Rapid responses of root traits and productivity to phosphorus and cation additions in a tropical lowland forest in Amazonia

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Total word count: 6,155
Word count for each section: Introduction: 1,414 Material and Methods: 1,668; Results: 805; Discussion: 2,268.
Number of figures: Four figures not coloured.
Number of tables: One table.
Supporting information: One file.
Heading: Fine root responses to nutrient manipulation in Amazonian forests
Social media: @Lay_Lugli, @amazonfertilisation, @ExeterGeography, @Ascom_Inpa

Summary

- Soil nutrient availability can strongly affect root traits. In tropical forests, phosphorus (P) is often considered the main limiting nutrient for plants. However, support for the P paradigm is limited, and N and cations might also control tropical forests functioning.
- We used a large-scale experiment to determine how the factorial addition of nitrogen (N), P and cations affected root productivity and traits related to nutrient acquisition strategies (morphological traits, phosphatase activity, arbuscular mycorrhizal colonisation and nutrient contents) in a primary rainforest growing on low-fertility soils in Central Amazonia after one year of fertilisation.
- Multiple root traits and productivity were affected. Phosphorus additions increased annual root productivity and root diameter, but decreased root phosphatase activity. Cation additions increased root productivity at certain times of year, also increasing root diameter and mycorrhizal colonisation. P and cation additions increased their element concentrations in root tissues. No responses were detected with N addition.
- Here we show that rock-derived nutrients determine root functioning in low-fertility Amazonian soils, demonstrating not only the hypothesised importance of P, but also highlighting the role of cations. The changes in fine root traits and productivity indicate that even slow-growing tropical rainforests can respond rapidly to changes in resource availability.

Key words: Amazon rainforest; arbuscular mycorrhiza; fine root productivity; large-scale nutrient fertilisation experiment; multiple nutrient limitation; phosphatase enzyme; root morphology.

Introduction

Tropical rainforests are the most diverse and productive terrestrial ecosystem on Earth (Beer *et al.*, 2010) representing a terrestrial carbon (C) sink of 2.89 ± 0.6 Pg C per year (Pan *et al.*, 2011), with the Amazon forest alone storing about one quarter of global terrestrial C sinks (Le Quéré *et al.*, 2018). Moreover, tropical net primary production (NPP) may be further stimulated under atmospheric CO_2 enrichment (Kimball & Idso, 1983; Ainsworth & Long, 2004; Norby *et al.*, 2005). Future CO_2 uptake could, however, ultimately be controlled by the amount of available nutrients in the soil to support new growth (Hungate *et al.*, 2006; Fleischer *et al.*,

2019) as well as by how efficiently plants can acquire and use nutrients. In temperate forests, nitrogen (N) is usually considered to limit plant growth, whereas phosphorus (P), or other rock-derived elements are considered more likely to be the limiting nutrient in tropical lowland forests (Walker & Syers, 1976; Vitousek & Sanford, 1986; Wardle, 2004). Phosphorus and cations, are supplied to soil predominantly by weathering of the parent material (Walker & Syers, 1976), and are essential in several metabolic process of plants, such as ATP production, stability of cells and enzyme activation (Aerts & Chapin, 1999; Lambers *et al.*, 2006; Hawkesford *et al.*, 2012). Approximately 60% of the Amazonian forests grow in highly-weathered soils, characterised by very low concentrations of rock-derived P and cations, with evidence for P affecting plant growth (Aragão *et al.*, 2009; Quesada *et al.*, 2010, 2012). However, even in tropical forests, N availability may be important in controlling key aspects of forest function (Wright *et al.*, 2011; Wright, 2019), and/or greater N availability could help alleviate limitation by other elements (Chen *et al.*, 2020). Therefore, there remain major gaps in our understanding of the role different elements play in controlling tropical forest function, especially in Amazonia.

Plants can adapt their root morphological, physiological, biochemical and molecular properties to optimise nutrient acquisition (Chapin, 1980; Bloom *et al.*, 1985; Aerts, 1999; Raghothama, 1999; Addo-Danso *et al.*, 2020). Because of the low mobility of P in soils, roots usually move towards P, getting thinner and longer to facilitate the exploration of greater soil volume in P patches (Hodge, 2004; Lambers *et al.*, 2008; Metcalfe *et al.*, 2008; McCormack & Iversen, 2019). Alternatively, roots displaying more conservative morphological features (*i.e.* lower specific root length - SRL, greater diameter) may invest more in mycorrhizal associations to meet nutrient demands (Hodge, 2004; Comas *et al.*, 2014; Eissenstat *et al.*, 2015; Liu *et al.*, 2015; Kong *et al.*, 2016; Ma *et al.*, 2018). The very fine hyphal network typical of arbuscular mycorrhizas (AM) allows the fungi to forage for P away from P-depleted zones around roots, resulting in high inorganic P uptake in exchange for photosynthetically fixed C from the host plant (Hodge, 2004; Smith & Read, 2010; Eissenstat *et al.*, 2015). There is also evidence for the role of AM in acquiring other elements, such as Ca, Mg, K and sulphur (Siqueira *et al.*, 1998; Zangaro *et al.*, 2003) and micronutrients such as zinc and copper (Smith & Read, 2010). The main source of P in low-fertility tropical soils is, however, bound in organic compounds or occluded in secondary minerals (Walker & Syers, 1976; Cross & Schlesinger, 1995; Quesada *et al.*, 2010) and, consequently, they need to be degraded before being

assimilated by roots (Lambers *et al.*, 2006). The hydrolysis of organic P happens mainly through the activity of phosphatase enzymes released by microbes and plant roots (Hinsinger, 2001; Treseder & Vitousek, 2001; Vance *et al.*, 2003; Olander & Vitousek, 2004). Therefore, strong investment in the production of phosphatase enzymes that can become bound to root surfaces or released into the soil matrix may be necessary to mine organic P in these forests (Liu *et al.*, 2015; Kong *et al.*, 2016; Lugli *et al.*, 2020).

Plant trait-based approaches are especially useful tools to increase understanding of plant function in species-rich environments, such as tropical forests. Although tropical trees may use a range of complementary adaptations to optimise P-uptake (Zemunik *et al.*, 2015; Lugli *et al.*, 2020), it remains uncertain how plastic these strategies are in response to short-term changes in the availability of different nutrients. Root functional traits are considered to represent a balance between maximising the acquisition of limiting resources and minimising the costs of root tissue construction and maintenance (Bloom *et al.*, 1985; Aerts & Chapin, 1999; Wurzburger & Wright, 2015; McCormack & Iversen, 2019). For example, about 20% of plant C could be transferred to AM fungi associates, whilst root exudates (*i.e.* organic acids, enzymes) can represent up to half of belowground C allocation (Bago *et al.*, 2003; Lynch *et al.*, 2005; Parniske, 2008). Therefore, trade-offs between uptake strategies are likely, with plant investment in root biomass and nutrient uptake strategies usually increasing with decreasing supply of the limiting nutrient (Bloom *et al.*, 1985). In naturally P-poor soils in Central Amazon, Lugli *et al.* (2020) demonstrate that due to the different levels of soil P availability in different pools (*i.e.* organic and inorganic P), plants need to invest in multiple P-uptake mechanisms.

Nutrient manipulation experiments greatly contribute to directly testing for nutrient limitation in terrestrial ecosystems (Cleveland *et al.*, 2011; Sullivan *et al.*, 2014; Wright *et al.*, 2018). Although the hypothesis of P-limitation in tropical forests is widely accepted, clear evidence from large-scale experiments is variable and limited (Yavitt *et al.*, 2011; Mirabello *et al.*, 2013; Wurzburger & Wright, 2015; Alvarez-Clare & Mack, 2015; Wright, 2019). In a recent meta-analysis, Wright (2019) compiled data from 48 nutrient manipulation experiments in tropical forests and concluded that N and P limitation are widespread, but no evidence was found for a greater role for P than N, and it is uncertain how other nutrients, including cations, affect these ecosystems. Furthermore, root responses are particularly poorly understood, with nutrient addition experiments in Central America tending to have not measured productivity

responses and having observed contrasting changes in standing stocks and root traits. For example, after two years of nutrient addition, root biomass (<2 mm diameter) decreased with K addition but increased with P addition for thicker roots (2-5 mm diameter) in Panama (Yavitt *et al.*, 2011), and no root biomass responses were detected in Costa Rica (Alvarez-Clare & Mack, 2015). In these same experiments, changes in fine root morphology following P addition were observed in Panama, with roots becoming less dense and with greater specific root length (Wurzburger & Wright, 2015), whilst increased root nutrient concentrations were detected in Costa Rica (Alvarez-Clare & Mack, 2015). However, current experiments in Neotropical forests are located on natural soils with total P concentrations ranging from 400-1,600 mg kg^{-1} (Wright *et al.*, 2011; Alvarez-Clare *et al.*, 2013). In contrast, in the dominant soil type across Amazonia, the world's largest tropical forest, total P ranges from 100-200 mg kg^{-1} (Quesada *et al.*, 2010). Given the range of responses observed in these Neotropical studies and the differing soil fertilities, we clearly cannot extrapolate to how fine root traits and productivity are controlled by soil nutrient status in Amazonian forests.

We used the first large-scale nutrient manipulation experiment installed in Central Amazonian forests (the Amazon Fertilisation Experiment; AFEX) to determine whether key nutrient uptake mechanisms adopted by fine roots were altered by the factorial addition of N, P and cations (Ca, Mg and K) in low-fertility soils. Our study quantified the short-term responses in the first year of manipulations, thus investigating how rapidly roots can respond to the addition of the different nutrients. We hypothesized that given the low availability of P in soils at our site, there would be a strong and immediate effect of P addition on root traits and productivity, but that N addition would have limited impacts. This is based on the high C-costs of production and maintenance of fine roots as well as allocation towards nutrient uptake strategies. Thus, we expected that fertilisation would decrease plant investment in such traits. Consequently, we predicted that with P addition alleviating belowground P limitation, there would be decreased root productivity, together with a reduction in root phosphatase activities and AM colonisation, with morphological changes reflecting shifts from acquisitive to more conservative traits, decreasing, for example, SRL and SRA and increasing tissue density and mean diameter. Furthermore, due to the very low concentrations of cations in Central Amazonian soils, we also expected that cations would trigger changes in root traits, shifting from acquisitive to more conservative morphological traits, but with no effect on root phosphatase activity.

Material and methods

Site description and experimental design

This study was carried out within the AFEX experiment in Central Amazonia, installed ca. 70 km north of Manaus/Amazonas, Brazil in the area of the Biological Dynamics of Forest Fragments Project (BDFFP) Reserve at ZF-3, a collaborative project between the National Institute for Amazonian Research (INPA) and the Smithsonian Institute (STRI). Mean air temperature is 26 ºC and mean annual precipitation is 2,400 mm (Araújo *et al.*, 2002). The vegetation is an old growth, lowland *terra firme* forest, associated with clay-rich (75%) Ferralsols and very low total P content (~ 85 mg kg^{-1} for the 0-30 cm soil depth). AFEX is composed of thirty-two 50 m x 50 m plots separated at least 50 m from each other and distributed in four blocks. Each of the four blocks (installed at least 300 m apart) includes eight plots representing seven nutrient addition treatments and one control applied in a factorial design: control (with no addition of nutrients), N, P, cations (Ca, Mg, K), N+P, N+cations, P+cations, and N+P+cations. All plots (n=4 for each treatment and control) were established in areas with similar soil, vegetation, and terrain, being restricted to plateaus.

Nutrient additions are split into three equal applications over the course of each wet season, with nutrients added every year since 2017 at the following total rates: (1) N: 125 kg ha^{-1} yr^{-1} as Urea; (2) P: 50 kg ha^{-1} yr^{-1} as triple superphosphate, and (3) Cations: 160 kg ha^{-1} yr^{-1} as dolomitic limestone for Ca and Mg, plus 50 kg ha^{-1} yr^{-1} as potassium chloride for K. Aiming to make our data comparable to other nutrient fertilisation experiments, the amount and rates of nutrients added to our site follow rates proposed by Wright *et al.* (2011) in Panama. Dry fertilisers were applied to the soil surface by hand covering the whole plot area (50 m x 50 m), including the surface of the ingrowth cores. Our results represent the root responses to the first year of nutrient additions, and thus also investigate how rapidly trees can respond to changes in soil fertility.

Fine root productivity

Key monitoring measurements were limited to the central 30 m x 30 m (900 m^2 area) of each plot. In each plot (n=32), five 12 cm-diameter, 30 cm-deep, root-free ingrowth cores (2 cm plastic mesh) were installed in August 2017 in the central 30 m x 30 m plot area. Ingrowth cores were collected every three months after installation and the five core replicates were

homogenised in the field by plot and by soil depth (0-10 and 10-30 cm; *N*=64) in each collection. Fine roots (< 2mm in diameter living roots) produced in the first year of nutrient addition (four ingrowth core campaigns from August 2017-September 2018) were used to determine productivity. All fine roots from the two soil depths were manually extracted during a period of 60 minutes in four intervals of 15 minutes and root-free soil reinserted into the existing holes (Metcalfe *et al.*, 2007). After sampling, roots were washed and cleaned by gently brushing to remove soil particles. The cumulative root biomass sampled at each time point (one sample for every 15 minutes = four samples) was used to estimate the amount of roots that would be sampled after the 60 minutes sampling collection (Metcalfe *et al.*, 2007). We tested four different types of curves (logarithmic curve, Michaelis-Menten asymptotic curve, power law curve and asymptotic exponential curve) to extrapolate to the amount of roots that would be sampled during 180 minutes, choosing the curve that resulted in the best model fit (Michaelis-Menten asymptotic curve; Equation 1).

$$y = \frac{\alpha * x}{\beta + x} \qquad \text{Equation 1.}$$

where y is total fine root biomass estimated in each sample after 180 minutes of sampling; x is accumulated time (15 to 180 minutes), α and β are fitted parameters from the equation for each plot and depth.

Fine root productivity was calculated as dry mass of roots produced per day for the entire ingrowth core sample and by depth (0-10 and 10-30 cm). Root net primary productivity was calculated summing the biomass of fine roots produced in each ingrowth core census and was expressed in Mg ha^{-1} $year^{-1}$.

Root morphology

Subsamples of fine roots from the ingrowth core campaign held in February 2018 (newly produced roots < 3 months old) were used to determine morphological traits. Fine roots from both soil depths (0-10 and 10-30 cm) were cleaned and fresh root samples (<2 mm diameter) were spread homogeneously in a plastic tray with approximately one quarter of the root biomass picked randomly for the subsequent scanning (Holdaway *et al.*, 2011). Roots were scanned at 600 dpi and images analysed using WinRHIZO (WinRHIZO Regular 2015, Regent Instruments, Canada) to provide root mean diameter, total length, area and volume, then

samples were dried at 60 ºC for 72 hours to determine dry root mass. These were used to determine specific root length (SRL), specific root area (SRA), root tissue density (RTD) and mean root diameter (Metcalfe *et al.*, 2008). SRL (cm g^{-1}) was calculated as root length per unit root dry mass, SRA (cm^2 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 phosphatase activity

Root subsamples collected in February 2018 were analysed for root-surface potential acid phosphomonoesterase activity (phosphatase). Phosphatase was measured within 3 days of root sampling using triplicate subsamples per plot and per soil depth (0-10 and 10-30 cm) using a fluorimetric microplate assay (Turner & Romero, 2010; German *et al.*, 2011) as described in Lugli *et al.* (2020). About 10 mg of the root sample (washed, fresh weight basis) were incubated with Methylumbelliferyl-phosphate (MUF), which was used as an analogue substrate for the enzyme acid phosphomonoesterase. In addition, sample, buffer and substrate blanks were prepared. Samples were incubated for 30 min at ~ 25 ºC while gently shaking, then 50 µL of 1 M NaOH were added to all samples and standard vials to terminate the reaction. Aliquots of the sample solution were pipetted into a black 96-well microplate and 20 min after termination, fluorescence was read on a fluorometer (Tecan Infinite® 200 PRO, Grödig, Austria), at 365 nm excitation and 450 nm emission. Roots were removed from vials, rinsed with Milli-Q water, scanned and subsequently dried at 60 ºC for 72 hours. Root phosphatase activity per plot and depth was expressed in µmol MUF g^{-1} root dry mass h^{-1}.

Mycorrhizal colonisation

To determine AM colonisation, roots collected in February 2018 were subsampled, cleaned and scanned, and segments were stored in 50% ethanol. Only root fragments from the 0-10 cm soil layer were used for AM analyses. The clearing and staining processes were adapted for tropical roots based on Brundrett *et al.* (1984) and Wurzburger and Wright (2015). Briefly, roots were cleared using a 2.5% KOH solution and autoclaved at ~ 120 °C for ± 10 minutes, then placed in alkaline H_2O_2 solution for further bleaching for ± 30 minutes. Before staining, roots were acidified in 2% HCl solution for 30 minutes and were then added to a beaker with Trypan Blue 0.05% until constantly blue. Roots were rinsed in tap water and ten uniformly stained 1 cm root fragments per plot were mounted on slides to quantify total root length

colonised by AM fungi (40 x optical) (McGonigleE *et al.*, 1990). Mycorrhizal colonisation was assessed as the percentage of the total root points along the root length that had any mycorrhizal fungi structures.

Nutrient concentration in fine roots

To ensure there was enough material for nutrient analysis, root material <2 mm diameter from all four collections spanning the first year of fertilisation (August 2017-September 2018) was bulked. Dried and ground roots from each collection were composited by plot and soil depth. Analyses were performed at the Soil and Plant laboratory (LTSP) at the National Institute of Amazonian Research (INPA) in Manaus, Brazil, and followed established methods that have also been used to characterise variability in the plant and soil variables across the Amazon basin (Quesada *et al.*, 2010). Carbon and N contents were determined using an automatic C and N analyser (VARIO MAX CHN Element Analyzer) (Nelson and Sommers, 1996). Concentrations of P and cations in roots were analysed by nitroperchloric digestion described by Malavolta *et al.*, (1989). Phosphorus concentrations were determined by colorimetry (Anderson and Ingram, 1993), and quantified by spectrophotometry (UV-120-01, Shimadzu, Kyoto, Japan). Ca, Mg and K were determined by atomic absorption spectrophotometry (AAS, 1100 B, Perkin-Elmer, Ueberlingen, Germany).

Statistical analyses

Linear mixed-effect models were used to test the effect of added nutrients and their interaction in the factorial design N*P*cations. The presence/absence of each of the main nutrients were used as a fixed factor and the four blocks as random factor. All models were run in the R packages 'lme4' and 'lmerTest' (Bates *et al.*, 2014; Kuznetsova *et al.*, 2017). Full factorial models were simplified using backward elimination performed by the *step* function in 'lmerTest' package. The significant model was then re-run and only the significant effects of nutrient additions are reported. Since no significant interaction effects were detected between the different nutrients added, results are shown for single nutrient additions only, following Wright et al., (2011). To graphically assess the effect of specific nutrients, all plots where a specific nutrient was not added (*i.e.* –P; n=16) are compared to all plots where that nutrient was added (*i.e.* +P; n=16) (Wright et al. 2011). Results are shown for the whole soil core and for both soil depths separately, but since our aim was to detect the effect of the addition of different

nutrients, depth was not used as a factor in the statistical models and differences between depths themselves are therefore not discussed in detail (Supporting Information; Table S1 and S2). Data were checked for normality and variance homogeneity and the selection for the best model was made based on functions from 'LMERConvenienceFunctions' R package (Tremblay & Ransijn, 2015). All analyses were conducted in R version 3.4.4 (R Core Team, 2018).

Results

Root productivity

After one year of nutrient addition, mean fine root productivity across all control plots (n=4) was 2.98 ± 0.33 Mg ha^{-1} year^{-1} (0-30 cm soil depth). Total root productivity for the 0-30 cm soil depth, significantly increased by 23% in P-addition plots compared to plots without added P (-P: 3.50 ± 0.30 *versus* +P: 4.31 ± 0.33 Mg ha^{-1} year^{-1}; $F_{1,24}$=4.67, p=0.04; Fig. 1). The significant increase in mean root productivity with P addition for the whole core was mainly driven by changes in the 0-10 cm soil layer (-P: 2.03 ± 0.15 *versus* +P: 2.64 ± 0.20 Mg ha^{-1} year^{-1}; $F_{1,24}$=6.62, p=0.017), with no significant effect in the 10-30 cm layer with the addition of any nutrient (Fig. 1). No significant effects were found for total root productivity with the addition of N or cations (Fig. 1). Although the addition of cations did not significantly affect annual root productivity, there were short-term effects of cations at certain times of the year. No interactions among nutrient treatments were found for root productivity in any sampling time. When analysing root productivity for the 3-month interval used for our root trait analyses (November 2017 – February 2018), the addition of cations increased fine root productivity by 52% for the whole 0-30 cm soil layer ($F_{1,26}$=8.28, p=0.008) and this increase was mainly driven by a significant effect detected for the 0-10 cm layer ($F_{1,26}$=12.32, p=0.002; Supporting Information Fig. S1).

Root morphological traits

Mean root diameter (0-30 cm) across control plots (n=4) was 0.99 ± 0.03 mm, SRL 1,310 ± 76 cm g^{-1}, SRA 311 ± 14 cm^2 g^{-1} and RTD 0.15 ± 0.007 g cm^{-3}. In plots where P was added, root diameter significantly increased in the 0-10 cm soil layer when compared to plots without P addition ($F_{1,26}$=4.78, p=0.038; Table 1), with no changes for the full 0-30 cm layer ($F_{1,25}$=3.61, p=0.07). The addition of cations increased mean root diameter from 1.03 to 1.12 mm for the

whole 0-30 cm soil layer ($F_{1,25}$=8.55, p=0.007). The same trend was found for the 0-10 cm ($F_{1,26}$=3.78, p=0.06) and 10-30 cm ($F_{1,27}$=3.36, p=0.08) soil layer. For mean root diameter, the addition of N did not result in any changes for any soil layer. The addition of N, P and cations separately had no effect on SRL, SRA or RTD (Table 1).

Root phosphatase activity

Mean root phosphatase activity across control plots (n=4) was 40.80 ± 6.74 μmol g^{-1} h^{-1} for the 0-30 cm soil layer. Compared to plots without P, the addition of P significantly decreased root phosphatase activity only in the top 10 cm by 23% (-P: 41.84 ± 2.70 *versus* +P: 31.97 ± 2.95 μmol g^{-1} h^{-1}; $F_{1,27}$=7.30, p=0.01; Fig. 2). No significant changes in root phosphatase activity were detected with the addition of N, P or cations for the whole core (0-30 cm), although a decline of root phosphatase activity was captured with P addition (-P: 38.90 ± 2.52 *versus* +P: 33.21 ± 3.07 μmol g^{-1} h^{-1}; $F_{1,27}$=3.45, p=0.07; Fig. 2). When analysing soil layers separately, the addition of N or cations did not affect root phosphatase activity.

Mycorrhizal colonisation

Mean total root AM colonisation in control plots was 38.46 ± 4.75% for the 0-10 cm soil layer. The addition of cations increased total AM colonisation from 41.90% in plots where cations were not added to 50.40% with cation addition ($F_{1,27}$= 4.57, p=0.042; Fig. 3). Neither the addition of N nor the addition of P significantly affected root AM colonisation. No significant effects of nutrient addition were detected when analysing AM structures separately (Supporting Information; Table S3).

Nutrient concentration in fine roots

Mean C and N concentrations in roots growing in control plots were 43.82 ± 0.19 and 0.74 ± 0.13 %, and mean P, Ca, Mg and K concentrations were 0.46 ± 0.02, 0.92 ± 0.09, 0.84 ± 0.12, 2.80 ± 0.20 g kg^{-1} for the whole 0-30 cm soil layer. In plots where P was added, concentrations of P and Ca increased in roots growing in the 0-10, 10-30 and for the mean 0-30 cm soil layer (Fig. 4). Concentrations of P in roots more than doubled with P addition ($F_{1,27}$= 40.97, p<0.001), whilst Ca concentrations increased by at least 50% ($F_{1,26}$= 17.08, p=0.0003). The addition of cations significantly increased Ca, Mg and K concentrations in roots. Ca concentrations increased about 30% in plots where cations were added, being significantly

366 higher only for the 0-10 cm ($F_{1,25}$= 4.29, p=0.048; Fig. 4) and mean 0-30 cm soil layer ($F_{1,26}$=
367 4.67, p=0.04; Fig. 4). Mg concentrations increased by more than 50% ($F_{1,26}$= 23.81, p<0.0001
368 for the 0-30 cm layer), with K concentrations increasing by 20-30% with cations addition
369 ($F_{1,27}$= 7.02, p=0.013 for the 0-30 layer; Fig. 4). The addition of N did not significantly affect
370 the concentrations of nutrients in roots one year after fertilisation commenced.

371

Discussion

372 Here we demonstrate experimental support for the hypothesis that rock-derived nutrients play
373 a more important role than N in controlling fine root functional traits in highly weathered,
374 ancient soils, such as those found in most Amazonian forests. Phosphorus addition had major
375 impacts on root productivity and functional traits analysed here, but cation additions also
376 affected root dynamics. The addition of N, as expected, did not affect root productivity or any
377 root trait analysed here. Overall, the results demonstrate that trees in these slow-growing forests
378 show high plasticity in response to shifts in P and cation availability.

379

380 Fine root productivity was stimulated by short-term P addition

381 Due to the high costs of construction and maintenance of fine acquisitive roots (McCormack
382 *et al.*, 2015), we expected that P addition would decrease fine root productivity as a sign of
383 alleviation of P limitation. Our results demonstrate that in the short-term, P addition increased
384 root productivity by 23%, suggesting that, contrary to what we expected, the construction costs
385 of short-lived acquisitive roots might be less than the maintenance costs of long-lived fine
386 roots. The increase in root productivity in our study is not consistent with the lack of responses
387 (Alvarez-Clare & Mack, 2015) and declines in root biomass (Yavitt *et al.*, 2011) observed in
388 previous fertilisation experiments in tropical forests, nor observed variation in root productivity
389 between soil types with contrasting fertility in the Colombian Amazon (Jiménez *et al.*, 2009),
390 but are in more agreement with the study of Waring *et al.*, (2019) in a tropical dry secondary
391 forest. The response also contrasts with the reductions in root productivity and C allocation
392 belowground following alleviation of N limitation in temperate and boreal forests (Janssens *et*
393 *al.*, 2010; Peng *et al.*, 2017). However, our results are actually consistent with large-scale
394 spatial patterns observed within Amazonia; higher fine root productivity has been observed in
395 more fertile soils of the Western Amazon basin than in low-fertility soils in the Central portion
396 of the basin (Aragão *et al.*, 2009). The agreement between our results and the broader spatial

patterns in Amazonia (Aragão *et al.*, 2009) may suggest a common response to greater P and cation concentrations across natural gradients and in response to experimental manipulation. At this stage, however, it is not clear if the increase in root productivity in our study site after one year of P additions is transient and could change with chronic nutrient enrichment and how these responses, together with turnover rates, will affect partitioning of plant biomass allocation and stocks between above and belowground compartments (Ostertag, 2001; Jiménez *et al.*, 2009; Wurzburger & Wright, 2015). Nonetheless, our results demonstrate a rapid change in productivity rates in response to P additions in Central Amazonia, pointing to an increased role of direct root nutrient uptake in a more P-fertile system.

A trend towards greater root productivity with cation addition was also observed, with the increase in productivity being greater in some of the sampling points but not overall. In Panama, four years of K additions elicited changes in fine root dynamics, decreasing root stocks while increasing root turnover (Yavitt *et al.*, 2011). Therefore, despite its potential importance, it remains less clear the extent to which the availability of specific cations controls root productivity in tropical forests and how such responses would change in the short and longer term.

Phosphorus and cations additions cause rapid increase in average root diameter

Together with other factors, soil fertility is expected to control the expression of fine root morphological traits (Valverde-Barrantes *et al.*, 2013, 2017; Freschet *et al.*, 2017; Addo-Danso *et al.*, 2020). Hence, we hypothesised that the addition of nutrients would alleviate limitation, resulting in a shift from acquisitive to more conservative root traits, decreasing, for example, SRL and SRA and increasing RTD and mean diameter. Root diameter increased ~10% with cation and P addition, but no responses were detected for SRL, SRA and RTD in our fertilisation experiment. The direct effect of P addition on root diameter is, however, not conclusively demonstrated in our study, since our P fertiliser (triple superphosphate) includes ~15% of Ca in its composition and root diameter also increased in plots where we added cations. Therefore, we cannot exclude the possibility that the responses in both treatments were driven by Ca. Contrary to our findings, Wurzburger & Wright, (2015) reported root morphological traits shifting toward more acquisitive roots with K, P and NPK additions, with lower tissue density and higher specific length after 14 years of nutrient manipulation in Panama. Such contrasting responses compared to our study could also be attributed to

differences in root age, with our results representing < 3 month old fine roots, whilst Wurzburger & Wright, (2015) studied mixed-age roots sampled from standing stocks.

The addition of P and cations could have favoured the root production of some species with naturally thicker roots in our study site, but since our measurements refer to the community-level, we cannot determine the species-specific effect in our results. Also, roots can maximize nutrient uptake employing very contrasting root morphologies (Chen *et al.*, 2016) diluting the signal at the community level. Although small diameter roots are more efficient in exploring larger soil volumes in terms of plant biomass investment per unit volume of soil (Bates & Lynch, 2001; Hodge, 2004; Liu *et al.*, 2015), the increase in root diameter detected here could also provide increased mechanical protection against pathogens and herbivores (Laliberté *et al.*, 2015; Valverde-Barrantes *et al.*, 2017) and increased number or size of root cortical cells which could consequently increase levels of mycorrhizal colonisation (Brundrett, 2002; Guo *et al.*, 2008; Comas *et al.*, 2014). Since nutrient concentrations in root tissues increased following fertilisation, thicker diameter roots could be related to increased nutrient uptake through AM networks, either as a result of greater nutrient delivery per unit root length colonised or due to greater AM colonisation (see below; Eissenstat & Yanai, 1997; Eissenstat *et al.*, 2000; McCormack & Iversen, 2019).

Reduction in fine root phosphatase activity with P addition

A strong line of evidence for the role of P in controlling nutrient uptake strategies used by plants in our study is the significant decrease in root phosphatase activity with short-term P addition. A previous study demonstrated that root-surface phosphatase potential activity was a prevalent mechanism adopted by fine roots in Central Amazonian forests (Lugli *et al.*, 2020). Our results, therefore, support the idea that the exudation of phosphatase by plants is an important avenue for P acquisition in soils with low P availability in Central Amazonia (Guilbeault-Mayers *et al.*, 2020; Lugli *et al.*, 2020), and its rapid reduction suggests that this is indeed a resource-costly strategy. Together with the increase in fine root productivity captured here, the decrease in phosphatase potential activity point to a possible shift in soil P sources in our system, from organic to inorganic P, benefiting root foraging (*i.e.* direct root nutrient uptake or AM colonisation) over mining strategies. In soils with low P concentrations, plants tend to be efficient in acquiring P, which is usually accompanied with higher root phosphatase activity (Raghothama & Karthikeyan, 2005; Kitayama, 2013). However, the negative relationship

between root-surface phosphatase potential activity and P availability captured in previous soil gradient studies (*e.g.* Kitayama, 2013; Nasto *et al.*, 2014; Ushio *et al.*, 2015) could also be a result of differences in plant species composition and soil physical properties. By controlling such factors in our large-scale experiment, we demonstrate that plants can rapidly detect increased P availability, changing their investment in key root traits. The addition of N and cations, on the other hand, did not affect root-surface phosphatase potential activity rates, suggesting there had been no increase in P limitation following the addition of other nutrients.

Increase in AM colonisation with cations addition

We expected that the addition of nutrients would decrease root AM colonisation levels, under the assumption that with greater nutrient availability, plants would not invest as much in the fungal symbiosis to acquire nutrients. In contrast, we observed AM colonisation increasing with cation additions, suggesting that plants could be relying on the association with AM fungi to acquire cations or other nutrients. Long-term addition of P, but not K, increased AM colonisation in standing-stock roots growing in forests in Panama (Wurzburger & Wright, 2015). Although the major benefit of AM fungi symbiosis has been considered the translocation of P to the host plant (Smith & Read, 2010), AM fungi also have been shown to acquire other macro and micronutrients such as N, Ca, Mg, K and S in pioneer and early successional tree species (Siqueira *et al.*, 1998; Zangaro *et al.*, 2003). Moreover, the higher levels of AM colonisation found here could be related to thicker root diameter detected in plots where cations were added (Table 1; Supporting Information Fig. S2). Trees with thicker absorptive roots would benefit more from AM fungi increasing their nutrient foraging capacity (Eissenstat *et al.*, 2015; Liu *et al.*, 2015; Kong *et al.*, 2016; Chen *et al.*, 2016). In contrast, trees with with thinner roots may take up nutrients directly from the soil solution or rely on phosphatase activity, thus using complementary mechanisms to acquire nutrients (Lugli *et al.*, 2020). Nevertheless, the higher investment in AM fungi with cation addition detected in this Central Amazon forest, could also suggest AMs benefit plants by increasing the uptake of other macro and micronutrients. Alternatively, it has been suggested that greater investment in AM fungi can alter the microbial community in the rhizosphere and decrease plant susceptibility to pathogens (Koide, 1991; Herre *et al.*, 2007; Laliberté *et al.*, 2015).

Stimulation of fine root nutrient concentrations

The addition of P and cations increased the concentrations of most elements in fine roots. Due the chemical composition of the fertiliser used in our P treatment (triple superphosphate: about 45% of P_2O_5 and 15% of Ca), the addition of P not only increased P concentrations in fine roots but also of Ca (Wright, 2019). It is important to highlight that even with no changes in AM colonisation and lower levels of root-bound phosphatase activity, the addition of P increased both P and Ca concentrations in roots. This points to either i) a greater role of direct root nutrient uptake or ii) increased nutrient uptake efficiency per unit AM and/or per unit phosphatase exuded in our study site. Such trends are likely due to higher nutrient availability in the soil solution after fertilisation and a change of P source from primarily organic to inorganic P. The higher concentrations of Ca, Mg and K in fine roots after cations addition, demonstrates that we successfully increased cation availability and could also be a result of the higher levels of AM colonisation detected in our study site (Siqueira *et al.*, 1998; Zangaro *et al.*, 2003). The addition of N, however, did not affect the concentration of N or any other element in fine roots, suggesting that the extra N added to these already N-rich soils was not taken up by plants and/or that N concentrations in the root could be already at their optimal levels, with N being retranslocated to other plant tissues (Wurzburger & Wright, 2015). Therefore, plants growing in this Central Amazon forest strongly respond to the alleviation of rock-derived nutrient limitation and increase nutrient uptake, with a potential role for AM fungi driving some of these responses.

Implications for root functioning in Amazonian forests

By analysing a range of key root traits and root productivity, our study supports the hypothesis that P availability controls root functioning in Central Amazon forests, but the responses to cations also suggests that the role of rock-derived elements other than P has previously been underestimated. We found partial support for our hypothesis that nutrient addition would shift root traits from an acquisitive to a more conservative strategy. With P addition, we did find evidence for reduced investment in P acquisition, with reduced investment in mining P via phosphatases, and there was equivocal evidence for increases in root diameter. On the other hand, in contrast to our hypothesis, total root productivity increased suggesting direct root foraging for available P had become a more important strategy. With cations, we did observe a shift towards more conservative root traits with greater average root diameter, but AM

524 colonisation increased suggesting a change in nutrient acquisition strategy rather than an
525 overall shift to less acquisitive root traits. Direct comparisons with other studies in tropical
526 forests are complicated because there is limited information on root productivity responses to
527 nutrient manipulation, and traits have tended not been measured on roots of a known age.
528 However, previous tropical nutrient manipulation experiments, installed in relatively more
529 fertile soils in Central America, did not find strong support for P controlling root traits and fine
530 root biomass, and only one studied the effect of cations (K, only) (Yavitt *et al.*, 2011;
531 Wurzburger & Wright, 2015; Alvarez-Clare & Mack, 2015). Based on the different responses
532 among fertilisation experiments, we suggest that soil nutrient availability may be even more
533 important in determining fine root dynamics in Amazonian forests than Central American
534 forests. Nevertheless, we stress the importance of continuous monitoring in long-term
535 manipulation experiments, to determine whether responses persist with chronic nutrient
536 addition. Overall, our findings increase understanding of the plasticity of belowground plant
537 traits and the factors controlling these responses, demonstrating that multiple nutrients shape
538 belowground processes in Central Amazonian forests and that even slow-growing tropical
539 rainforest in low fertility soils can respond very rapidly to nutrient additions. Phosphorus and
540 cation availability, and changes in resource allocation to nutrient acquisition by Amazonian
541 trees, thus, will likely play a key role in determining responses to future environmental change
542 in these forests.
543

Acknowledgments

544 The authors would like to thank Jefferson "Pedrinho" Cruz, Alexandre dos Santos and Benedito
545 Sergio da Silva for helping in the field sampling and Adriana da Conceição for helping
546 processing root samples, Cilene Palheta and the Laboratorio Tematico de Microscopia Optica
547 e Eletronica (LTMOE), Laboratorio Tematico de Plantas e Solos (LTSP) at INPA, Manaus and
548 the BDFFP (Biological Dynamics of Forest Fragments Project) for their help during the field
549 campaign and lab analyses. We are very thankful to Bruno Takeshi for his help with the project
550 logistics. This research was supported by the Brazilian National Council for Scientific and
551 Technological Development (CNPq) through the Science Without Borders Program grant
552 number 206826/2014-1 and UK's Natural Environment Research Council (NERC), grant
553 number NE/L007223/1; C. A. Q. acknowledges the support from CNPq/LBA 68/2013 research
554 grant 458020/2013-3. P. M. also acknowledges support from ARC, DP170104091 and L. F.

555 acknowledges the support of the European Research Council grant ERC-SyG-2013-610028.
556 This paper is number 801 on the BDFFP series.
557

Author Contributions

558 LFL, KMA, LMM and IPH designed the research; LFL, JSR, RVA, MP, RLA, KMA, ALC,
559 HFVC, RP, LF, JLC, NPM, ACMM, STS and KJS performed the research, assisting with field
560 sampling, logistics and/or laboratory analyses; IPH, LMM, CAQ, LEOCA, LFL and PM and
561 wrote the grants that funded this research; LFL, KMA and JSR analysed the data; LFL, IPH,
562 KMA, LMM, RLA, ALC, HFVC, LF, JLC, LEOCA, PM, OJVB, and CAQ commented on the
563 manuscript. All authors approved the manuscript.
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References

566 **Addo-Danso SD, Defrenne CE, McCormack ML, Ostonen I, Addo-Danso A, Foli EG,**
567 **Borden KA, Isaac ME, Prescott CE. 2020**. Fine-root morphological trait variation in tropical
568 forest ecosystems: an evidence synthesis. *Plant Ecology* **221**: 1–13.

569 **Aerts R. 1999**. Interspecific competition in natural plant communities: mechanisms, trade-
570 offs and plant-soil feedbacks. *Journal of Experimental Botany* **50**: 29–37.

571 **Aerts R, Chapin FS. 1999**. The Mineral Nutrition of Wild Plants Revisited: A Re-
572 evaluation of Processes and Patterns. In A. H. Fitter and D. G. Raffaelli (Ed.): *Advances in*
573 *Ecological Research*. (Vol. **30**, pp. 1-67). London, UK: Academic Press.

574 **Ainsworth EA, Long SP. 2004**. What have we learned from 15 years of free-air CO2
575 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy
576 properties and plant production to rising CO2. *New Phytologist* **165**: 351–372.

577 **Alvarez-Clare S, Mack MC. 2015**. Do Foliar, Litter, and Root Nitrogen and Phosphorus
578 Concentrations Reflect Nutrient Limitation in a Lowland Tropical Wet Forest? (L
579 Schwendenmann, Ed.). *PLOS ONE* **10**: e0123796.

580 **Alvarez-Clare S, Mack MC, Brooks M. 2013**. A direct test of nitrogen and phosphorus
581 limitation to net primary productivity in a lowland tropical wet forest. *Ecology* **94**: 1540–1551.

582 **Aragão LEOC, Malhi Y, Metcalfe DB, Silva-Espejo JE, Jiménez E, Navarrete D,**
583 **Almeida S, Costa ACL, Salinas N, Phillips OL, *et al.* 2009**. Above- and below-ground net
584 primary productivity across ten Amazonian forests on contrasting soils. *Biogeosciences* **6**:
585 2759–2778.

586 **Araújo AC, Nobre AD, Kruijt B, Elbers JA, Dallarosa R, Stefani P, Randow C von,**
587 **Manzi AO, Culf AD, Gash JHC, *et al.* 2002**. Comparative measurements of carbon dioxide
588 fluxes from two nearby towers in a central Amazonian rainforest: The Manaus LBA site.
589 *Journal of Geophysical Research* **107**: 8090.

590 **Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers**
591 **PJ, Shachar-Hill Y. 2003**. Carbon Export from Arbuscular Mycorrhizal Roots Involves the
592 Translocation of Carbohydrate as well as Lipid. *Plant physiology* **131**: 1496–1507.

593 **Bates TR, Lynch JP. 2001**. Root hairs confer a competitive advantage under low
594 phosphorus availability. *Plant and Soil* **236**: 243–250.

595 **Bates D, Mächler M, Bolker B, Walker S. 2014**. Fitting Linear Mixed-Effects Models

using lme4. URL: *arXiv preprint arXiv:1406.5823.* Accessed in July 2018.

Beer C, Reichstein M, Tomelleri E, Ciais P, Jung M, Carvalhais N, Rodenbeck C, Arain MA, Baldocchi D, Bonan GB, *et al.* 2010. Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. *Science* **329**: 834–838.

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

Brundrett MC. **2002**. Coevolution of roots and mycorrhizas of land plants. *New Phytologist* **154**: 275–304.

Brundrett MC, Piché Y, Peterson RL. **1984**. A new method for observing the morphology of vesicular–arbuscular mycorrhizae. *Canadian Journal of Botany* **62**: 2128–2134.

Chapin FS. **1980**. The Mineral Nutrition of Wild Plants. *Annual Review of Ecology and Systematics* **11**: 233–260.

Chen J, Groenigen KJ, Hungate BA, Terrer C, Groenigen J, Maestre FT, Ying SC, Luo Y, Jørgensen U, Sinsabaugh RL, *et al.* 2020. Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. *Global Change Biology*: gcb.15218.

Chen W, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM. **2016**. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences* **113**: 8741–8746.

Cleveland CC, Townsend AR, Taylor P, Alvarez-Clare S, Bustamante MMC, Chuyong G, Dobrowski SZ, Grierson P, Harms KE, Houlton BZ, *et al.* 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. *Ecology Letters* **14**: 939–947.

Comas LH, Callahan HS, Midford PE. **2014**. Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. *Ecology and Evolution* **4**: 2979–2990.

Cross AF, Schlesinger WH. **1995**. A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* **64**: 197–214.

Eissenstat DM, Kucharski JM, Zadworny M, Adams TS, Koide RT. **2015**. Linking root traits to nutrient foraging in arbuscular mycorrhizal trees in a temperate forest. *New Phytologist* **208**: 114–124.

628 **Eissenstat DM, Wells CE, Yanai RD, Whitbeck JL**. **2000**. Building roots in a changing
629 environment: implications for root longevity. *New Phytologist* **147**: 33–42.
630 **Eissenstat DM, Yanai RD**. **1997**. The Ecology of Root Lifespan. In M. Begon and A. H.
631 Fitter (Ed.): *Advances in ecological research* (Vol. **27**, pp. 1-60). San Diego, CA, USA:
632 Academic Press.
633 **Fleischer K, Rammig A, De Kauwe MG, Walker AP, Domingues TF, Fuchslueger L,**
634 **Garcia S, Goll DS, Grandis A, Jiang M, *et al.* 2019**. Amazon forest response to CO2
635 fertilization dependent on plant phosphorus acquisition. *Nature Geoscience* **12**: 736–741.
636 **Freschet GT, Valverde-Barrantes OJ, Tucker CM, Craine JM, McCormack ML,**
637 **Violle C, Fort F, Blackwood CB, Urban-Mead KR, Iversen CM, *et al.* 2017**. Climate, soil
638 and plant functional types as drivers of global fine-root trait variation (JC Cahill, Ed.). *Journal*
639 *of Ecology* **105**: 1182–1196.
640 **German DP, Weintraub MN, Grandy AS, Lauber CL, Rinkes ZL, Allison SD**. **2011**.
641 Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology*
642 *and Biochemistry* **43**: 1387–1397.
643 **Guilbeault-Mayers X, Turner BL, Laliberté E**. **2020**. Greater root phosphatase activity
644 of tropical trees at low phosphorus despite strong variation among species. *Ecology*. e03090.
645 **Guo D, Li H, Mitchell RJ, Han W, Hendricks JJ, Fahey TJ, Hendrick RL**. **2008**. Fine
646 root heterogeneity by branch order: exploring the discrepancy in root turnover estimates
647 between minirhizotron and carbon isotopic methods. *New Phytologist* **177**: 443–456.
648 **Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P**.
649 **2012**. Functions of Macronutrients. In P. Marschner (Ed): *Marschner's mineral nutrition of*
650 *higher plants* (pp. 135-189). London, UK: Academic Press.
651 **Herre EA, Mejía LC, Kyllo DA, Rojas E, Maynard Z, Butler A, Van Bael SA**. **2007**.
652 Ecological implications of anti-pathogen effects of tropical fungal endophytes and
653 mycorrhizae. *Ecology* **88**: 550–558.
654 **Hinsinger P**. **2001**. Bioavailability of soil inorganic P in the rhizosphere as affected by
655 root-induced chemical changes: a review. *Plant and Soil* **237**: 173–195.
656 **Hodge A**. **2004**. The plastic plant: root responses to heterogeneous supplies of nutrients.
657 *New Phytologist* **162**: 9–24.
658 **Holdaway RJ, Richardson SJ, Dickie IA, Peltzer DA, Coomes DA**. **2011**. Species- and
659 community-level patterns in fine root traits along a 120 000-year soil chronosequence in

660 temperate rain forest. *Journal of Ecology* **99**: 954–963.
661 **Hungate BA, Johnson DW, Dijkstra P, Hymus G, Stiling P, Megonigal JP, Pagel AL,**
662 **Moan JL, Day F, Li J, *et al.* 2006**. Nitrogen cycling during seven years of atmospheric CO2
663 enrichment in a scrub oak woodland. *Ecology* **87**: 26–40.
664 **Janssens IA, Dieleman W, Luyssaert S, Subke J-A, Reichstein M, Ceulemans R, Ciais**
665 **P, Dolman AJ, Grace J, Matteucci G, *et al.* 2010**. Reduction of forest soil respiration in
666 response to nitrogen deposition. *Nature Geoscience* **3**: 315–322.
667 **Jiménez EM, Moreno FH, Peñuela MC, Patiño S, Lloyd J**. **2009**. Fine root dynamics
668 for forests on contrasting soils in the Colombian Amazon. *Biogeosciences* **6**: 2809–2827.
669 **Kimball BA, Idso SB**. **1983**. Increasing atmospheric CO2: effects on crop yield, water use
670 and climate. *Agricultural Water Management* **7**: 55–72.
671 **Kitayama K**. **2013**. The activities of soil and root acid phosphatase in the nine tropical
672 rain forests that differ in phosphorus availability on Mount Kinabalu, Borneo. *Plant and Soil*
673 **367**: 215–224.
674 **Koide RT**. **1991**. Nutrient supply, nutrient demand and plant response to mycorrhizal
675 infection. *New Phytologist* **117**: 365–386.
676 **Kong DL, Wang JJ, Kardol P, Wu HF, Zeng H, Deng XB, Deng Y**. **2016**. Economic
677 strategies of plant absorptive roots vary with root diameter. *Biogeosciences* **13**: 415–424.
678 **Kuznetsova A, Brockhoff PB, Christensen RHB**. **2017**. lmerTest Package: Tests in
679 Linear Mixed Effects Models. *Journal of Statistical Software* **82**(13), pp.1-26.
680 **Laliberté E, Lambers H, Burgess TI, Wright SJ**. **2015**. Phosphorus limitation, soil-
681 borne pathogens and the coexistence of plant species in hyperdiverse forests and shrublands.
682 *New Phytologist* **206**: 507–521.
683 **Lambers H, Rave J, Shave G, Smith S**. **2008**. Plant nutrient-acquisition strategies change
684 with soil age. *Trends in Ecology & Evolution* **23**: 95–103.
685 **Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ**. **2006**. Root Structure
686 and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and
687 Physiological Traits. *Annals of Botany* **98**: 693–713.
688 **Liu B, Li H, Zhu B, Koide RT, Eissenstat DM, Guo D**. **2015**. Complementarity in
689 nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14
690 coexisting subtropical tree species. *New Phytologist* **208**: 125–136.
691 **Lugli LF, Andersen KM, Aragão LEOC, Cordeiro AL, Cunha HF V., Fuchslueger L,**

Meir P, Mercado LM, Oblitas E, Quesada CA, *et al.* 2020. Multiple phosphorus acquisition strategies adopted by fine roots in low-fertility soils in Central Amazonia. *Plant and Soil* **450**: 49–63.

Lynch JP, Ho MD, Phosphorus L. 2005. Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant and Soil* **269**: 45–56.

Ma Z, Guo D, Xu X, Lu M, Bardgett RD, Eissenstat DM, McCormack ML, Hedin LO. 2018. Evolutionary history resolves global organization of root functional traits. *Nature* **555**: 94–97.

Malavolta E, Vitti GC, Oliviera SA. 1989. *Avaliação do estado nutricional das plantas: princípios e aplicacoes.* Associação Brasileira para Pesquisa da Potassa e do Fosfato, Piracicaba, SP (Brasil). Piracicaba, SP (Brasil).

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari H-S, Hobbie EA, Iversen CM, Jackson RB, *et al.* 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* **207**: 505–518.

McCormack ML, Iversen CM. 2019. Physical and Functional Constraints on Viable Belowground Acquisition Strategies. *Frontiers in Plant Science* **10**: 1–12.

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

Metcalfe DB, Meir P, Aragão LEOC, da Costa ACL, Braga AP, Gonçalves PHL, de Athaydes Silva Junior J, de Almeida SS, Dawson LA, Malhi Y, *et al.* 2008. The effects of water availability on root growth and morphology in an Amazon rainforest. *Plant and Soil* **311**: 189–199.

Metcalfe DB, Williams M, Aragão LEOC, da Costa ACL, de Almeida SS, Braga AP, Gonçalves PHL, de Athaydes J, Junior S, Malhi Y, *et al.* 2007. A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. *New Phytologist* **174**: 697–703.

Mirabello MJ, Yavitt JB, Garcia M, Harms KE, Turner BL, Wright SJ. 2013. Soil phosphorus responses to chronic nutrient fertilisation and seasonal drought in a humid lowland forest, Panama. *Soil Research* **51**: 215.

Nasto MK, Alvarez-Clare S, Lekberg Y, Sullivan BW, Townsend AR, Cleveland CC.

2014. Interactions among nitrogen fixation and soil phosphorus acquisition strategies in lowland tropical rain forests (N Johnson, Ed.). *Ecology Letters* **17**: 1282–1289.

Norby RJ, DeLucia EH, Gielen B, Calfapietra C, Giardina CP, King JS, Ledford J, McCarthy HR, Moore DJP, Ceulemans R, *et al.* 2005. Forest response to elevated CO2 is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences* **102**: 18052–18056.

Olander LP, Vitousek PM. **2004**. Biological and Geochemical Sinks for Phosphorus in Soil from a Wet Tropical Forest. *Ecosystems* **7**: 404–419.

Ostertag R. **2001**. Effects of Nitrogen and Phosphorus Availability on Fine-Root Dynamics in Hawaiian Montane Forests. *Ecology* **82**: 485.

Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, *et al.* 2011. A Large and Persistent Carbon Sink in the World's Forests. *Science* **333**: 988–993.

Parniske M. **2008**. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nature Reviews Microbiology* **6**: 763–775.

Peng Y, Guo D, Yang Y. **2017**. Global patterns of root dynamics under nitrogen enrichment. *Global Ecology and Biogeography* **26**: 102–114.

Le Quéré C, Andrew RM, Friedlingstein P, Sitch S, Hauck J, Pongratz J, Pickers PA, Korsbakken JI, Peters GP, Canadell JG, *et al.* 2018. Global Carbon Budget 2018. *Earth System Science Data* **10**: 2141–2194.

Quesada CA, Lloyd J, Schwarz M, Patiño S, Baker TR, Czimczik C, Fyllas NM, Martinelli L, Nardoto GB, Schmerler J, *et al.* 2010. Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences* **7**: 1515–1541.

Quesada CA, Phillips OL, Schwarz M, Czimczik CI, Baker TR, Patiño S, Fyllas NM, Hodnett MG, Herrera R, Almeida S, *et al.* 2012. Basin-wide variations in Amazon forest structure and function are mediated by both soils and climate. *Biogeosciences* **9**: 2203–2246.

Raghothama KG. **1999**. PHOSPHATE ACQUISITION. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 665–693.

Raghothama KG, Karthikeyan AS. **2005**. Phosphate Acquisition. *Plant and Soil* **274**: 37–49.

Siqueira JO, Carneiro MAC, Curi N, Rosado SC da S, Davide AC. **1998**. Mycorrhizal colonization and mycotrophic growth of native woody species as related to successional groups

in Southeastern Brazil. *Forest Ecology and Management* **107**: 241–252.

Smith SE, Read D. **2010**. *Mycorrhizal symbiosis*. New York, NY, USA: Academic Press.

Sullivan BW, Alvarez-Clare S, Castle SC, Porder S, Reed SC, Schreeg L, Townsend AR, Cleveland CC. **2014**. Assessing nutrient limitation in complex forested ecosystems: alternatives to large-scale fertilization experiments. *Ecology* **95**: 668–681.

Team RC. **2018**. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL *http://www.R-project.org/.* Accessed in January 2018.

Tremblay A, Ransijn J. **2015**. Model selection and post-hoc analysis for (G)LMER Models. : R package version 2.10.

Treseder KK, Vitousek PM. **2001**. Effects of soil nutrient availability on investment in acquisition of n and p in hawaiian rain forests. *Ecology* **82**: 946–954.

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

Turner BL, Romero TE. **2010**. Stability of hydrolytic enzyme activity and microbial phosphorus during storage of tropical rain forest soils. *Soil Biology and Biochemistry* **42**: 459–465.

Valverde-Barrantes OJ, Freschet GT, Roumet C, Blackwood CB. **2017**. A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants (L Poorter, Ed.). *New Phytologist* **215**: 1562–1573.

Valverde-Barrantes OJ, Smemo KA, Feinstein LM, Kershner MW, Blackwood CB. **2013**. The distribution of below-ground traits is explained by intrinsic species differences and intraspecific plasticity in response to root neighbours (D Guo, Ed.). *Journal of Ecology* **101**: 933–942.

Vance CP, Uhde-Stone C, Allan DL. **2003**. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157**: 423–447.

Vitousek PM, Sanford RL. **1986**. Nutrient Cycling in Moist Tropical Forest. *Annual Review of Ecology and Systematics* **17**: 137–167.

Walker TW, Syers JK. **1976**. The fate of phosphorus during pedogenesis. *Geoderma* **15**: 1–19.

Wardle DA. **2004**. Ecosystem Properties and Forest Decline in Contrasting Long-Term Chronosequences. *Science* **305**: 509–513.

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

Wright SJ. **2019**. Plant responses to nutrient addition experiments conducted in tropical forests. *Ecological Monographs* **89**: 1–18.

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

Wright SJ, Yavitt JB, Wurzburger N, Turner BL, Tanner EVJ, Sayer EJ, Santiago LS, Kaspari M, Hedin LO, Harms KE, *et al.* 2011. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* **92**: 1616–1625.

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

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

Zangaro W, Nisizaki SMA, Domingos JCB, Nakano EM. **2003**. Mycorrhizal response and successional status in 80 woody species from south Brazil. *Journal of Tropical Ecology* **19**: 315–324.

Zemunik G, Turner BL, Lambers H, Laliberté E. **2015**. Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature Plants* **1**: 15050.

Tables

Table 1 Mean root diameter, specific root length (SRL), specific root area (SRA) and root tissue density (RTD) ± standard errors with and without the addition of N, P and cations in two soil depths (0-10 cm and 10-30 cm) and for the mean 0-30 cm depth. *n*= 16 per treatment per depth. Significant effects of the N, P and cations by depth (*e.g.* -P *versus* +P) are indicated by * and **, representing probability at the 5 and 1 % levels, respectively.

Nutrient	Depth	Diameter (mm)	SRL (cm g^{-1})	SRA (cm^2 g^{-1})	RTD (g cm^{-3})
-N	0-10 cm	1.05 ± 0.03	1,150 ± 56	290 ± 11.14	0.16 ± 0.005
	10-30 cm	1.06 ± 0.03	1,260 ± 69	308 ± 10.01	0.15 ± 0.004
	0-30 cm	1.05 ± 0.02	1,200 ± 53	299 ± 9.30	0.15 ± 0.004
+N	0-10 cm	1.09 ± 0.03	1,310 ± 102	320 ± 18.68	0.15 ± 0.004
	10-30 cm	1.11 ± 0.03	1,220 ± 82	308 ± 16.19	0.15 ± 0.007
	0-30 cm	1.10 ± 0.02	1,267 ± 64	313 ± 11.01	0.15 ± 0.003
-P	0-10 cm	1.02 ± 0.04	1,250 ± 94	304 ± 15.89	0.16 ± 0.005
	10-30 cm	1.07 ± 0.03	1,220 ± 61	305 ± 10.66	0.15 ± 0.005
	0-30 cm	1.05 ± 0.02	1,240 ± 60	304 ± 9.71	0.15 ± 0.005
+P	0-10 cm	1.11 ± 0.02*	1,210 ± 75	306 ± 15.81	0.15 ± 0.005
	10-30 cm	1.09 ± 0.03	1,260 ± 88	310 ± 15.74	0.15 ± 0.006
	0-30 cm	1.10 ± 0.02	1,230 ± 58	308 ± 10.94	0.15 ± 0.004
-Cations	0-10 cm	1.03 ± 0.03	1,260 ± 83	308 ± 14.63	0.15 ± 0.005
	10-30 cm	1.04 ± 0.03	1,330 ± 68	322 ± 13.20	0.14 ± 0.005
	0-30 cm	1.03 ± 0.03	1,290 ± 57	315 ± 10.60	0.15 ± 0.004
+Cations	0-10 cm	1.11 ± 0.03	1,200 ± 87	302 ± 16.95	0.15 ± 0.006
	10-30 cm	1.13 ± 0.03	1,150 ± 76	293 ± 12.61	0.15 ± 0.006
	0-30 cm	1.12 ± 0.02**	1,180 ± 57	297 ± 9.57	0.15 ± 0.003

825 **Figures**

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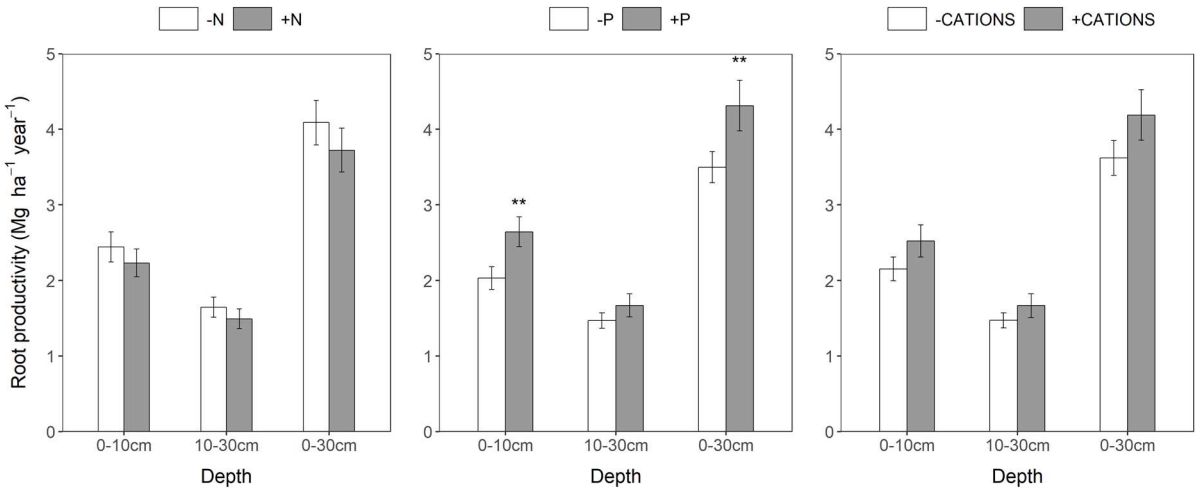

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830 Fig. 1. Fine root productivity in Mg ha^{-1} $year^{-1}$ for the 0-10 and 10-30 cm soil depths and sum
831 of the whole soil core (0-30 cm) with and without the addition of N, P and cations in a lowland
832 tropical forest in Central Amazon, Brazil. Each panel contrasts 16 plots with and without the
833 addition of nutrients in each depth. Means ±1SE (n=16) are presented. Significant effects of
834 the N, P and cations are indicated by ** representing probability at the 1% level.

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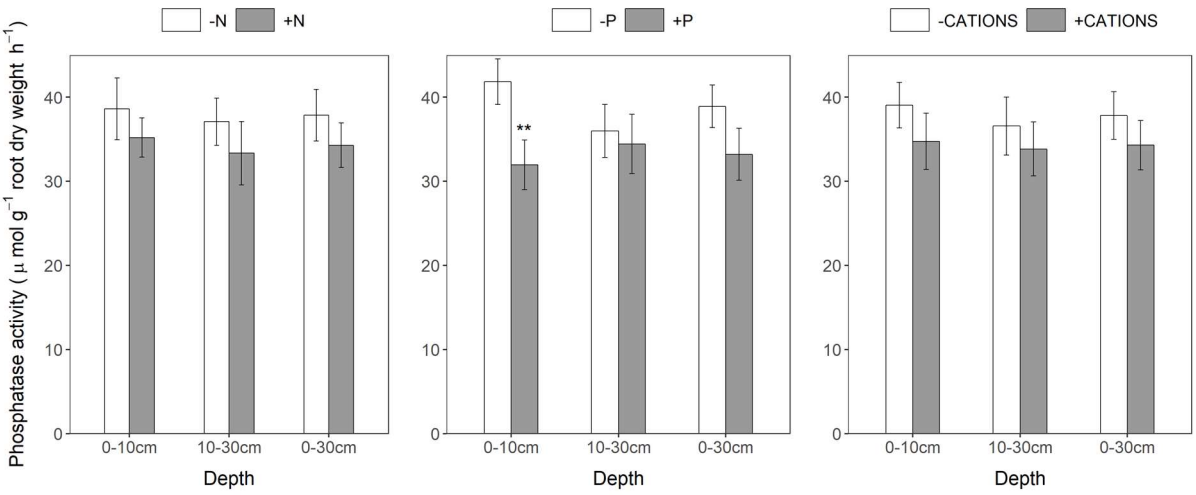
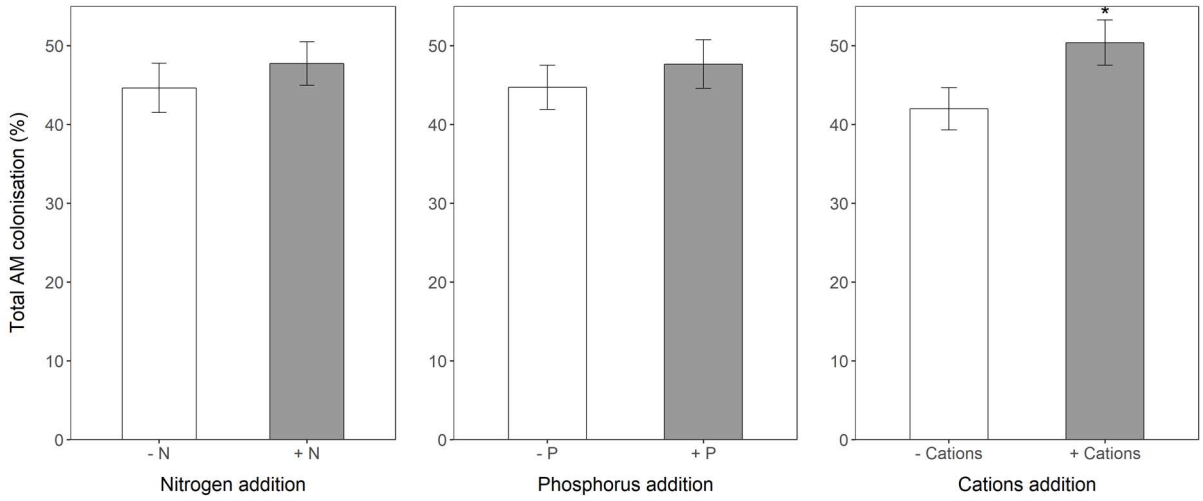


Fig. 2. Mean root phosphatase activity in µmol g^{-1} root dry weight $hour^{-1}$ for the 0-10 and 10-30 cm soil depths and mean of the whole soil core (0-30 cm) with and without the addition of N, P and cations in a lowland tropical forest in Central Amazon, Brazil. Each panel contrasts 16 plots with and without the addition of nutrients in each depth. Means ±1SE (n=16) are presented. Significant effects of the N, P and cations are indicated by ** representing probability at the 1% level.



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849 Fig. 3. Total root arbuscular mycorrhizal colonisation in % root length for roots from the 0-10
850 cm soil layer with and without the addition of N, P and cations in a lowland tropical forest in
851 Central Amazonia, Brazil. Each panel contrasts 16 plots with and without the addition of
852 nutrient. Means ±1SE (n=16) are presented. Significant effects of the N, P and cations are
853 indicated by * representing probability at the 5% level.

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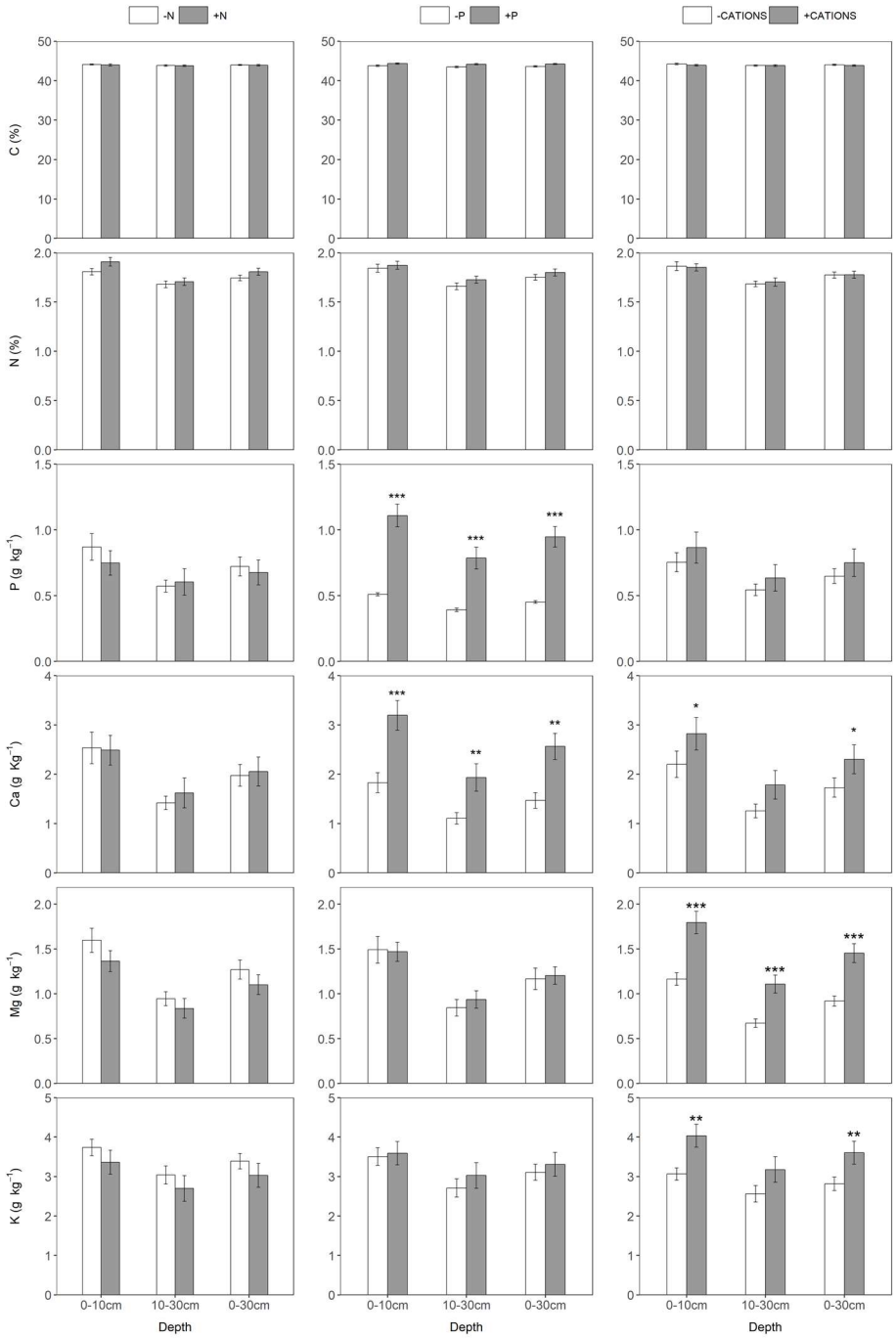

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857 Fig. 4. Element concentrations in fine root tissues for the 0-10 and 10-30 cm soil depths and
858 mean of the whole soil core (0-30 cm) with and without the addition of N, P and cations in a
859 lowland tropical forest in central Amazon, Brazil. Concentrations of C and N are given as %
860 and concentrations of P, Ca, Mg and K are given in g kg^{-1}. Each panel contrasts 16 plots per
861 depth with and without the addition of nutrient. Means ±1SE (n=16) are presented. Significant
862 effects of the N, P and cations are indicated by *, **, and ***, representing probability at the
863 5, 1, and 0.1 % levels, respectively.