variable}) \sim \text{P} \times \text{N} + (1|\text{Topo}) + (1|\text{Season})$. Full factorial models were simplified using backward elimination performed by the step function in 'lmerTest' package (Kuznetsova et al. 2015,

2017). The significant model was then re-run, and only the significant effects of nutrient additions and/or interactions were reported. When significant interactions between N and P addition occurred, we performed a pairwise comparison using the emmeans function (Lenth et al. 2019) and reported Bonferroni-adjusted p-values.

Since natural root trait distribution was consistently different between sites (Table 1), we discussed the effects of nutrient addition at each site separately, but we refrained from discussing differences between topographic levels and seasons due to our limited statistical power (no significant differences in root traits between seasons). Data from root biomass are shown as the sum of soil layers (0–15 cm depth) per plot, while data for morphological traits and element concentrations are shown as weighted means using root biomass of each layer to produce values for the 0–15 cm depth per plot. As a result, the sample size was 24 for each site (means of five points per plot in 12 plots and two seasons). We then represented the effect of nutrient addition by comparing plots where a specific nutrient was not added (i.e. $-\text{N}$; $n = 12$) and plots where that nutrient was added (i.e. $+\text{N}$; $n = 12$), or when significant, the interaction among N and P (i.e. $+\text{N}-\text{P}$ versus $-\text{N}-\text{P}$; $n = 6$) for each site separately (Lugli et al. 2021).

We used radar plots to visualise and discuss the trait space occupied by the combined response of all traits per site. Since all traits were in different units, we scaled them using the scale function in R, which normalises the data by subtracting from each value the sample mean and dividing it by the sample standard deviation per site and trait. The magnitude of post-scaling values indicates the number of standard deviations from the mean. All plots were created using the 'ggplot2' R package (Wickham et al. 2016). Data were checked for normality and variance homogeneity and all analyses were run using R ver. 4.1.2 (www.r-project.org).

Results

Soil element concentrations

Soil C, N and P concentrations were significantly lower in Paracou than in Nouragues (Table 1), but, after three years of nutrient addition, soil C concentrations in the upper 15 cm soil layer did not change in either site (Table 2). In contrast, soil N concentrations significantly increased (around 20%) in $+\text{N}$ plots for both Paracou ($F_{1,19} = 2.93$, $p = 0.008$) and Nouragues ($F_{1,20} = 4.65$, $p = 0.043$). Total P concentrations in soils almost doubled with $+\text{P}$ in both Paracou ($F_{1,18} = 63.39$, $p = 2.6^{-7}$), and Nouragues ($F_{1,20} = 31.82$, $p = 1.60^{-5}$). No significant interactions between N and P additions were captured for total soil element concentrations.

Root biomass, nutrient contents and stocks

Root biomass in the upper 15 cm of soil was naturally higher in Paracou than in Nouragues (Table 1), but neither the addition of N or P changed root biomass in Paracou

Table 1. Details of study site location, climate, tree species diversity and analysis of study site natural differences in soil properties and fine root biomass and traits. Mean \pm SE are presented. Tree species abundance and richness data from [Peguero et al. \(2023\)](#) (n = 24 plots; 12 plots per site). Soil and root data refer to samples from control-plots only (n = 6; three control plots per site in two seasons) collected in 2019. Study site differences in soil and root control-data were tested using site as a fixed effect term and topography and season as random effect factor. Statistical significance is denoted as . $p < 0.1$, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	Paracou	Nouragues
Lat/long coordinates	05°16'38"N, 52°55'38"W	04°04'53"N, 52°42'13"W
Main soil type (FAO)	Acrisols	Acrisols
Mean annual temperature	25.8°C	25.2°C
Mean annual precipitation	3150 mm	2874 mm
Dry season	3 months	3 months
Tree abundance per hectare	24.94 \pm 6.3	19.63 \pm 6.4
Tree species richness per hectare	8.77 \pm 2.9	10.33 \pm 3.9
Soil bulk density (g cm ⁻³)	1.18 \pm 0.12	0.94 \pm 0.12 .
Total soil C (%)	1.99 \pm 0.25	3.74 \pm 0.24 ***
Total soil N (%)	0.13 \pm 0.02	0.25 \pm 0.02 ***
Total soil P (mg kg ⁻¹)	62.99 \pm 27.81	114.16 \pm 26.27
Fine root biomass (Mg ha ⁻¹)	7.47 \pm 0.98	3.34 \pm 0.93 **
Root diameter (mm)	0.71 \pm 0.03	0.60 \pm 0.03 *
Specific root length (cm g ⁻¹)	503.93 \pm 66.23	793.65 \pm 60.46 *
Specific root area (cm ² g ⁻¹)	112.49 \pm 10.14	155.20 \pm 9.26 *
Root tissue density (g cm ⁻³)	0.49 \pm 0.02	0.43 \pm 0.02 .
Root N (%)	1.26 \pm 0.09	1.39 \pm 0.09 **
Root P (mg kg ⁻¹)	218.57 \pm 18.02	260.83 \pm 16.45
Root tissue N:P	58.74 \pm 6.34	54.63 \pm 6.08

(Fig. 1). In Nouragues, no effects of +N were detected, but root biomass significantly increased by 20% in +P plots (from 4.39 \pm 0.32 to 5.27 \pm 0.30 Mg ha⁻¹; $F_{1,20} = 5.04$, $p = 0.036$; Fig. 1).

Fine root C and N contents for the 0–15 cm layer did not change with nutrient addition for Paracou or Nouragues, also leading to no changes in root C:N ratios at both sites (Table 3). However, fine root P content was significantly higher in +P plots than in -P for both Paracou and Nouragues. In Paracou, +P resulted in root P content three times higher in comparison to -P ($F_{1,21} = 122.64$, $p = 3.15 \times 10^{-10}$), leading also to a significant decline in root C:P and N:P ratios (Table 3). In Nouragues, root P content significantly increased almost threefold in +P plots compared to -P ($F_{1,18} = 268.27$, $p = 2.93 \times 10^{-12}$), significantly reducing root C:P and N:P ratios (Table 3). In contrast, +N significantly decreased root P content by around 25% ($F_{1,18} = 29.85$, $p = 3.44 \times 10^{-5}$), significantly increasing root C:P and N:P ratios compared to -N plots (Table 3). Such antagonistic responses of N and P addition affecting root P content in Nouragues lead to a significant interaction between N and P treatments ($F_{1,18} = 15.71$,

$p = 0.0009$), where the effect of +P decreased when added in combination with N (Fig. 2).

Combining root biomass and element contents in fine roots, we calculated fine root nutrient stocks. Similar to patterns found for root biomass, C and N stocks in Paracou were not affected by nutrient addition (Table 4). P stocks in roots in Paracou significantly increased almost fourfold in +P plots ($F_{1,20} = 104.54$, $p = 2.17 \times 10^{-9}$) but declined about 25% in +N plots ($F_{1,20} = 5.92$, $p = 0.02$), with no significant interaction between N and P. In contrast, in Nouragues, +P significantly increased C ($F_{1,18} = 6.79$, $p = 0.017$), and N ($F_{1,18} = 5.74$, $p = 0.027$) stored in roots compared to -P (Table 4).

Moreover, there was a significant interaction between N and P affecting C and N stocks in roots in Nouragues (Fig. 3a–b), indicating overall greater stocks of C and N in roots when P was added alone. In Nouragues, P stocks in roots were 3.5 times higher in +P plots ($F_{1,19} = 204.76$, $p = 1.26 \times 10^{-11}$) but declined about 30% in +N plots ($F_{1,19} = 28.07$, $p = 4.10 \times 10^{-5}$), with a significant interaction between N and P ($F_{1,19} = 30.85$, $p = 2.34 \times 10^{-5}$), pointing to overall greater P stocks in roots when P was added alone (Fig. 3c).

Table 2. Mean and standard errors of carbon (C), nitrogen (N) and total phosphorus (P) concentrations in soils in Paracou (PAR) and Nouragues (NOU) for the mean 0–15 cm soil layer after three years of nutrient addition in French Guiana. Values in bold mean statistically significant differences ($p < 0.05$) between treatments with and without a specific nutrient addition per study site (n = 12 per treatment and site).

		Treatments			
		-N	+N	-P	+P
PAR	C (%)	2.02 \pm 0.13	2.19 \pm 0.09	2.11 \pm 0.09	2.10 \pm 0.13
	N (%)	0.13 \pm 0.007	0.15 \pm 0.007	0.14 \pm 0.007	0.14 \pm 0.008
	P (mg kg ⁻¹)	110.15 \pm 15.00	126.40 \pm 12.70	84.80 \pm 10.88	149.63 \pm 8.90
NOU	C (%)	3.63 \pm 0.18	4.07 \pm 0.33	4.09 \pm 0.33	3.61 \pm 0.18
	N (%)	0.24 \pm 0.013	0.28 \pm 0.018	0.28 \pm 0.020	0.25 \pm 0.012
	P (mg kg ⁻¹)	189.72 \pm 39.25	229.11 \pm 48.20	140.18 \pm 24.51	278.65 \pm 49.61

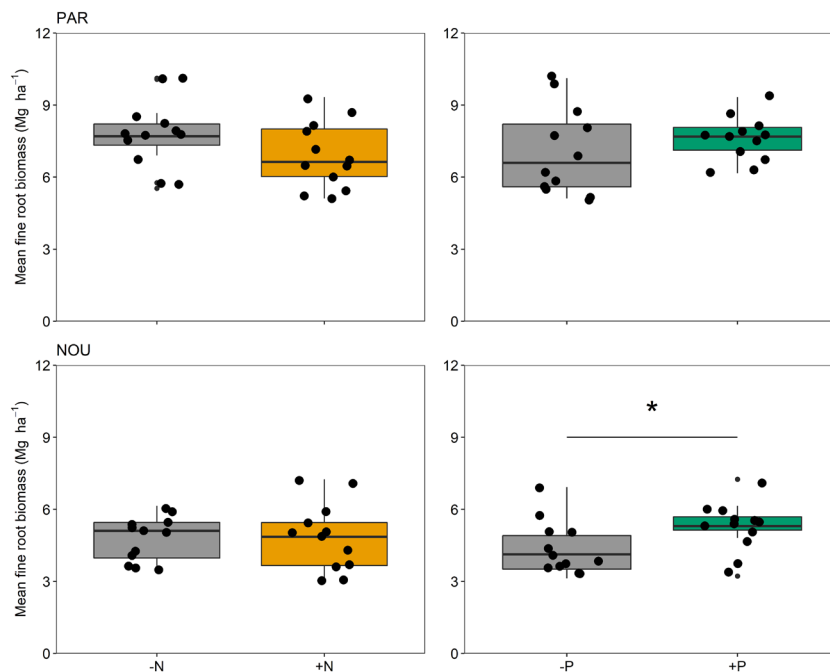


Figure 1. Fine root biomass stocks (Mg ha^{-1}) for the 0–15 cm soil layer in Paracou (PAR) and Nouragues (NOU) for the 0–15 cm soil layer with or without the addition of nitrogen (N) and phosphorus (P) in French Guiana. Only significant effects are shown and are indicated by *, ** and ***, representing p -values < 0.05, 0.01 and 0.001, respectively ($n = 12$ per treatment and site).

Since the P-fertiliser also contains significant amounts of cations (Ca, magnesium (Mg) and K), we tested the effects of +N and +P on cation content and stocks in fine roots (Supporting information). In Paracou, +P also increased root Ca content ($F_{1,20} = 38.34$, $p = 4.76 \times 10^{-6}$) and Ca and K stocks ($F_{1,22} = 34.03$, $p = 7.19 \times 10^{-6}$ and $F_{1,17} = 14.1$, $p = 0.0016$, respectively) whilst +N significantly decreased Mg and K contents ($F_{1,21} = 22.5$, $p = 0.00010$ and $F_{1,19} = 24.57$, $p = 8.75 \times 10^{-5}$, respectively) and stocks ($F_{1,19} = 22.28$, $p = 0.00015$ and $F_{1,17} = 64.93$, $p = 3.30 \times 10^{-7}$, respectively). In Nouragues, +P increased Ca contents and stocks in roots ($F_{1,18} = 4.63$, $p = 0.045$ and $F_{1,19} = 7.35$, $p = 0.013$, respectively). While, Mg and K contents remained unaffected, Mg and K

stocks significantly increased ($F_{1,20} = 10.28$, $p = 0.004$ and $F_{1,18} = 7.50$, $p = 0.013$, respectively; Supporting information). The addition of +N significantly decreased Ca, Mg and K contents ($F_{1,18} = 11.69$, $p = 0.003$, $F_{1,20} = 25.77$, $p = 5.76 \times 10^{-5}$ and $F_{1,20} = 8.14$, $p = 0.009$, respectively) and stocks in roots ($F_{1,19} = 6.46$, $p = 0.02$, $F_{1,20} = 16.41$, $p = 0.0006$ and $F_{1,18} = 6.96$, $p = 0.016$, respectively).

Root morphology

In Paracou, neither N nor P addition caused a significant change in any of the four root morphological traits measured after three years of fertilisation (Table 5). In Nouragues,

Table 3. Mean and standard errors of carbon (C; %), nitrogen (N; %) and phosphorus (P; mg kg^{-1}) contents, C:N, C:P and N:P ratios in roots in Paracou (PAR) and Nouragues (NOU) for the mean 0–15 cm soil layer with or without the addition of N and P in French Guiana. Values in bold mean statistically significant differences ($p < 0.05$) between treatments with and without a specific nutrient addition per study site ($n = 12$ per treatment and site).

		Treatments			
		-N	+N	-P	+P
PAR	C (%)	44.26 ± 0.21	44.61 ± 0.23	44.48 ± 0.27	44.39 ± 0.16
	N (%)	1.20 ± 0.04	1.23 ± 0.03	1.24 ± 0.03	1.19 ± 0.04
	P (mg kg^{-1})	519.95 ± 97.83	436.07 ± 69.50	216.82 ± 8.55	739.21 ± 47.37
	C:N	37.19 ± 1.26	36.62 ± 1.03	36.08 ± 0.91	37.73 ± 1.31
	C:P	1301 ± 226	1417 ± 237	2092 ± 95	626 ± 38
	N:P	36.06 ± 6.66	39.04 ± 6.70	58.40 ± 3.08	16.70 ± 1.02
NOU	C (%)	46.83 ± 0.95	46.72 ± 0.61	46.21 ± 0.49	47.34 ± 0.99
	N (%)	1.38 ± 0.04	1.40 ± 0.04	1.39 ± 0.03	1.39 ± 0.04
	P (mg kg^{-1})	542.02 ± 89.07	388.72 ± 55.53	235.59 ± 10.79	695.15 ± 48.66
	C:N	34.21 ± 1.05	33.68 ± 1.06	33.43 ± 0.86	34.46 ± 1.20
	C:P	1210 ± 199	1519 ± 207	2007 ± 94.37	722 ± 56.70
	N:P	35.50 ± 5.82	45.88 ± 6.75	60.52 ± 3.35	20.86 ± 1.27

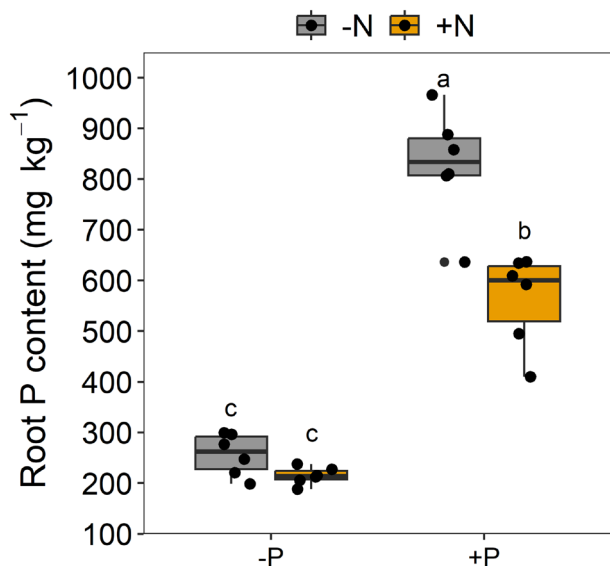


Figure 2. Effects of the interaction between the addition of phosphorus (P; x-axis) and nitrogen (N; coloured boxplots) affecting fine root P contents (mg kg^{-1}) in Nouragues (NOU) for the mean 0–15 cm soil layer ($n=6$ for each interaction). Differences between pairwise comparisons among treatment levels are denoted by letters. Means sharing the same letter are not significantly different ($p\text{-value} > 0.05$).

no significant differences were observed for mean RD with +N or +P (Table 5), but a significant interaction between N and P was detected ($F_{1,19}=8.73$, $p=0.008$), resulting in only a marginally non-significant difference between $-N-P$ and $+N-P$ (mean RD 0.59 ± 0.011 and 0.64 ± 0.016 respectively; $p=0.057$). For this site, +N alone marginally decreased SRL when compared to $-N$ plots ($F_{1,16}=4.18$, $p=0.056$), but also a significant interaction between N and P was detected ($F_{1,16}=19.38$, $p=0.0004$), with such a trend of decreasing SRL being stronger in $+N-P$ plots (Fig. 4a). For SRA, no significant changes were captured after +P, with only a marginal decrease with +N ($F_{1,18}=4.12$, $p=0.057$; Table 5), but with a significant interaction between both elements ($F_{1,18}=7.82$, $p=0.012$), also resulting in lower SRA in $+N-P$ plots (Fig. 4b). For RTD, +N significantly increased RTD in Nouragues ($F_{1,19}=5.76$, $p=0.027$; Table 5), with no effects of +P or the interaction NP.

When data were normalised to account for background differences at each site, we found that both sites responded

proportionally similarly to P addition (Fig. 5), mainly driven by changes in root tissue stoichiometry, followed by increasing root biomass, but little or no responses of root morphological traits. Since no changes in N content in roots were captured in our treatments, the strong responses of N:P ratios were solely driven by changes in P content in roots. Differently to patterns seen with P addition, N addition rather triggered changes in root morphological traits, that differed depending on site (Fig. 5) dominated by a tendency of reduction in root biomass in Paracou and changes in tissue construction (RTD) in Nouragues.

Discussion

In this study, we demonstrate that in the less fertile, lower species diversity forest in Paracou, fine root trait responses were significantly less plastic than in the more fertile, but also more diverse forest in Nouragues. Interestingly, at both sites, root traits responded diametrically to N versus P additions, with the magnitude of most responses to P addition in Nouragues being controlled by the interaction with N.

Root biomass stocks increased with P addition in the more fertile site

Fine root biomass tends to decrease with increasing fertility in natural forests, reflecting less investment in nutrient uptake strategies (Jiménez et al. 2009, Kochsiek et al. 2013, Cusack et al. 2021). Similar patterns were found in a large-scale fertilisation experiment in Panama where the addition of K and N+P+K decreased root biomass after 4 years and 14 years of experiment duration, respectively (Wright et al. 2011, Yavitt et al. 2011, Wurzbarger and Wright 2015). Contrary to these trends and our hypothesis, nutrient additions did not change root biomass in Paracou, and in Nouragues, root biomass even increased with P addition.

At the tree community-level, three years of N and P addition stimulated stem growth in this fertilisation experiment (Vallicrosa et al. 2023), but only in the more fertile site Nouragues (Supporting information) highlighting differences in whole-plant biomass with N and P at this site. In Paracou, however, we can conclude that neither the addition of N or P elicited whole-plant level responses, except for tissue stoichiometry (Table 3, Supporting information).

Table 4. Mean and standard errors of carbon (C; Mg ha^{-1}), nitrogen (N; kg ha^{-1}) and phosphorus (P; kg ha^{-1}) stocks in roots in Paracou (PAR) and Nouragues (NOU) for the mean 0–15 cm soil layer with or without the addition of N and P. Values in bold mean statistical significant differences ($p < 0.05$) between treatments with and without a specific nutrient addition per study site ($n=12$ per treatment and site).

		Treatments			
		-N	+N	-P	+P
PAR	C (Mg ha^{-1})	3.45 ± 0.18	3.10 ± 0.19	3.16 ± 0.24	3.39 ± 0.12
	N (kg ha^{-1})	93.89 ± 6.36	85.69 ± 5.96	88.59 ± 7.99	90.99 ± 3.87
	P (kg ha^{-1})	4.14 ± 0.83	3.12 ± 0.57	1.49 ± 0.08	5.78 ± 0.46
NOU	C (Mg ha^{-1})	2.26 ± 0.15	2.28 ± 0.18	$2.04 \pm 0.0.16$	2.50 ± 0.15
	N (kg ha^{-1})	65.69 ± 4.08	66.50 ± 4.41	60.29 ± 3.78	71.80 ± 3.96
	P (kg ha^{-1})	2.78 ± 0.54	1.83 ± 0.28	1.03 ± 0.09	3.58 ± 0.33

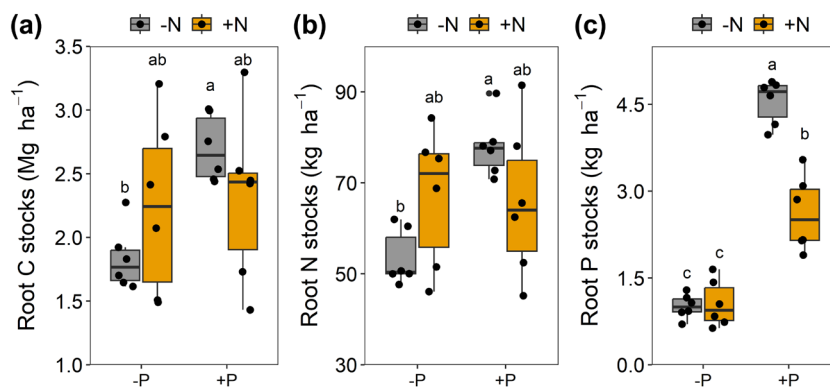


Figure 3. Interactive effects between the addition of phosphorus (P; x-axis) and nitrogen (N; coloured boxplots) affecting (A) fine root carbon (C; Mg ha^{-1}), (B) nitrogen (N; kg ha^{-1}) and (C) phosphorus (P; kg ha^{-1}) stocks in Nouragues (NOU) for the mean 0–15 cm soil layer ($n=6$ for each interaction). Differences between pairwise comparisons among treatment levels are denoted by letters. Means sharing the same letter are not significantly different (p -value > 0.05).

In Nouragues, a coordinated whole plant response to P resulted in greater investment in stems and fine roots up to 15 cm depth. Nonetheless, N addition resulted in greater stem growth with no changes in root biomass in Nouragues, indicating preferential aboveground biomass allocation when N is plenty (Supporting information). While most fine root biomass in these forests is typically located in superficial soil layers (Cordeiro et al. 2020), long-term nutrient addition could also impact roots deeper than 15 cm.

Recent studies have also pointed to differential nutrient limitation among plant functional types (Zalamea et al. 2016, Toro et al. 2022, Vallicrosa et al. 2023). The distinct soil conditions in Paracou and Nouragues that, therefore, promote different tree communities (Peguero et al. 2023), might influence some of the contrasting effects that we captured with nutrient addition among sites (Vallicrosa et al. 2023). Aboveground, slow stem growth and lower species richness in Paracou (Peguero et al. 2023) could help explain the lack of responses in root biomass. In contrast, faster tree growth and higher species richness could drive stronger community-level root responses in Nouragues. Nonetheless, we lack direct evidence of whether tree species with more acquisitive traits are more or less plastic to changes in soil nutrient concentrations, especially in their root trait expression.

Since our P fertiliser has cations in its composition and that +P also resulted in higher Ca concentrations in fine roots in both study sites, we cannot discard the possibility that Ca might be shaping belowground plant dynamics. Similarly, in other long-term manipulation experiments, K also appears as a limiting element to plant function in tropical forests (reviewed by Wright et al. 2019), although the direction of responses found in our study was contrary to the ones already reported from other manipulation experiments. In central Amazon, a combination of cations (Ca, Mg, K) addition increased short-term root productivity, but such effect did not last, with P alone resulting in stronger responses for both belowground (Lugli et al. 2021) and aboveground productivity (Cunha et al. 2022). Although cations might also be limiting in very weathered soils in tropical forests, we cannot, up to now, explicitly determine the effect of Ca alone on tree growth and trait expression. Nonetheless, we demonstrated that already after three years of nutrient addition, we were able to capture belowground plant community responses even in such hyper-diverse forests that could point to 1) luxurious root biomass production due to high availability of P; 2) lower root mortality or greater root longevity and/or 3) increased limitation by other resources, such as other soil nutrients or water, where

Table 5. Mean and standard errors of root diameter (RD; mm), specific root length (SRL; m g^{-1}), specific root area (SRA; $\text{cm}^2 \text{g}^{-1}$) and root tissue density (RTD; g cm^{-3}) in Paracou (PAR) and Nouragues (NOU) for the 0–15 cm soil layer with or without the addition of nitrogen (N) and phosphorus (P). Values in bold mean statistically significant differences ($p < 0.05$) between treatments with and without a specific nutrient addition per study site ($n=12$ per treatment and site).

		Treatments			
		-N	+N	-P	+P
PAR	RD (mm)	0.70 ± 0.02	0.73 ± 0.02	0.73 ± 0.02	0.70 ± 0.02
	SRL (m g^{-1})	5.33 ± 0.27	4.88 ± 0.18	5.05 ± 0.24	5.15 ± 0.25
	SRA ($\text{cm}^2 \text{g}^{-1}$)	118.19 ± 4.54	112.37 ± 3.50	114.43 ± 3.70	116.14 ± 4.53
	RTD (g cm^{-3})	0.48 ± 0.01	0.49 ± 0.02	0.48 ± 0.01	0.49 ± 0.02
NOU	RD (mm)	0.61 ± 0.01	0.62 ± 0.01	0.62 ± 0.01	0.61 ± 0.01
	SRL (m g^{-1})	7.35 ± 0.28	6.76 ± 0.32	6.91 ± 0.35	7.20 ± 0.26
	SRA ($\text{cm}^2 \text{g}^{-1}$)	147.54 ± 4.50	135.95 ± 6.08	139.67 ± 6.05	143.82 ± 5.54
	RTD (g cm^{-3})	0.44 ± 0.01	0.48 ± 0.02	0.47 ± 0.01	0.46 ± 0.02

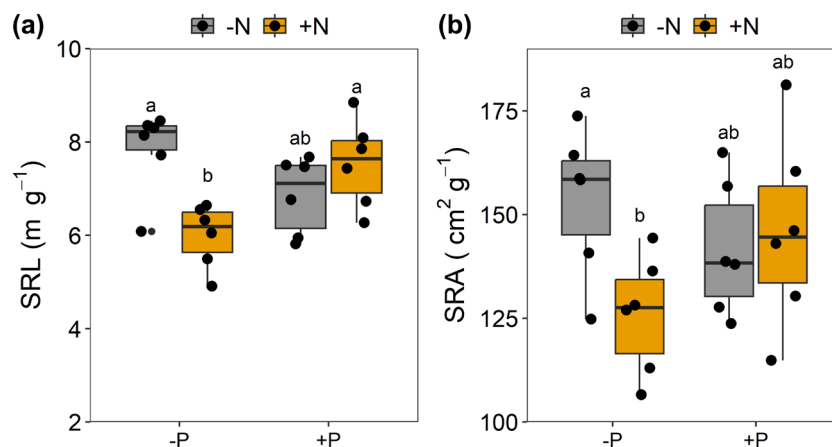


Figure 4. Interactive effects between the addition of phosphorus (P; x-axis) and nitrogen (N; coloured boxplots) affecting (A) specific root length (SRL; m g^{-1}) and (B) specific root area (SRA; $\text{cm}^2 \text{g}^{-1}$) in Nouragues (NOU) for the mean 0–15 cm soil layer ($n=6$ for each interaction). Differences between pairwise comparisons among treatment levels are denoted by letters. Means sharing the same letter are not significantly different ($p\text{-value} > 0.05$).



Figure 5. Radar plot showing the trait-space occupied when combining the response of fine root biomass and traits (0–15 cm) in this study per site and per treatment. Since all traits were in different units, they are scaled to normalise the data by subtracting from each value the sample mean ($n=24$ per site) and dividing it by the sample standard deviation for each trait. ‘RB’ refers to fine root biomass (Mg ha^{-1}); ‘RD’ refers to fine root diameter (mm); ‘SRL’ refers to specific root length (m g^{-1}); ‘SRA’ refers to specific root area ($\text{cm}^2 \text{g}^{-1}$); ‘RTD’ refers to root tissue density (g cm^{-3}) and ‘N:P’ refers to the ratio of concentrations and N and P in fine roots. Top two panels show traits from Paracou (PAR) and bottom two panels show traits from Nouragues (NOU). Colours indicate the presence and absence of N and P, as described in the figure. Values above 0 are above the mean, and values below 0 are below the mean, with their magnitudes indicating the number of standard deviations away from the mean. Significant effects reported in previous tables and figures comparing the trait response with and without a specific nutrient addition are indicated by *, ** and ***, representing $p\text{-values} < 0.05$, 0.01 and 0.001, respectively ($n=12$ per treatment and site).

increasing root biomass would translate into increased access to such limited resources.

Fine root traits: from acquisitive with P addition to conservative with N addition

At a global scale, root tissue N and P concentrations tend to be positively correlated with soil nutrients (Wang et al. 2019). In our manipulation experiment in French Guiana, three years of N and P addition increased the content of these two elements in soils for both Paracou and Nouragues (Table 2), however, N content in roots did not follow such increase, whereas P contents in roots were higher in +P plots in both study sites, similar to results found in other tropical nutrient manipulation experiments (Wurzburger and Wright 2015, Lugli et al. 2021). Nonetheless, N and P addition resulted in higher N and P contents in leaves, respectively, in Paracou, whilst in Nouragues, adding N and P together increased P contents but not N contents in leaves (Supporting information). Such divergent responses among nutrients and plant tissues could suggest that there might be a preferential N allocation aboveground, especially in the lower fertility site, Paracou (Peguero et al. 2023).

Root P concentrations increased threefold in +P plots compared to -P plots, suggesting that P is not only being used for biomass production, but roots could also be acting as P storage tissues. For instance, we found a positive correlation between total P stocks in soils and roots for both sites (Supporting information). In Paracou, despite soil P stocks being lower than in Nouragues, P stocks in roots were almost twice as high as in Nouragues (Supporting information), which could suggest that the role of root P storage might be more important in lower-fertility soils. In the long-term, such changes in root tissue quality, with general decreases in C:P and N:P ratios, could largely impact some ecosystem processes such as litter mineralisation and nutrient recycling (Sardans et al. 2017). Nevertheless, because of the different soil properties between Paracou and Nouragues, the mechanisms behind increased root P concentrations with nutrient addition might be different between these two sites (Soong et al. 2020). Since the addition of P also increased Ca and K concentrations in fine roots, we could likewise hypothesise that in Paracou, multiple rock-derived nutrients could be limiting trees, although not severely enough to affect fine root biomass or morphological traits.

It is interesting to point out that +P did not significantly change the expression of fine root morphological traits in either of our study sites, indicating that the sole increase of soil P availability after fertilisation might have enabled a higher root P uptake per unit of absorptive surface area (Grau et al. 2017, Van Langenhove et al. 2020b), similar to another site in central Amazonia (Lugli et al. 2021). Our results corroborate previous findings from the same experiment in Paracou, where added P was very rapidly assimilated by plants, whereas no changes in root N assimilation rates occurred with N addition (Van Langenhove et al. 2020b). Here, we also speculate that as a result of growing in a

naturally 'more favourable' environment at higher soil fertility, roots showed more acquisitive features in Nouragues (Table 1), such as lower RD, higher SRL and SRA, that they might deploy to acquire P using the 'do-it-yourself' strategy (Bergmann et al. 2020, Laughlin et al. 2021). Contrarily, in the lower-fertility site Paracou, roots displayed more conservative traits (Table 1), such as thicker RD and lower SRL and SRA, and therefore trees might rely more on mycorrhizal associations for nutrient acquisition (Bergmann et al. 2020, Soong et al. 2020, Reichert et al. 2022).

The addition of N, on the other hand, triggered contrasting responses in root traits compared to P addition, partially corroborating our initial hypothesis that +N could exacerbate P limitation. Although N addition did not change N concentrations in roots nor root biomass stocks for either of the sites, fine root morphology tended to shift towards more conservative traits (increasing RD and RTD), but only in the already fertile site of Nouragues, where N addition also stimulated stem growth (Vallicrosa et al. 2023). Moreover, we captured a significant interaction between N and P in Nouragues, and morphological traits shifted only when P was not added in combination with N (e.g. decreasing SRL and SRA in +N-P but not in +N+P). As a result, root morphological traits that were once acquisitive started to shift to similar values as found in less fertile soils in Paracou, where roots displayed more conservative traits. Such coarser and shorter roots (when not associated with mycorrhizas) are generally linked to lower absorptive capacity, but they could also result in lower costs for plants if root lifespan increases and respiration rates decrease (McCormack et al. 2015). Here we suggest that N addition could be detrimental for plants in the already N-rich site in Nouragues, enforcing limitations by other elements (Vitousek et al. 2010). For instance, we noticed that in Nouragues, the addition of N decreased root P and cation concentration, as well as increased root C:P and N:P, which could suggest that with extra N in the system, higher amounts of root biomass were produced with less nutrients. This points to an even tighter P (and/or cations) cycling when compared to plots where P was added, showing that with N addition, plants may have built root systems aiming to increase their lifespan and decrease their metabolic costs at the expense of their absorptive capacity.

Conclusions

Our results suggest that both N and P play an important role in old-growth tropical forests in Amazonia, but in contrasting ways. Although an increasing amount of evidence indicates that either P-only or a combination of elements (N+P+K) control tropical forest belowground functioning, here we demonstrate how both N and P could have antagonistic effects on root parameters in two forests in French Guiana. The complex mosaic of soil types along Amazon regions, varying in fertility, physical properties, age and parent material, underscores the significance of understanding the role of nutrient limitation within diverse forest

ecosystems. We suggest that even without changes in root morphological traits, increasing root nutrient content and root biomass indicate the presence of other physiological mechanisms controlling P acquisition efficiency. On the other hand, the addition of N in N-rich forests caused shifts from acquisitive to more conservative root traits (i.e. from thin and long to short and coarse roots). Such changes in root biomass and traits could impact nutrient cycling in the soil–plant continuum and alter carbon stocks and fluxes in these forests. Long-term monitoring combined with an integrated perspective of the above and belowground system would be informative to track the persistence of such effects shown here and the coordination (or lack thereof) among different plant tissues. With increasing atmospheric CO₂, the potential ability of forests to adapt in the face of soil nutrient limitation might be influenced and even limited by the overall plasticity of traits from the tree communities that evolved in environments with different soil physical and chemical properties.

Speculations and alternative viewpoints

Soil nutrients significantly influence tree abundance, taxonomic composition, and community functional trait space. Nonetheless, translating community-level differences across sites into responses to experimental fertilisation is challenging. Previous research suggests that tree species with more nutrient-acquisitive traits tend to be distributed across wider ranges of edaphic properties, while nutrient-conservative species are confined to nutrient-poor environments. Fertilisation experiments in tropical forests indicate that nutrient-poor specialists could exhibit lower responsiveness, particularly in root morphological traits. The lower response of root traits at a community level observed here in the poorer sites, coupled with the greater stem growth only in the more fertile site (where species with acquisitive traits are selected), suggest that tree species adapted to nutrient-poor soils have a limited capacity to respond to changes in nutrient availability. Whether tree species with more acquisitive traits present higher adaptability to variation in soil nutrient concentrations through fine-tuning the expression of their root traits remains an exciting question to be addressed. Testing these contrasting alternatives for the phenotypic expression of belowground traits would contribute to our understanding of the environmental factors influencing the basic mechanisms driving tree community assembly and improve our predictions of the magnitude of forests' responses to global change drivers.

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Author contributions

Laynara Figueiredo Lugli: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Validation (lead); Visualization (lead); Writing – original draft (lead). **Lucia Fuchslueger:** Conceptualization (supporting); Data curation (supporting); Investigation (equal); Methodology (equal); Writing – review and editing (supporting). **Helena Vallicrosa:** Data curation (supporting); Formal analysis (supporting); Investigation (supporting); Writing – review and editing (supporting). **Leandro Van Langenhove:** Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Methodology (supporting). **Christian Ranits:** Investigation (supporting). **Pere Roc Fernandez Garberi:** Investigation (supporting). **Lore Verryct:** Investigation (supporting). **Oriol Grau:** Investigation (supporting). **Laëtitia Bréchet:** Investigation (supporting); Methodology (supporting); Resources (supporting); Writing – review and editing (supporting). **Guille Peguero:** Investigation (supporting); Writing – review and editing (supporting). **Joan Llusia:** Investigation (supporting). **Romà Ogaya:** Investigation (supporting). **Laura Marquez:** Investigation (supporting). **Miguel Portillo-Estrada:** Investigation (supporting); Resources (supporting). **Irene Ramirez-Rojas:** Writing – review and editing (supporting). **Elodie Courtois:** Funding acquisition (supporting); Resources (supporting). **Clement Stahl:** Funding acquisition (supporting); Resources (supporting); Writing – review and editing (supporting). **Jordi Sardans:** Funding acquisition (supporting); Resources (supporting). **Josep Penuelas:** Funding acquisition (lead); Resources (supporting); Writing – review and editing (supporting). **Erik Verbruggen:** Methodology (supporting); Resources (supporting); Writing – review and editing (supporting). **Janssens Ivan:** Funding acquisition (lead); Methodology (supporting); Project administration (lead); Resources (lead); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: <https://datadryad.org/stash/dataset/doi:10.5061/dryad.v15dv4231> (Lugli et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

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