# Direct evidence for phosphorus limitation on Amazon forest productivity

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The productivity of rainforests growing on highly weathered tropical soils is expected to be limited by phosphorus availability[1]. Yet, controlled fertilization experiments have been unable to demonstrate a dominant role for phosphorus in controlling tropical forest net primary productivity. Recent syntheses have demonstrated that responses to nitrogen addition are as large as to phosphorus[2], and adaptations to low phosphorus availability appear to enable net primary productivity to be maintained across major soil phosphorus gradients[3]. Thus, the extent to which phosphorus availability limits tropical forest productivity is highly uncertain. The majority of the Amazonia, however, is characterized by soils that are more depleted in phosphorus than those in which most tropical fertilization experiments have taken place[2]. Thus, we established a phosphorus, nitrogen and base cation addition experiment in an old growth Amazon rainforest, with a low soil phosphorus content that is representative of approximately 60% of the Amazon basin. Here we show that net primary productivity increased exclusively with phosphorus addition. After 2 years, strong responses were observed in fine root (+29%) and canopy productivity (+19%), but not stem growth. The direct evidence of phosphorus limitation of net primary productivity suggests that phosphorus availability may restrict Amazon forest responses to $CO_2$ fertilization[4], with major implications for future carbon sequestration and forest resilience to climate change.

The inclusion of nutrient cycling in Earth systems models has substantially reduced predictions of future carbon uptake by vegetation[4–7] under conditions of increased atmospheric $CO_2$. Furthermore, fundamental differences between the cycles of nitrogen and rock-derived elements such as phosphorus, mean that phosphorus limitation may place a greater constraint on plant responses to $CO_2$ fertilization than nitrogen limitation[8,9]. During soil development[10], the weathering of rocks or parent material provides the major source of phosphorus for initial vegetation development. Over millions of years, however, the parent material is gradually depleted, and available phosphorus, as well as rock-derived base cations such as calcium, magnesium and potassium may be lost through leaching or made unavailable through occlusion by iron and aluminium oxides, with organic forms of phosphorus becoming key pools in depleted and highly weathered systems[10,11]. Meanwhile, nitrogen tends to accumulate over time, with inputs from biological fixation and atmospheric deposition exceeding nitrogen losses[12]. For these reasons, a long-standing paradigm in tropical ecology (the 'phosphorus paradigm') has been that forest productivity on highly weathered soils, such as in those in central Amazonia, is limited primarily by plant available phosphorus[13], with a potential secondary role of other rock-derived elements. Supporting this paradigm, seminal forest ecology studies have demonstrated very low levels of phosphorus and base cations in plant tissues in Amazonia[14], and high carbon:phosphorus ratios in litterfall of tropical forest more generally[1]. Greater wood productivity has also been observed in forests growing on fertile soils in western Amazonia when compared to less fertile sites

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in central and eastern portions of the Amazon basin, with the strongest relationships being with total soil phosphorus[15,16]. However, across the Amazon basin, climatic and edaphic factors covary[17], influencing species distributions, standing forest biomass and turnover rates[16]. Thus, directly determining the extent to which soil fertility controls tropical forest growth and the elements that are most important, remains a key knowledge gap[18], and addressing this is critical for understanding forest growth dynamics and predicting responses to $CO_2$ fertilization[19].

By minimizing confounding factors, manipulation experiments can identify directly which specific elements limit forest productivity[20]. Although no large-scale nitrogen, phosphorus and base cation experiment has been carried out in Amazonia until now, a recent synthesis study argued that there is as much evidence for nitrogen limitation of tropical forest productivity as there is for phosphorus[2]. For example, in Costa Rica, phosphorus additions did not elicit any changes in litterfall and fine root productivity in two years after fertilization[21], and in Panama, an increase in litter production with phosphorus addition was evident only eight years after fertilization[22], with initial responses being stronger for nitrogen additions, at least in the rainy season[23]. Critically, previous nutrient-manipulation studies in primary tropical rainforests have taken place mainly where total soil phosphorus contents are much higher than in central and eastern Amazonia (443–1,600 mg kg$^{-1}$ versus 70–120 mg kg$^{-1}$ in typical Amazon Ferralsols). In Amazonia, fertilization experiments have been carried out in secondary forests, but little evidence for strong phosphorus limitation has been observed[24,25], with nitrogen availability found to be important during initial forest recovery[26,27]. There have been fertilization experiments in forests growing on soils with phosphorus as low as in Amazonia in Cameroon[28] and Borneo[29]. These studies have also generally failed to provide clear support for the phosphorus paradigm, with no positive effects of phosphorus addition being observed[28], or with responses to nitrogen being at least as large as those to phosphorus[29]. However, the tree communities were very different to those found across Amazonia, with fundamental differences in nutrient uptake strategies, including contrasting mycorrhizal associations. Therefore, although previous fertilization studies strongly question the ubiquity of phosphorus limitation in tropical forests, their results cannot be extrapolated to Amazonian forests, especially those growing on low-fertility soils in central and eastern regions of the basin.

To address this major knowledge gap, in 2017, we set up a large-scale fully factorial nitrogen, phosphorus and base cation addition experiment in lowland tropical evergreen rainforest near Manaus, Brazil (the Amazon Fertilisation Experiment (AFEX)), manipulating 8 hectares of forest across 32 plots in 4 blocks[30]. The Ferralsols of the study site have low concentrations of total phosphorus and base cations that are characteristic of up to 60% of Amazon forest soils[31] (Fig. 1). To determine directly which nutrients control Amazon forest productivity, we measured the responses of fine root, stem wood and litterfall production between 2017 and 2019 (Methods), making nearly 1,500 measurements of canopy production, quantifying root productivity every 3 months across 160 locations and measuring the growth of 4,849 trees. Notably, our base cation treatment added the same amount of calcium as in the super-triple phosphate that was used in the phosphorus addition treatment. Thus, comparisons between these treatments ensure that the effects of phosphorus can be isolated.

Annual net primary productivity (NPP) increased rapidly with the addition of phosphorus in a Central Amazon forest. After 2 years of phosphorus addition, annual NPP significantly increased by 1.16 Mg C ha$^{-1}$ yr$^{-1}$ (15.6%; with phosphorus addition (+P): 8.60 ± 0.33 Mg C ha$^{-1}$ yr$^{-1}$ versus without phosphorus addition (−P): 7.44 ± 0.21 Mg C ha$^{-1}$ yr$^{-1}$; $F_{1,27}$ = 9.56, $P$ = 0.005) (Fig. 2a), owing to greater canopy and fine root productivity. No significant effects of nitrogen and base cation addition were observed on total NPP or any of its components measured. The increase in NPP may have been driven by the increase in phosphorus availability stimulating GPP[32], and/or through reductions in autotrophic respiration[33]. Forests growing on high-fertility soils may produce biomass more efficiently and thus show greater carbon use efficiency[34] (the ratio of net carbon gain to gross carbon assimilated). Although the direct causes of changes are not yet clear, our results clearly demonstrate that NPP in this forest is limited by phosphorus alone. The observed increase in NPP with phosphorus addition, and the lack of any nitrogen response, contrasts strongly with a meta-analysis based on previous tropical forest fertilization studies[2], with the lower levels of soil phosphorus in Amazonia probably explaining this contrast (Fig. 1). We have previously observed that base cation addition affects root morphology and mycorrhizal colonization[30]. Thus, whereas base cation availability does not appear to limit NPP, it seems to influence key belowground processes.

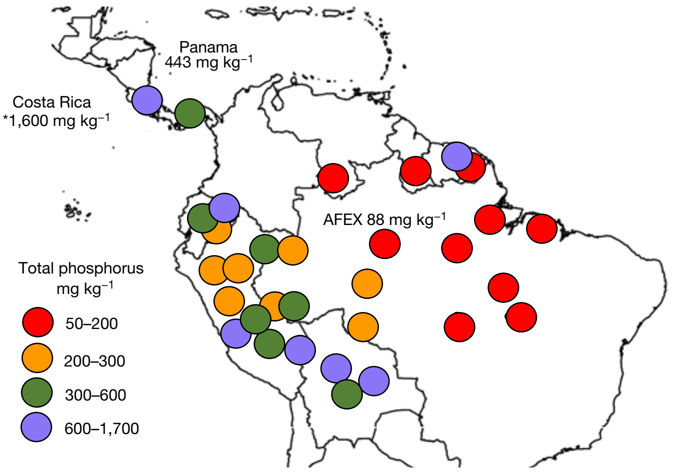

**Fig. 1 | Total soil phosphorus measured in primary forest plots across the Amazon basin, showing the low phosphorus concentration at our site and across central and eastern Amazonia.** The fertility gradient across the Amazon basin. Red circles show the lowest total phosphorus concentration and purple circles show the highest. The 2 large-scale fertilization experiments in Central American terra firme tropical forest are also shown, highlighting the 5–18-fold greater total phosphorus concentrations than in the central Amazon basin. Total phosphorus concentrations are derived from Quesada & Lloyd[49], except those for Costa Rica[21] and Panama[40]. Values are for 0–30 cm soil depth, except where indicated by the asterisk (0–10 cm soil depth).

We observed a substantial 0.83 Mg C ha$^{-1}$ yr$^{-1}$ (19%; +P: 5.19 ± 0.15 Mg C ha$^{-1}$ yr$^{-1}$ versus −P: 4.36 ± 0.12 Mg C ha$^{-1}$ yr$^{-1}$; $F_{1,30}$ = 18.3, $P$ < 0.001) (Fig. 2b), increase in canopy productivity. Investment in leaf production provides a return revenue stream of photosynthate that can promote NPP of other tissues and can be used to acquire other limiting resources[35] such as light and nutrients. We observed weak evidence towards higher leaf area index (LAI) with phosphorus addition over the first 1.5 years of the experiment (3.6% increase: +P: 5.75 ± 0.10 versus −P: 5.55 ± 0.15; $F_{1,27}$ = 1.76, $P$ = 0.20) (Extended Data Fig. 1), which may have had minor contributions to enhanced rates of carbon gain. The increase in litterfall productivity at our site appears to result from a decrease in leaf life span, which was estimated to have decreased by 10–20% following phosphorus addition (+P: 1.03 ± 0.04 yr versus −P: 1.15 ± 0.05 yr; $F_{1,30}$ = 4.08, $P$ = 0.05 and +P: 1.15 ± 0.05 yr versus −P: 1.56 ± 0.07 yr; $F_{1,27}$ = 28.4, $P$ = 0.0000127 for fresh and litter leaves, respectively; Methods) (Extended Data Fig. 2). Therefore, the increases in leaf turnover appear to be important in driving the greater canopy productivity in response to phosphorus addition, and so far no substantial LAI increment was observed.

Fine root productivity responded strongly to phosphorus addition, increasing by 0.35 Mg C ha$^{-1}$ yr$^{-1}$, and had the strongest relative increase of 29.4% in the top 30 cm of soil (+P: 1.54 ± 0.09 Mg C ha$^{-1}$ yr$^{-1}$ versus −P: 1.19 ± 0.06 Mg C ha$^{-1}$ yr$^{-1}$; $F_{1,30}$ = 9.24, $P$ = 0.005) (Fig. 2b). The overall

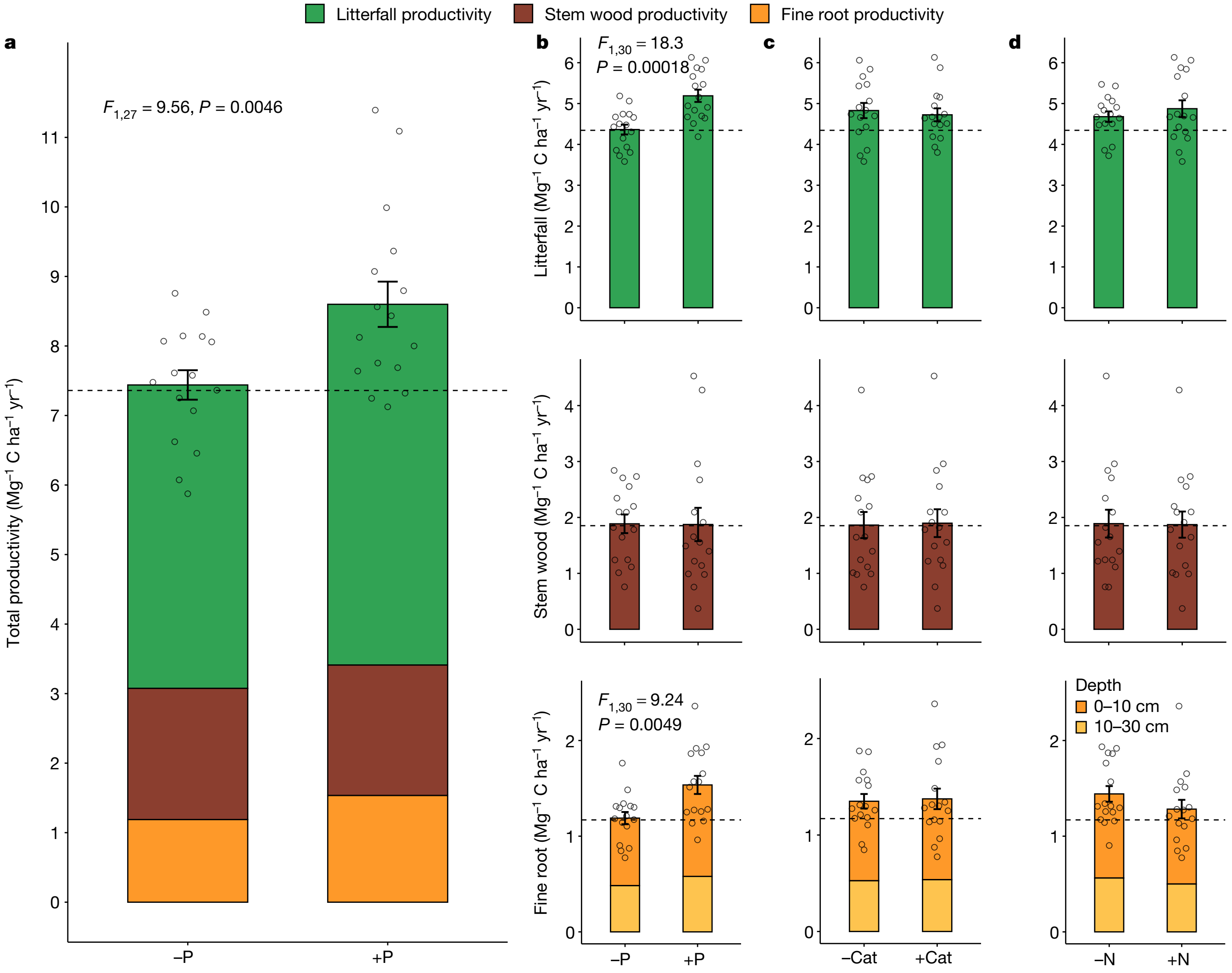

**Fig. 2 | The effect of nitrogen, phosphorus and base cation availability on total NPP and its components. a**, The responses of total NPP, representing the sum of NPP components. Only the statistically significant phosphorus effects are shown for total NPP, as nitrogen, base cation and their interactions had no effect (Supplementary Tables 2–4). **b**–**d**, The individual components of NPP. **b**, Litterfall productivity showed an increase with phosphorus addition (Supplementary Tables 6–8). In stem wood productivity, there was no effect of any nutrient addition (Supplementary Tables 32 and 33). Fine root productivity (0–30 cm) showed an increase with phosphorus addition only (**b**) (Supplementary Tables 21–23). Fine root productivity was higher at both 0–10 cm and 10–30 cm with phosphorus addition, but the mean was significant only for the 0–10 cm layer. Data are means ±s.e.m., $n = 16$ plots. Dotted lines represent mean values for the control plots (no nutrients added; $n = 4$ plots). Linear mixed models were used to evaluate responses in total NPP and its components to added nutrients, where nutrient additions and their interactions were fixed effects and block was a random effect with the general full model formula lmer(response ~ Nitrogen × Phosphorus × Cations + (1|Block)). Only phosphorus addition remained in significant models after model simplification. All differences in mean values between plots with and without added nutrients with $P < 0.01$ are indicated. Cation (cat) (**c**) and nitrogen (**d**) panels for NPP components are shown for comparison only.

increase in fine root productivity over 2 years of fertilization, was greater[30] compared to observations during the first 12 months (23.4%). Fine root productivity increased significantly in the top 10 cm of soil depth (+P: 0.96 ± 0.05 Mg C ha$^{-1}$ yr$^{-1}$ versus −P: 0.71 ± 0.04 Mg C ha$^{-1}$ yr$^{-1}$; $F_{1,30} = 12.9$, $P = 0.001$) (Supplementary Tables 25–27), but below 10 cm, although fine root productivity was around 20% greater following phosphorus addition, this difference was not statistically significant (+P: 0.58 ± 0.04 Mg C ha$^{-1}$ yr$^{-1}$ versus −P: 0.48 ± 0.03 Mg C ha$^{-1}$ yr$^{-1}$; $F_{1,30} = 3.56$, $P = 0.069$) (Supplementary Tables 29 and 30). The greater fine root productivity in the upper soil layer may be owing to the low mobility of phosphorus in the soil[36], with most of the added phosphorus being likely to remain in the top 10 cm, where it can be rapidly taken up by roots[30,37,38] or soil microbes. In a nearby site, at least 40% of fine root productivity was shown to occur[39] below 30 cm. Thus, although it is unlikely that reductions in productivity below 30 cm could have compensated for the increased root growth near the surface, across the full rooting depth, the overall stimulation of fine root production will probably have been lower than 29%.

There is very limited information on fine root productivity responses to nutrient addition in old growth tropical rainforests. In a fertilization experiment in Panama, although fine root productivity was not measured directly, potassium addition induced significant changes, decreasing fine root standing biomass, increasing fine root turnover and reducing root tissue density, leading to shifts toward the construction of fine roots with a more acquisitive strategy[40,41]. In one of the few studies that measured root productivity responses to large-scale nutrient additions in the tropics, in a secondary seasonally dry tropical forest (approximately 30 years old) in Costa Rica, the addition of phosphorus did stimulate root productivity 1 year after fertilization, but this appeared to be at the expense of aboveground tissue production, with no overall effect of nutrient addition on total productivity[42]. The clear increase in fine root productivity in our experiment also contrasts strongly with results observed in temperate forests, where reductions in root productivity and soil respiration (less heterotrophic and autotrophic respiration) have generally been observed following experimental fertilization and alleviation of nitrogen limitation[43].

No significant effects of the nutrient addition were detectable on stem wood productivity (phosphorus: $F_{1,24} = 0.001$, $P = 0.97$; cations: $F_{1,27} = 0.01$, $P = 0.92$; nitrogen: $F_{1,26} = 0.003$, $P = 0.96$). Mean stem wood productivity was 1.85 ± 0.39 Mg C $ha^{-1}$ $yr^{-1}$ (diameter at breast height (DBH) > 10 cm). Whereas plants that grow in high-fertility soils can increase the concentration of nutrients in tissues, with the potential to promote growth[44], species in low-fertility sites may be adapted to allocate nutrients to tissues with higher phosphorus demand (more active), prioritizing roots and leaves, increasing photosynthetic and metabolic capacities, promoting ion uptake, tissue growth and maintenance[45]. In addition, the advantage of higher woody biomass production occurs only if it provides a competitive advantage over neighbouring trees (competition for light) or decreases the risk of mortality[46]. The rapid responses to phosphorus addition observed for the canopy and fine roots are important and enhance our understanding of nutrient limitation in Amazon forests, but longer-term monitoring of the experiment is required to determine whether the responses of different NPP components and resource allocation change over time, and whether a stem wood productivity response becomes apparent.

While attributing variation in forest productivity to phosphorus availability across fertility gradients in Amazonian has proved challenging owing to confounding variation in tree species composition and both climatic and soil physical factors, our results suggest that phosphorus availability may be critical in controlling geographical variation in canopy and fine root productivity across the basin. Along a natural soil fertility gradient spanning the Amazon Basin, fine root productivity, measured in the top 30 cm and extended to 1 m depth, increased on average by around 28% and canopy productivity also increased by around 28% from east (less fertile soils) to west[47] (high-fertility soils). Thus, after 2 years of phosphorus addition, the 29.4% stimulation in fine root productivity in our experiment is similar to the difference in fine root productivity between Amazon regions with contrasting soil fertility (Extended Data Table 1). The observed 19% increase in canopy productivity with phosphorus addition (Fig. 2b) is lower than the 28% greater litterfall production in fertile western forests of the basin (Peru and Colombia), compared with low-fertility sites in central and eastern Amazonia[47] (Brazil) (Extended Data Table 1). This may be explained by spatial variability representing the combination of direct phosphorus effects as well as changes in the species present, with a greater dominance of fast-growing species with lower wood density in the western Amazon[16]. However, overall, the similar magnitudes of the responses observed in our experiment—in which confounding variations in climatological variables, other edaphic factors and species present have been minimized—to the patterns observed across major soil fertility gradients, strongly suggest that phosphorus availability is a critical in controlling geographical variation in fine root and canopy productivity across the basin.

Direct demonstration of limitation by phosphorus, rather than nitrogen, of NPP in a central Amazon forest has major implications for predicting forest responses to climate change and rising atmospheric $CO_2$. In contrast to the nitrogen cycle, the phosphorus cycle has no major gaseous phase, and aqueous losses are low[9]. Therefore, although ecosystem nitrogen stocks can increase under elevated $CO_2$ if rates of biological fixation increase or aqueous or gaseous losses are reduced[8], in ecosystems with highly weathered soils there is little opportunity for total phosphorus stocks to change, owing the lack of inputs and outputs[9]. For this reason, phosphorus limitation may place a stronger constraint on forest responses to rising atmospheric $CO_2$ than nitrogen limitation, questioning the potential for current high rates of carbon uptake in Amazonia to be maintained. Recent model projections have demonstrated that the inclusion of phosphorus in dynamic global vegetation models reduce predictions of carbon uptake and biomass production in Amazon forests[4], decreasing forest carbon sink and contributing to more rapid global climate change[7]. Furthermore, because the resistance of tropical forests to climate change depends on their ability to respond positively to rising $CO_2$ levels, if the responses to increased $CO_2$ are limited by phosphorus availability, Amazon forests growing in low-fertility soils may be more vulnerable than currently recognized[48]. Testing this suggestion directly with experimental manipulations of atmospheric $CO_2$ in tropical rainforests remains an urgent research priority, with the AmazonFACE (https://amazonface.inpa.gov.br/en/index.php) experiment aiming to do just that. Overall, in contrast to recent meta-analyses and the results from experiments in different tropical regions, our results provide direct evidence for phosphorus availability controlling forest productivity in the low-fertility soils that characterize central and eastern Amazonia, with no evidence for a role of nitrogen. This new understanding of the role of nutrient limitation in Amazon forests has critical implications for current and future mitigation policies required to avoid the most dangerous consequences of climate change.

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-022-05085-2.

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# Methods

## Site

This research was part of AFEX, a large-scale fertilization experiment installed in a lowland tropical forest, 80 km north of Manaus, Brazil, in Central Amazonia (2° 30′ S, 60° W) at one of the continuous old growth evergreen forests of the Biological Dynamics of Forest Fragments Project (BDFFP)[51]. The experimental site is located in terra firme forest and has a high species diversity[52], with about 280 plant species (≥10 cm DBH) per hectare. The dominant tree families in our site are Lecythidaceae, Sapotaceae, Fabaceae and Burseraceae, and the most abundant species are *Micrandropsis scleroxylon*, *Protium hebetatum*, *Eschweilera wachenheimii*, *Scleronema micranthum* and *Eschweilera truncata*.

The mean annual air temperature[53] is *c.* 26 °C, and the mean annual precipitation is 2,400 mm with a dry season from June to October, when monthly precipitation[54] can reach less than 100 mm. Aboveground biomass[55] was estimated to be 322 ± 54 Mg ha$^{-1}$ (tree individuals ≥10 cm DBH) with mean wood density of 0.67 g cm$^{-3}$. Local soils are geric Ferrasols (World Reference Base Soil Classification) (also known as Oxisols (US Department of Agriculture Soil Taxonomy))[15,31]. The soils are deep (≥400 cm) with good particle aggregation, friable and with low subsoil bulk density[56] (0.8–1.2 g cm$^{-3}$), typically acidic (pH approximately 4.1), with low concentrations of nutrients such as P (total P = 87.5 mg kg$^{-1}$), exchangeable Ca (0.034 cmolc kg$^{-1}$), and exchangeable K (0.066 cmolc kg$^{-1}$). The soil texture of the site is 7.69% sand, 14.75% silt and 77.55% clay.

## Experimental design

AFEX[30] comprises 32 plots, 50 m × 50 m each, distributed across 4 blocks separated by at least 200 m. Each of the 4 blocks comprises 8 plots, which are separated by at least 50 m, representing 8 treatments applied in a fully factorial randomized block design: control (with no addition of nutrients), N, P, cations (Ca, Mg, K), N + P, N + cations, P + cations and N + P + cations.

Fertilization consists of 125 kg ha$^{-1}$ yr$^{-1}$ of N as urea ($CO(NH_2)_2$), 50 kg ha$^{-1}$ yr$^{-1}$ of P as triple superphosphate ($Ca(H_2PO_4)_2$) and base cations with 160 kg ha$^{-1}$ yr$^{-1}$ as dolomitic limestone ($CaMg(CO_3)_2$ for Ca and Mg plus 50 kg ha$^{-1}$ yr$^{-1}$ as potassium chloride (KCl) for K. Annual doses of N, P and K are similar to the Panama fertilization experiment, in order to facilitate comparisons[22], and the addition rates of Ca within the base cation treatment equals the addition rate of Ca in the triple superphosphate, allowing us to directly determine the effect of the added P. Nutrient additions are split into three equal applications over the course of each wet season, with nutrients added every year since May 2017. The results presented here correspond to forest growth after two years of field measurements.

## Fine root productivity

The productivity of fine roots was measured every three months using the ingrowth core method as described in detail in Lugli et al.[30]. In each plot, the five ingrowth cores were bulked into a composite sample per plot, divided into depths of 0–10 cm and 10–30 cm, and roots were removed from the soil core by hand in the field over a period of 60 min, which was split into 15-min time intervals. Subsequently, fine roots (<2 mm diameter) were cleaned, dried at 60 °C until constant mass and weighed.

Different curve types were fitted to the first 60 min of manual root extraction and used to predict the pattern of extraction[30,57] up to 180 min.

We used the period from November 2017 to September 2019, comprising 2 years of data collection (year 1: November 2017 to September 2018 and year 2: December 2018 to September 2019, in a total of 8 ingrowth core collections). Total fine root productivity (0–30 cm) was summed for both years and the annual mean root productivity was obtained dividing the root productivity by 2. To convert root productivity from biomass to C, we used C data from the root tissues carried out in the study area[30], in which the average C concentration was 43.94%. Fine root productivity was expressed in Mg C ha$^{-1}$ yr$^{-1}$.

## Stem wood productivity

To calculate stem wood productivity, the stem diameter of all identified trees with a DBH ≥ 10 cm were recorded annually at the end of the wet season (May) from 2017–2019. An allometric equation specific for tropical moist forest[58] was applied to convert tree DBH (cm) (*D*), species wood density (g cm$^{-3}$) (WD) and a bioclimatic parameter (*E*) in woody biomass. The equation is expressed as:

$$\begin{aligned}\mathrm{AGB} = \exp(&-2.024 - 0.896E + 0.920\ln(\mathrm{WD}) + 2.795\ln(D)\\ &- 0.0461\,[\ln(D)]^2)\end{aligned}$$

This is a modified version of equation (7) from Chave et al.[58] given by the biomass package, where woody biomass can be inferred in the absence of height measurements. The bioclimatic parameter (*E*) is a measure of environmental stress[58] related to climatic water deficit, temperature seasonality and precipitation seasonality, inferred when the site coordinates were given (2° 40′ S, 60° W).

Wood density was estimated for each species from the getWoodDensity function from the R biomass package using the global wood density database as a reference[59,60], ideally assigned to species, but to genus level where species-level wood density data were not available. Of the total number of individuals, 55.1% of the wood densities were obtained at the species level, 37.1% at the genus level and for the remaining 7.9% of the individuals, we assumed the average wood density of the plot, because species was not identified or was absent in the database.

Stem wood productivity was calculated as the change in stem biomass of surviving trees added to the biomass of the recruited individuals divided by the census length. For 4,600 tree individuals, we selected a census length of 2 years (2017–2019) and for 249 trees where 1 census was missing (for example, tree not measured in 2017, recruited in 2018 census or measurement error), annual productivity was calculated using one year interval (2017–2018 or 2018–2019). Recruitment was the inclusion of new individuals who reached 10 cm of DBH in the 2019 inventory (42 trees). 22 trees with DBH > 15 cm in 2019 that were not measured in at least 2 censuses were not considered in the analyses. For 38 trees that died in 2019, productivity was calculated by the difference in biomass between 2018 and 2017.

The change in biomass was then summed over all trees with ≥10 cm DBH in each plot (2,500 m$^2$) and extrapolated to estimate the change in biomass per hectare. To convert biomass values into C, we assumed that dry stem biomass[61] corresponds to 50% C and stem wood productivity was expressed in Mg C ha$^{-1}$ yr$^{-1}$. To avoid or minimize potential errors, we used some parameters to check for quality control of the data. We used data that fell inside both of the following criteria: diametric growth smaller than 4 cm yr$^{-1}$ and a negative growth limit of −0.5 cm across the census intervals. Small negative DBH increments were included to accommodate measurement error and also because trees may shrink by a small amount owing to hydrostatic effects in times of drought[62].

## Litterfall productivity

Litterfall production was estimated by sampling litterfall every 15 days in 5 litter traps (0.25 m$^2$) placed 1 m above the ground within the central area of each plot (30 × 30 m). Litterfall includes leaves, twigs and thin branches with diameter <2 cm, reproductive material (flowers, fruits and seeds), residues (other fractions not identified) and insect frass that was oven-dried at 65 °C to constant mass and weighed.

We used data from the census of July 2017 to June 2019, where this period comprises 2 years. Litterfall productivity in g m$^{-2}$ day$^{-1}$ was extrapolated to Mg ha$^{-1}$ yr$^{-1}$ and the average was obtained considering two years of collection (Moraes, A. M. et al., manuscript in preparation; Supplementary Material). Litter material was estimated to be 50% C, based on mean values in our site, to convert biomass productivity into C productivity and it was also expressed in Mg C $ha^{-1}$ $yr^{-1}$.

### Leaf area index

A LAI-2200C (LI-COR Biotechnology) was used to measure LAI inside the central 30 m × 30 m of each plot. Sixteen measurement points were made in each plot, on a grid with an even spacing of 10 m. Measurements made on these 16 points per plot were averaged to represent plot means. The data were collected from 06:00 h to 17:00 h, avoiding recording data between 12:00 h and 14:00 h, to avoid direct sun. The LAI-2200C requires an above-canopy reading for reference, and in our case the optical sensor was placed in a clearing to log automatically while the operator collected manually below the canopy. The sensors were always placed in the same compass direction (both in the west in the morning and east in the afternoon) and we used a view cap of 45° in the sensors to remove the operator from the sensor's view. The sensors were matched before the data collection. The raw data were analysed using the FV2200 software, where LAI was obtained ($m^2$ one sided foliage area per $m^2$ ground area) and computed with four rings. These 4 rings read radiation at 4 angles: 7°, 23°, 38° and 53°. The data were collected during 10 to 13 October 2017, 22 to 25 March 2018, 7 to 10 August 2018 and between 29 October and 2 November 2018. LAI was based on these four collections, and was transformed to a single value representing the mean LAI over one year.

### Total productivity

We calculated total productivity using the following equation:

$$\mathrm{NPP}_{\mathrm{total}} = \mathrm{NPP}_{\mathrm{fineroots}} + \mathrm{NPP}_{\mathrm{stem}} + \mathrm{NPP}_{\mathrm{litterfall}}$$

All terms are expressed in Mg C $ha^{-1}$ $yr^{-1}$.

### Leaf residence time

This parameter was calculated by dividing the leaf biomass by annual leaf fall productivity (from July 2017 to July 2018) in Mg dry biomass $ha^{-1}$ $yr^{-1}$ (ref. [63]). Leaf biomass was calculated by dividing the mean LAI of four campaigns (10 to 13 October 2017, 22 to 25 March 2018, 7 to 10 August 2018 and between 29 October and 2 November 2018) by specific leaf area (SLA). The SLA was included in 2 approaches: (1) obtained from a census in October 2018, from about 8 individuals per plot from canopy dominant trees (−P: 83.36 ± 1.83 $cm^2$ $g^{-1}$ and +P: 88.02 ± 2.49 $cm^2$ $g^{-1}$, −cations: 85.61 ± 2.25 $cm^2$ $g^{-1}$ and +cations: 85.77 ± 2.28 $cm^2$ $g^{-1}$, −N: 85.54 ± 2.67 $cm^2$ $g^{-1}$ and +N: 85.85 ± 1.76 $cm^2$ $g^{-1}$, based on mean values in our site; Andersen, K. M. et al., unpublished results) 2) Obtained from sampling in litter traps (−P: 162.50 ± 26 g $m^{-2}$ and +P: 128.75 ± 11 g $m^{-2}$). Transformations from leaf mass per unit area (LMA) to SLA were made when necessary. The numerator, leaf biomass in g $m^{-2}$ was extrapolated to Mg $ha^{-1}$. The denominator, leaf fall productivity was based on 24 collections, and was transformed to a single value representing the mean leaf fall productivity over 1 year.

### Data analyses

Linear mixed models were used to test the effect of added nutrients and their interaction in the factorial design N × P × base cations. The model simplification method used to find the best model was the step function in the lmerTest package, based on the drop1 function, which systematically drops fixed factors in order of the model hierarchy[64]. We started with the full model including all nutrients and their interaction, and followed a stepwise backward elimination on non-significant effects based on chi-square test comparing two consecutive models. When dropping interaction effects significantly changed the model fit, they were retained in the model and the elimination process was completed. When all fixed effects were dropped from the model, the intercept was accepted as the final model. A probability < 0.05 was adopted to determine significance. Results are reported for the best fit model in the text and figures. The denominator degrees of freedom was estimated using the Satterthwaite approximation. The four blocks were used as random factors and the response variables were fine root, stem wood, litterfall productivity, total productivity, LAI and leaf residence time. All models were run using lme4 and lmerTest R packages[65]. We tested the assumptions for normality and homogeneity of variance to meet assumptions for linear models, using the Shapiro–Wilk and Levene tests. Since no interactions between nutrients were found, all plots where a specific nutrient was not added (that is, −P ($n = 16$)) are compared to all plots where that nutrient was added[22,30] (that is, +P ($n = 16$)). Original datasets from this study are publicly available[66–69]. Compiled datasets and R scripts used for statistical analyses, figures and tables are available at https://github.com/kmander7/Paper-AFEX-NPP.

### Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

## Data availability

Data that support the findings of this study have been deposited in NERC Environmental Information Data Centre at https://doi.org/10.5285/b3a55011-bf46-40f5-8850-86dc8bc4c85d for root biomass, https://doi.org/10.5285/c2587e20-ba4a-4444-8ce9-ccdec15b0aa3 for tree census, https://doi.org/10.5285/c0294ec9-45d6-464c-b543-ce9ece9fd968 for litterfall production and https://doi.org/10.5285/6e70665f-b558-4949-b42a-49fbaec7e7cc for LAI. The Global Wood Density Database can be requested from https://doi.org/10.5061/dryad.234. Plot mean datasets for all response variables and AFEX plot treatment identifications are available at https://github.com/kmander7/Paper-AFEX-NPP.

## Code availability

The R code used to find the best model for each variable is available in the Supplementary Material. R scripts used to generate the Supplementary Material are available at https://github.com/kmander7/Paper-AFEX-NPP.

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**Author contributions** H.F.V.C., C.A.Q., I.P.H. and K.M.A. planned the study. H.F.V.C., R.D.P., A.M., M.P., J.S.R., B.B., A.L.C., S.D.C., S.T.d.S., F.A., L.S.S., G.R., R.L.d.A., A.C.S., B.T.T.P., A.C.M., L.F.L., E.O.M. and J.L.C. collected data and/or helped with project logistics. I.P.H., L.M.M., L.E.O.C.A., T.F.D., L.N., P.M. and C.A.Q. wrote the grants that funded this research. H.F.V.C., K.M.A. and I.A. organized the datasets. H.F.V.C., K.M.A., I.A. and A.M.M. conducted the statistical analyses. H.F.V.C., L.F.L., I.P.H., C.A.Q., L.M.M., S.G., I.A., K.M.A., F.D.S., T.F.D., A.L.C., P.M., R.D.P., R.L.d.A., L.E.O.C.A. and L.N. discussed the results and the structure of the paper and improved the manuscript.

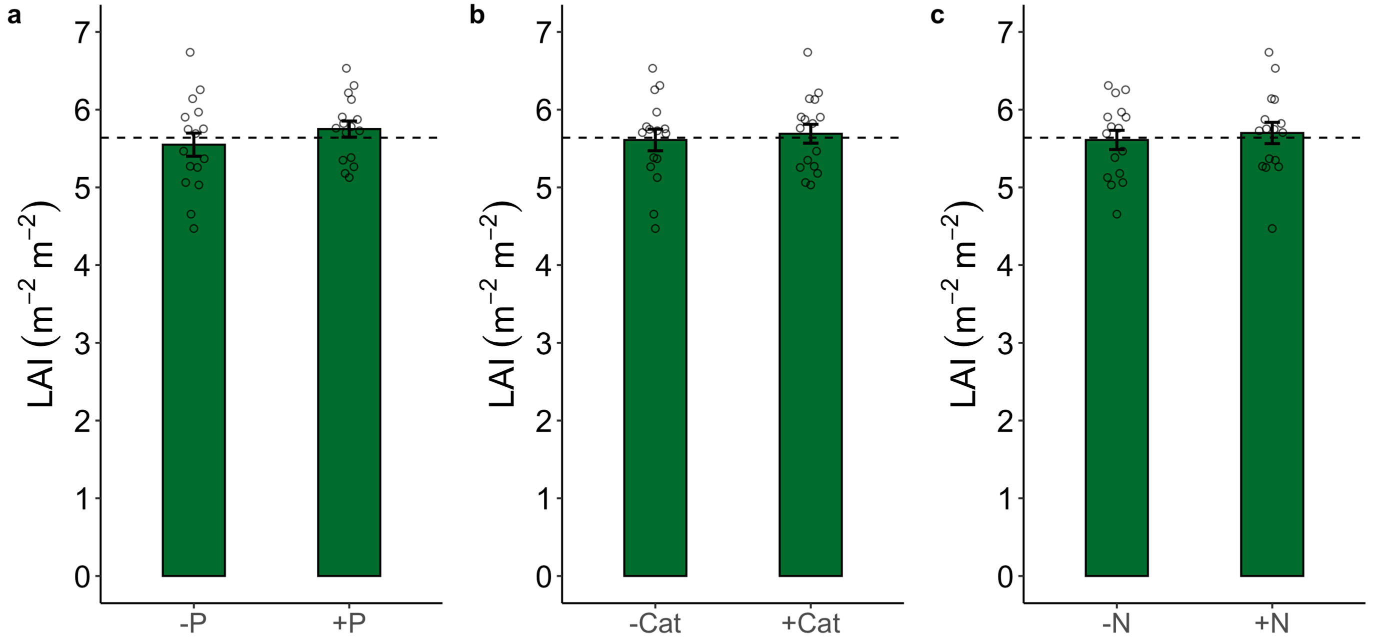
**Competing interests** The authors declare no competing interests.

**Additional information**

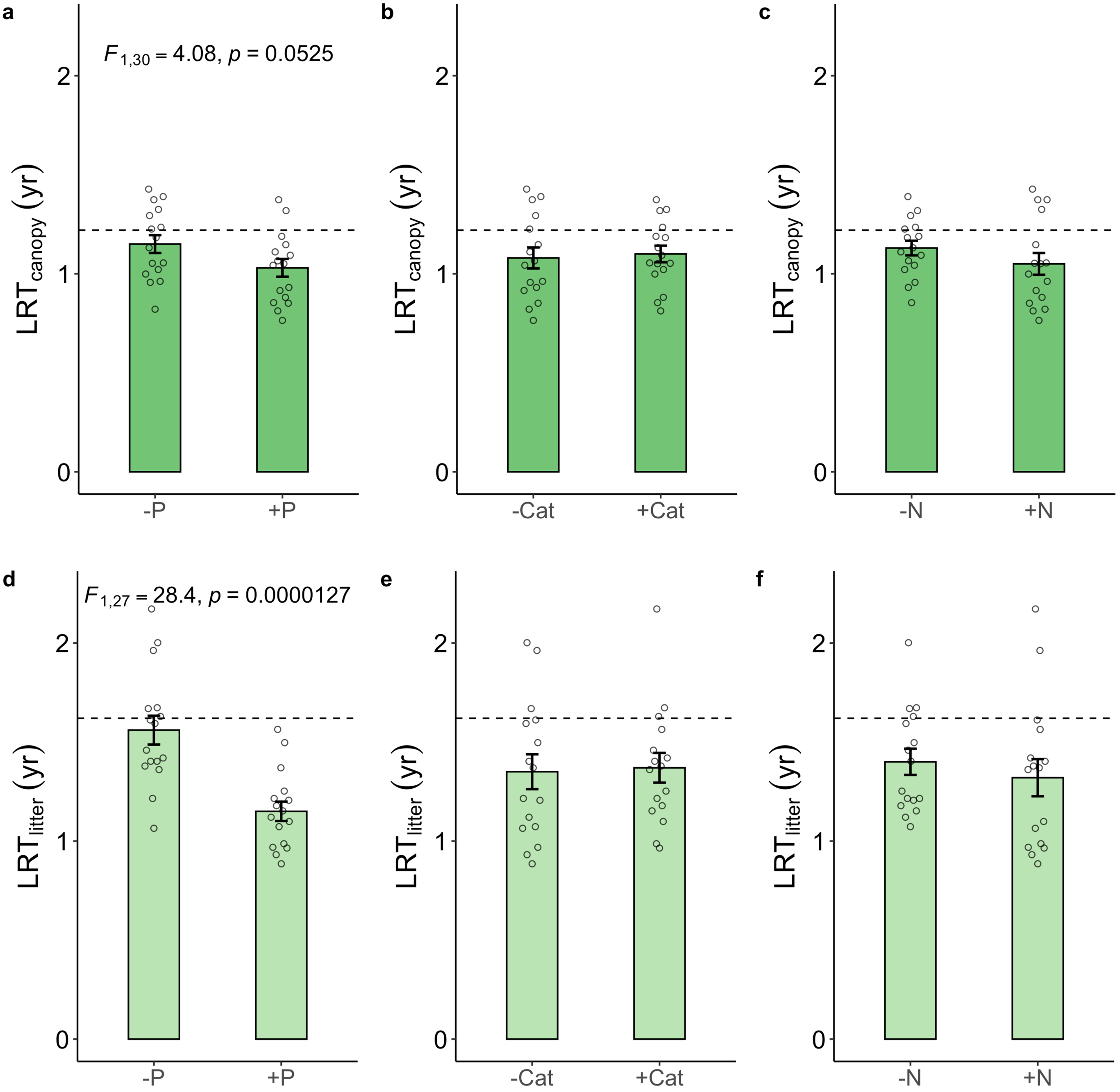
**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41586-022-05085-2.

**Correspondence and requests for materials** should be addressed to Hellen Fernanda Viana Cunha.

**Peer review information** *Nature* thanks Stuart Wright and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.



**Extended Data Fig. 1 | Nutrient addition effects on Leaf area index.** LAI was measured over four field campaigns across treatments in a lowland forest in Central Amazon. Each panel represents mean ± 1SE LAI with (+) or without (−) the addition of specific nutrients (phosphorus addition (a); base cation addition (b); nitrogen addition (c)), based on the average LAI across the four field campaigns, n = 16 plots. No significant differences among the means were detected in linear mixed models for any of the nutrients. The dotted lines represent the mean values for the control plots (no nutrients added; n = 4 plots) for comparison purposes.



**Extended Data Fig. 2 | Nutrient addition effects on Leaf residence time (LRT).** Leaf residence time (yr) across treatments in a lowland forest in Central Amazon. Two separate measures of specific leaf area were used in the leaf residence time calculations based on: 1) fresh canopy leaves of common families represented across all plots sampled for a photosynthesis campaign (**a**-**c**); 2) composite leaf litter collected in the plots (**d**–**f**). Leaf residence time showed a decrease with P addition only (**a**, **d**) for both LRT estimates, with cations (**b**, **e**) and N (**c**, **f**) being shown for comparison. Means ± 1SE are presented, n = 16 plots. Linear mixed models were performed to evaluate responses in leaf residence time to added nutrients. The dotted lines represent the mean values for the control plots (no nutrients added; n = 4 plots) for comparison purposes.

**Extended Data Table 1 | NPP comparisons along the Basin**

| | Soil parameters | | | NPP components (Mg C ha$^{-1}$ yr$^{-1}$) | | |
|---|---|---|---|---|---|---|
| **Country** | **Ptotal (mg kg$^{-1}$)** | **N (%)** | **SB (cmol$_c$ kg$^{-1}$)** | **Canopy** | **Fine roots** | **Stem** |
| **Our site (control)** | 87.5[a] | 0.19[a] | 0.16[a] | 4.3 | 2.0 | 1.9 |
| **Eastern sites** | | | | | | |
| Brazil (CAX03) | 37.4 [b] | 0.06[b] | 0.02 | 3.5 | 4.0 | 2.6 |
| Brazil (MAN05) | 79.5 | 0.11 | 0.19 | 3.6 | 2.8[c] | 2.6 |
| Brazil (CAX06) | 178.5 | 0.13 | 0.41 | 3.8 | 3.9 | 1.7 |
| Mean | 98.5 | 0.10 | 0.21 | 3.6 | 3.6 | 2.3 |
| **Western sites** | | | | | | |
| Peru (TAM05) | 256.3 | 0.16 | 0.22 | 5.6 | 6.8 | 2.8 |
| Colombia (AGP02) | 286.7 | 0.16 | 1.02 | 3.7 | 2.2 | 3.8 |
| Peru (TAM06) | 528.8 | 0.17 | 4.99 | 4.6 | 4.8 | 2.6 |
| Mean | 357.2 | 0.16 | 2.08 | 4.6 | 4.6 | 3.1 |
| Magnitude of difference % | | | | 27.8 | 27.8 | 34.8 |

The soil data without letter are derived from Quesada et al. 2010 (ref 15).

[a] Values for our site are from AFEX data for the soil depth 0-30 cm.

[b] Values are derived from Girardin et al. 2016 (ref 50).

[c] Values are fine root productivity (0-90 cm depth) reported for Manaus using minirhizotrons[39].

Total P (mg kg$^{-1}$), N (%) and sum of base cations (SB in cmol$_c$ kg$^{-1}$ refer to the sum of Ca+Mg+K+Na), canopy, fine roots and stem wood net primary productivity (Mg C ha$^{-1}$ yr$^{-1}$), from low-fertility soils in eastern Amazonian sites (CAX 03, MAN 05, CAX 06) and more fertile soils in western sites (TAM 05, AGP 02, TAM 06) according to their total soil P concentrations. Components of net primary productivity are derived from Aragão et al. 2009. Aragão et al. 2009 presents fine root productivity to 1 m, so we have extended our data to 1 m by dividing by 0.6, based on the study of Cordeiro et al. 2020 that demonstrated that 40% of fine root productivity was located below 30 cm at a nearby site on the same soil type. The percentage indicates the magnitude of differences between more fertile and least fertile sites.

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| ☐ | ☒ | A description of all covariates tested |
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*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

| | |
|---|---|
| Data collection | Leaf area index - FV 2200 ver 2.1.1 |
| Data analysis | R version 3.6.3 (2020-02-29)<br>biomass package ver 2.1.7<br>lmerTest package ver 3.1-3<br>lme4 package ver 1.1-28 |

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Data that support the findings of this study have been deposited in NERC Environmental Information Data Centre at (https://doi.org/10.5285/b3a55011-

bf46-40f5-8850-86dc8bc4c85d) for fine root biomass, at (https://doi.org/10.5285/c2587e20-ba4a-4444-8ce9-ccdec15b0aa3) for tree census, at (https://doi.org/10.5285/c0294ec9-45d6-464c-b543-ce9ece9fd968) for litterfall production, at (https://doi.org/10.5285/6e70665f-b558-4949-b42a-49fbaec7e7cc) for leaf area index. Global wood density database can be requested from http://datadryad.org//. Compiled datasets and R scripts used for statistical analyses, figures and tables are available at https://github.com/kmander7/Paper-AFEX-NPP.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | *Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data where this information has been collected, and consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.* |
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| Recruitment | *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.* |
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# Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| | |
|---|---|
| Study description | The Amazon Fertilization Experiment (AFEX) is a large scale soil nutrient manipulation study to examine how nutrient availability affects key components of the carbon cycle. This paper aims to determine which nutrient controls Amazon forest productivity. AFEX is composed of thirty two 50 m x 50 m plots distributed across four blocks separated by at least 200 m. Each of the four blocks comprises 8 plots, which are separated by at least 50 m, representing 8 treatments applied in a fully factorial design: control (with no addition of nutrients), N,P, CATIONS (Ca, Mg, K), N+P, N+CATIONS, P+CATIONS and N+P+CATIONS. |
| Research sample | Root and litterfall were collected in five samples per plot to estimate root and litterfall production. Each tree > 10 cm DBH was measured in each plot. We used the mean of these collections per plot, which represent 748 tree species across the 8 ha. |
| Sampling strategy | Sampling strategy was based on standardized protocols by rainfor (http://www.rainfor.org/pt/manuais/em-campo) and Wright et al. 2011 for fertilisation experiment. |
| Data collection | The author and field assistants collected the data in teams. The authors who collected the data are: Hellen Fernanda Viana Cunha, Raffaello Di Ponzio, Anna Martins Moraes, Maria Pires, Jéssica Schmeisk Rosa, Barbara Brum, Amanda L.Cordeiro, Sara Deambrozi Coelho, Sheila Trierveiler de Souza, Felipe Antonieto, Lara Siebert Silva, Gyovanni Ribeiro, Rafael L. de Assis, Ana Cláudia Salomão, Ana Caroline Miron, Erick Oblitas Mendoza, Kelly M. Andersen and Laynara Figueiredo Lugli. Root production was estimated using ingrowth cores, litterfall was estimated using litterfall traps and wood productivity estimated by changes in DBH. Leaf area index was estimated using the LAI-2200 C. Leaf residence time was derived from literfall productivity, LAI and specific leaf area. |
| Timing and spatial scale | The authors and field assistance collected data in teams with roots collected four times per year (started in November 24, 2017 and stoped in September 12, 2019. Litterfall collected bi weekely (started in July 19, 2017 and stoped in July 11, 2019). Tree was measured every year (started in June 07, 2017 and stoped in May 14, 2019). Leaf are index was measured in 2017 and 2018 (started in October 10, 2017 and stoped November 02, 2018). |
| Data exclusions | 22 trees with DBH > 15 cm in 2019 that were not measured in at least two censuses were not considered in the analysis. |
| Reproducibility | The data reported here are from the first two years with all values within a similar range confirming reproducibility of the data. The original data, datasets to run the analysis and create the figures along the R code to produce these are freely available online. |

| | |
|---|---|
| Randomization | Nutrient addition treatments were randomly assigned to each plot within blocks. |
| Blinding | We used plot number and block numbers in place of treatments to reduce potencial bias. |

Did the study involve field work? ☒ Yes ☐ No

## Field work, collection and transport

| | |
|---|---|
| Field conditions | The mean annual air temperature is c. 26 °C, and the mean annual precipitation is 2400 mm with a dry season from June to October, when monthly precipitation can reach less than than 100 mm |
| Location | The research was part of the Amazon Fertilisation Experiment (AFEX), a large-scale fertilisation experiment installed in a lowland tropical forest, 80 km north of Manaus, in Central Amazonia (lat 2° 30' S, long 60° W) at one of the continuous old growth evergreen forests of the Biological Dynamics of Forest Fragments Project (PDBFF). |
| Access & import/export | Portaria n° 488, June 28, 2016 until 2018 |
| Disturbance | We used paths to minimize disturbance in the plots. Root ingrowth cores holes were filled with soil after final measurements. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study | |
|---|---|---|
| ☒ | ☐ | Antibodies |
| ☒ | ☐ | Eukaryotic cell lines |
| ☒ | ☐ | Palaeontology and archaeology |
| ☒ | ☐ | Animals and other organisms |
| ☒ | ☐ | Clinical data |
| ☒ | ☐ | Dual use research of concern |

### Methods

| n/a | Involved in the study | |
|---|---|---|
| ☒ | ☐ | ChIP-seq |
| ☒ | ☐ | Flow cytometry |
| ☒ | ☐ | MRI-based neuroimaging |