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INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA – INPA

Programa de Pós-Graduação em Ciências de Florestas Tropicais – PPG - CFT

PHOSPHORUS ADDITION DRIVES CARBON SINK, BUT MULTIPLE NUTRIENTS DRIVE CARBON LOSS

HELLEN FERNANDA VIANA CUNHA

Manaus, Amazonas

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Tese apresentada ao Programa de Pós-Graduação em Ciências de Florestas Tropicais como parte dos requisitos para obtenção do título de Doutora em Ciências de Florestas Tropicais.

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RESUMO. A produtividade da floresta e a respiração são componentes chaves do ciclo do carbono que podem ser afetadas pelos nutrientes. Este trabalho teve como objetivo identificar diretamente por meio de experimento de manipulação de nutrientes, que elementos específicos limitam esses processos. O experimento foi instalado em maio de 2017 em uma floresta tropical na Amazônia Central, as coletas abrangeram um período que vai de 2017 até 2019. A Produtividade Primária Líquida Total aumentou em 15,6% somente com a adição de fósforo, dirigida pelo aumento na produtividade da serapilheira (19%) e produtividade das raízes finas (29,4%). A adição de nutrientes não afetou a produtividade da madeira durante o período estudado. De forma contrária a NPP, os componentes da respiração responderam a várias combinações de nutrientes, uma interação entre fósforo e cátions foi observada na respiração das folhas e uma interação entre nitrogênio e cátions afetou a respiração autotrófica e heterotrófica do solo. A respiração do ecossistema não foi afetada pela adição de fósforo, uma vez que a respiração do tronco diminuiu em 12% com a adição de fósforo, e por outro lado a adição do mesmo elemento aumentou em 13% a respiração heterotrófica, de forma que os fluxos se compensaram. De forma geral, parece que as fortes respostas da produtividade à adição de fósforo, não vieram com altos custos respiratórios, pelo menos a curto prazo. Os resultados da presente tese demostram que Modelos de Sistema Terrestre devem incorporar o ciclo do fósforo e potencial limitação por fósforo para capturar o ciclo de carbono global.

Palavras chave: Experimento de fertilização na Amazônia, crescimento, nutrientes, fluxo respiratório.

ABSTRACT. Forest productivity and respiration are key components of the carbon cycle that can be affected by nutrients. This work aimed to directly identify through nutrient manipulation experiment, which specific elements limit these processes. The experiment was installed in May 2017 in a tropical forest in the Central Amazon, the collections covered a period from 2017 to 2019. Total net primary production increased by 15,6 % with phosphorus addition alone, driven by the increase in litter productivity (19%) and fine root productivity (29,4%). The addition of nutrients did not affect wood productivity during the studied period. In contrast to NPP, the components of respiration responded to various combinations of nutrients, an interaction between phosphorus and cations was observed in leaf respiration and an interaction between nitrogen and cation affected autotrophic and heterotrophic soil respiration. Ecosystem respiration was not affected by phosphorus addition, since stem respiration decreased by 12% with phosphorus addition, and on the other hand, the addition of the same element increased heterotrophic respiration by 13%, so that the flows compensated. In summary, it appears that rapid productivity responses did not come with a high autotrophic respiration cost. The results of the present thesis will demonstrate that Earth System Models must incorporate P cycles and potential P limitation to capture the global carbon cycle.

Keywords: Amazon fertilization experiment, growth, nutrients, respiratory flow.

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INTRODUÇÃO GERAL

O ciclo de CO_2 atmosférico é composto pelas emissões de CO_2, como o emitido pelos combustíveis fósseis (petróleo, carvão mineral, gás natural) e indústrias, e mudanças de uso da terra como o desmatamento, assim como a liberação de CO_2 por plantas e microrganismos. Por outro lado, o sequestro de CO_2 é proporcionado pela biosfera terrestre (~30%) e oceanos (~26%), enquanto uma parte permanece na atmosfera (44%) (Le Queré et al, 2016; Philips et al. 2017). As florestas tropicais respondem por 34% da produtividade primária bruta total global, contribuindo com 41 Pg C por ano do total de 122 Pg C por ano (Beer et al. 2010), desempenhando um papel significativo no ciclo de carbono global e na mitigação da mudança climática. Além disso, as florestas tropicais têm um estoque estimado de carbono de 471 Pg C, que corresponde a 55% do estoque de carbono global que é de 861 Pg C (Pan et al. 2011).

Os nutrientes influenciam o ciclo de carbono global. O nitrogênio geralmente limita a produtividade nas latitudes temperadas e boreais (Norby et al. 2010; Lambers et al. 2007) e, por esta razão, muitos Modelos de Sistema Terrestre (ESM – *Earth System Models*) têm incorporado o ciclo do nitrogênio e potencialmente a limitação por nitrogênio no ciclo de carbono (Nakhavali et al. 2022). No entanto, em ecossistemas tropicais, a disponibilidade de elementos derivados de rocha, como o fósforo, pode ser baixa enquanto a disponibilidade de nitrogênio tende a ser abundante (Lambers et al. 2007, Vitousek et al. 2010). Recentemente, os ESM têm incorporado o ciclo do fósforo para representar a potencial limitação por fósforo no ciclo de carbono global (Sun et al. 2017, Fleischer et al. 2019, Nakhavali et al. 2022). Todavia, até o momento, uma evidência direta da potencial limitação por fósforo, nos experimentos de fertilização conduzidos em florestas tropicais, não tinha sido encontrada, apenas fornecendo suporte parcial para a teoria do paradigma de limitação por fósforo (Wright, 2022).

A presente tese, tem como objetivo geral demonstrar experimentalmente a importância da limitação nutricional nos processos de produtividade e respiração em uma floresta madura na Amazônia Central. Para testar a limitação nutricional na produtividade e na respiração, o primeiro experimento de fertilização em grande escala com adição fatorial dos nutrientes nitrogênio, fósforo e cátions base foi instalada em uma floresta madura na Amazônia Central, que apresenta baixa disponibilidade de fósforo e cátions e é representativa de cerca de 60% dos solos inférteis da Amazônia. Os resultados estão descritos em dois capítulos. No capítulo 1 são apresentados os resultados que indicam que nutrientes controlam a produtividade primária líquida total e cada um de seus componentes (dossel, madeira e raízes) em uma floresta de terra firma na Amazônia Central. No capítulo 2 são apresentadas e discutidas a resposta da fertilização aos processos de respiração autotrófica (dossel, madeira e raízes) e heterotrófica (respiração dos microrganismos).

GENERAL INTRODUCTION

The atmospheric CO_2 cycle is composed of CO_2 emissions, such as those emitted by fossil fuels (oil, coal, natural gas) and industries, and changes in land use such as deforestation, as well as the release of CO_2 by plants and microorganisms. On the other hand, CO_2 sequestration is provided by the terrestrial biosphere (~30%) and oceans (~26%), while a part remains in the atmosphere (44%) (Le Queré et al, 2016; Philips et al. 2017). Tropical forests account for 34% of total gross primary productivity, contributing with 41 Pg C per year of the total 122 Pg C per year (Beer et al. 2010), playing a significant role in the global carbon cycle and mitigation of climate change. Furthermore, tropical forests have and estimated carbon stock of 471 Pg C, which corresponds to 55% of the global carbon stock of 861 Pg C (Pan et al. 2011).

Nutrients influence the global carbon cycle. Nitrogen generally limits productivity at temperate and boreal latitudes (Norby et al. 2010; Lambers et al. 2007) and for this reason many Earth System Models (ESM) have incorporated the nitrogen cycle and potentially nitrogen limitation in the carbon cycle. However, in tropical ecosystems, the availability of rock derived elements such as phosphorus can be low while nitrogen availability tends to be abundant (Lambers et al. 2007, Vitousek et al. 2010). Recently, ESM have incorporated the phosphorus cycle to account for potential phosphorus limitation in the global carbon cycle (Sun et al. 2017, Fleischer et al. 2019, Nakhavali et al. 2022). However, to date, direct evidence of potential phosphorus limitation in fertilization experiments conducted in tropical forests has not been found, providing only partial support for the phosphorus limitation paradigm theory (Wright, 2022).

This thesis has the general objective of demonstrating experimentally the importance of nutritional limitation in productivity and respiration processes in a mature forest in Central Amazonia. To test the nutritional limitation on productivity and respiration, the first large-scale fertilization experiment with factorial addition of nitrogen, phosphorus and base cations nutrients was installed in a mature forest in Central Amazonia, which has low availability of phosphorus and cations and is representative of about 60% of the infertile soils of the Amazon. The results are described in two chapters. In chapter 1, results are presented that indicate which nutrients control total net primary productivity and each of its components (canopy, wood and roots) in a *terra firme* forest in Central Amazonia. In chapter 2, the fertilization response to autotrophic (canopy, wood and roots) and heterotrophic (microorganism respiration) respiration processes are presented and discussed.

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Introdução geral

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CAPÍTULO 1

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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 (P) availability[1]. Yet, controlled fertilisation experiments have failed to demonstrate a dominant role for P in controlling tropical forest net primary productivity (NPP). Recent syntheses have demonstrated that responses to N addition are as large as to P [2], and adaptations to low P availability appear to allow NPP to be maintained across major soil P gradients [3]. Thus, the extent to which P availability limits tropical forest productivity is highly uncertain. The majority of the Amazonia, however, is characterised by soils even more depleted in P than where most tropical fertilisation experiments have previously taken place[2]. Thus, we established the first P, nitrogen (N), and base cation addition experiment in an old growth Amazon rainforest, with the site's low soil P content representative of ~60% of the basin. Here we show that NPP increased exclusively with P addition. After 2 years, strong responses were observed in fine root (+29%) and canopy productivity (+19%), but not stem growth. The direct evidence of P limitation of NPP suggests that P availability may restrict Amazon forest responses to CO_2 fertilisation[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 C uptake by vegetation under elevated atmospheric CO_2 ([4,5,6,7]). Furthermore, fundamental differences between the cycles of nitrogen (N) and rock-derived elements such as P, mean that P limitation may place a greater constraint on plant responses to CO_2 fertilisation than N limitation[8,9]. During soil development[10], the weathering of rocks or parent material provides the major source of P for initial vegetation development. Over millions of years, however, the parent material is gradually depleted, and available P, as well as rock-derived base cations such as calcium (Ca), magnesium (Mg) and potassium (K), may be lost via leaching or made unavailable through occlusion by iron and aluminium-oxides, with organic forms of P becoming key pools in depleted and highly weathered systems[10,11]. Meanwhile, N tends to accumulate over time, with inputs from biological fixation and atmospheric deposition exceeding N losses[12]. For these reasons, a long-standing paradigm in tropical ecology (the so-called P paradigm) has been that forest productivity on highly-weathered soils, such as in those in central Amazonia, is primarily limited by plant available P[13], with a potential secondary role of other rock-derived elements. Supporting this paradigm, seminal forest

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ecology studies demonstrated very low levels of P and base cations in plant tissues in Amazonia[14], and high C:P ratios in litterfall of tropical forest more generally[1]. In Amazonia, greater wood productivity has also been observed in forests growing on fertile soils in western Amazonia when compared to less fertile sites in central and eastern portions of the basin, with relationships with total soil phosphorus being strongest[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 which elements 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 fertilisation[19.]

By minimising confounding factors, manipulation experiments can identify directly which specific elements limit forest productivity[20]. Although no large-scale N, P and base cation experiment has been carried out in Amazonia until now, a recent synthesis study argued that there is as much evidence for N limitation of tropical forest productivity as there is for P (ref 2). For example, in Costa Rica, P additions did not elicit any changes in litterfall and fine root productivity in two years after fertilisation[21], and in Panama, an increase in litter production with P addition was evident only 8 years after fertilisation[22], with initial responses stronger for N additions, at least in the rainy season[23]. Critically, previous nutrient manipulation studies in primary tropical rain forests have mainly taken place where total soil P contents are much greater than in central and eastern Amazonia (~443-1600 mg kg^{-1} *versus* 70-120 mg kg^{-1} in typical Amazon Ferralsols). In Amazonia, fertilisation experiments have been carried out in secondary forests, but little evidence for strong P limitation has been observed[24,25], with N availability found to be important during initial forest recovery[26,27]. There have been fertilisation experiments in forests growing on soils with P as low as in Amazonia in Cameroon[28] and Borneo[29]. These studies have also generally failed to provide clear support for the P paradigm, with no positive effects of P addition being observed[28], or with responses to N being at least as large as those to P[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, while previous fertilisation studies strongly question the ubiquity of P 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 in lowland tropical evergreen rainforest near Manaus, Brazil, we set up a large-scale fully factorial N, P and base cation-addition experiment (the Amazon Fertilisation Experiment-AFEX), manipulating 8 hectares of forest across 32 plots in four blocks[30]. The Ferralsols of the study site have low concentrations of total P and base cations that are characteristic of up to 60% of Amazon forest soils[31] (Fig. 1). To determine directly which nutrient(s) control Amazon forest productivity, we measured the responses of fine root, stem wood, and litterfall production between 2017 and 2019 (see Methods), making nearly 1500 measurements of canopy production, quantifying root productivity every three months across 160 locations and measuring the growth of 4849 trees. Importantly, our base cation treatment added the same amount of calcium as in the super-triple phosphate that was used in the P addition treatment. Thus, comparisons between these treatments ensure that the effects of P can be isolated.

Annual NPP rapidly increased with the addition of P in a Central Amazon Forest. After two years of P addition, annual NPP significantly increased by 1.16 Mg C ha^{-1} yr^{-1}, or 15.6% (+P (with P addition): 8.60 ± 0.33 *versus* -P (without P addition): 7.44 ± 0.21 Mg C ha^{-1} yr^{-1}; $F_{1,27} = 9.56$, $p = 0.005$; Fig. 2a), due to greater canopy and fine root productivity. No significant effects of N 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 P availability stimulating GPP[32], and/or through reductions in autotrophic respiration[33]. It has been shown that forests growing on high fertility soils may produce biomass more efficiently and thus show greater carbon use efficiency (CUE, the ratio of net carbon gain to gross carbon assimilated, NPP/GPP)[34]. Although the direct causes of changes are not yet clear, our results clearly demonstrate that NPP in this forest is limited by P alone. The observed increase in NPP with +P, and the lack of any N response, strongly contrasts with a meta-analysis based on previous tropical forest fertilisation studies[2], with the lower levels of soil P in Amazonia likely explaining this contrast (Fig. 1). We have previously

observed that base cation addition affects root morphology and mycorrhizal colonisation[30]. Thus, while base cation availability did not appear to limit NPP, they do appear to influence key belowground processes.

We observed a substantial 0.83 Mg C ha^{-1} yr^{-1}, or 19% (+P: 5.19 ± 0.15 *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 P addition over the first 1.5 years of the experiment (3.6% increase: +P: 5.75 ± 0.10 *versus* -P: 5.55 ± 0.15 m^2 m^{-2}; $F_{1,27} = 1.76$, $p = 0.20$; Extended Data Figure 1), which may have had minor contributions to enhanced rates of C gain. The increase in litterfall productivity in our site appears to result from a decrease in leaf life span, which was estimated to have decreased by 10 to 20% following phosphorus addition (+P: 1.03 ± 0.04 *versus* -P: 1.15 ± 0.05 yr; $F_{1,30} = 4.08$, $p = 0.05$ and +P: 1.15 ± 0.05 *versus* -P: 1.56 ± 0.07; $F_{1,27} = 28.4$, $p = 0.0000127$, analysis based on fresh and litter leaves, respectively – see methods; Extended Data Figure 2). Therefore, the increases in leaf turnover appear important in driving the greater canopy productivity in response to P addition, and so far no substantial LAI increment was observed.

Fine root productivity responded strongly to P 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 *versus* -P: 1.19 ± 0.06 Mg C ha^{-1} yr^{-1}; $F_{1,30} = 9.24$, $p = 0.005$; Fig. 2b). The overall increase in fine root productivity over two years of fertilisation, was greater compared to observations during the first 12 months (23.4% ref 30). Fine root productivity increased significantly in the top 10 cm of soil depth (+P: 0.96 ± 0.05 *versus* -P: 0.71 ± 0.04 Mg C ha^{-1} yr^{-1}; $F_{1,30} = 12.9$, $p = 0.001$; Table S25-27), but below 10 cm, although fine root productivity was ~20% greater following P addition, this difference was not statistically significant (+P: 0.58 ± 0.04 *versus* -P: 0.48 ± 0.03 Mg C ha^{-1} yr^{-1}; $F_{1,30} = 3.56$, $p = 0.069$; Table S29-30). The greater fine root productivity in the upper soil layer may be due to the low mobility of P in the soil[36], with most of the added P 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 below 30 cm[39]. Thus, while 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 fertilisation experiment in Panama, while fine root productivity was not measured directly, K 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 tropical forest (~30 years) in Costa Rica, the addition of P did stimulate root productivity one year after fertilisation, 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 fertilisation and alleviation of N limitation[43].

No significant effects of the nutrient addition were detectable on stem wood productivity (P: $F_{1,24} = 0.001$, $p = 0.97$; cations: $F_{1,27} = 0.01$, $p = 0.92$; N: $F_{1,26} = 0.003$, $p = 0.96$). Mean stem wood productivity was 1.85 ± 0.39 Mg C ha^{-1} $year^{-1}$ (DBH > 10 cm). While 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 P demand (more active), prioritising 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 P 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 P availability across fertility gradients in Amazonian has proven challenging due to confounding variation in tree species composition and both climatic and soil physical factors, our results suggest that P 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 ~28% and canopy productivity also increased by ~28% from East (less fertile soils) to West (high-fertility soils)[47]. Thus, after two years of P addition, the 29.4% stimulation in fine root productivity in our experiment is comparable 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 P addition (Fig. 2b) is lower than the 28% greater litterfall production in fertile Western forests of the basin (Peru, Colombia), compared with low-fertility sites in Central and Eastern Amazonia (Brazil)[47] (Extended Data Table 1). This may be explained by spatial variability representing the combination of direct P 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 variation in climatological variables, other edaphic factors, and species present has been minimised, to the patterns observed across major soil fertility gradients, strongly suggest that P availability is a critical in controlling geographical variation in fine root and canopy productivity across the basin.

Direct demonstration of limitation by P, rather than N, 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 N cycle, the P cycle has no major gaseous phase, and aqueous losses are low[9]. Therefore, while ecosystem N 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 P stocks to change due the lack of inputs and outputs[9]. For this reason, P limitation may place a stronger constraint on forest responses to rising atmospheric CO_2 than N limitation, questioning the potential for current high rates of C uptake in Amazonia to be maintained. Recent model projections demonstrated that the inclusion of P in dynamic global vegetation models reduced predictions of C uptake and biomass production in Amazon forests[4], decreasing forest C 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 elevated CO_2 are limited by P availability, Amazon forests growing in low fertility soils may be more vulnerable than currently recognised[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 P availability controlling forest productivity in the low fertility soils that characterise central and eastern Amazonia, with no evidence for a role of N. 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.

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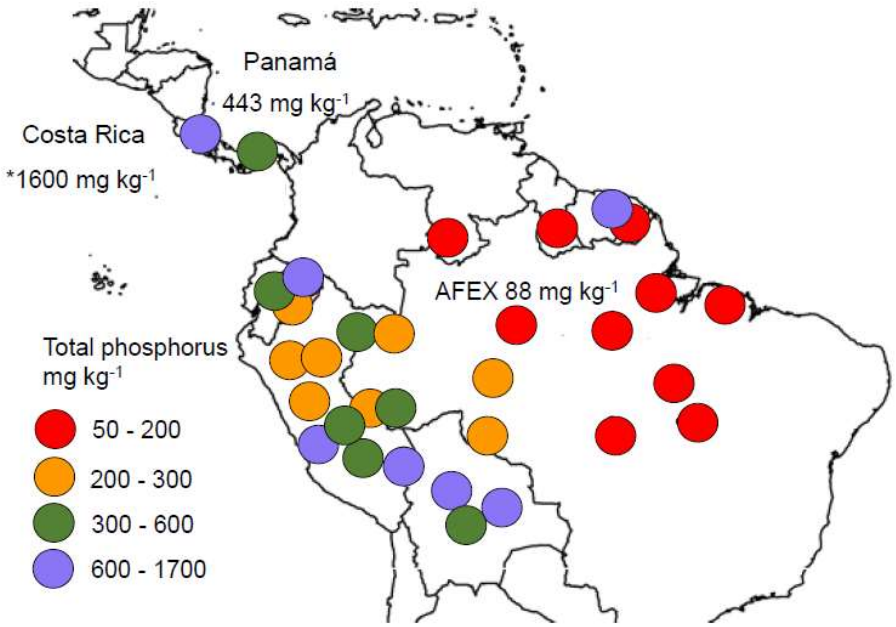

Figure 1| Total soil phosphorus measured in primary forest plots across the Amazon Basin, showing the low P concentration at our site and across central and eastern Amazonia. A fertility gradient across the basin is shown, with red circles showing the lowest concentration of total phosphorus and blue circles showing the highest concentration of total phosphorus. The two large scale fertilisation experiments in Central American *terra firme* tropical forest are also shown, highlighting the five to eighteen-fold greater total phosphorus concentrations than in central Amazon. Total phosphorus concentrations are derived from Quesada and Lloyd 2016 (ref 49), except the values of Costa Rica[21] and Panama[40]. *In Costa Rica, values are available only for the 0-10 cm soil depth. For the other sites, values are for 0-30 cm soil depth.

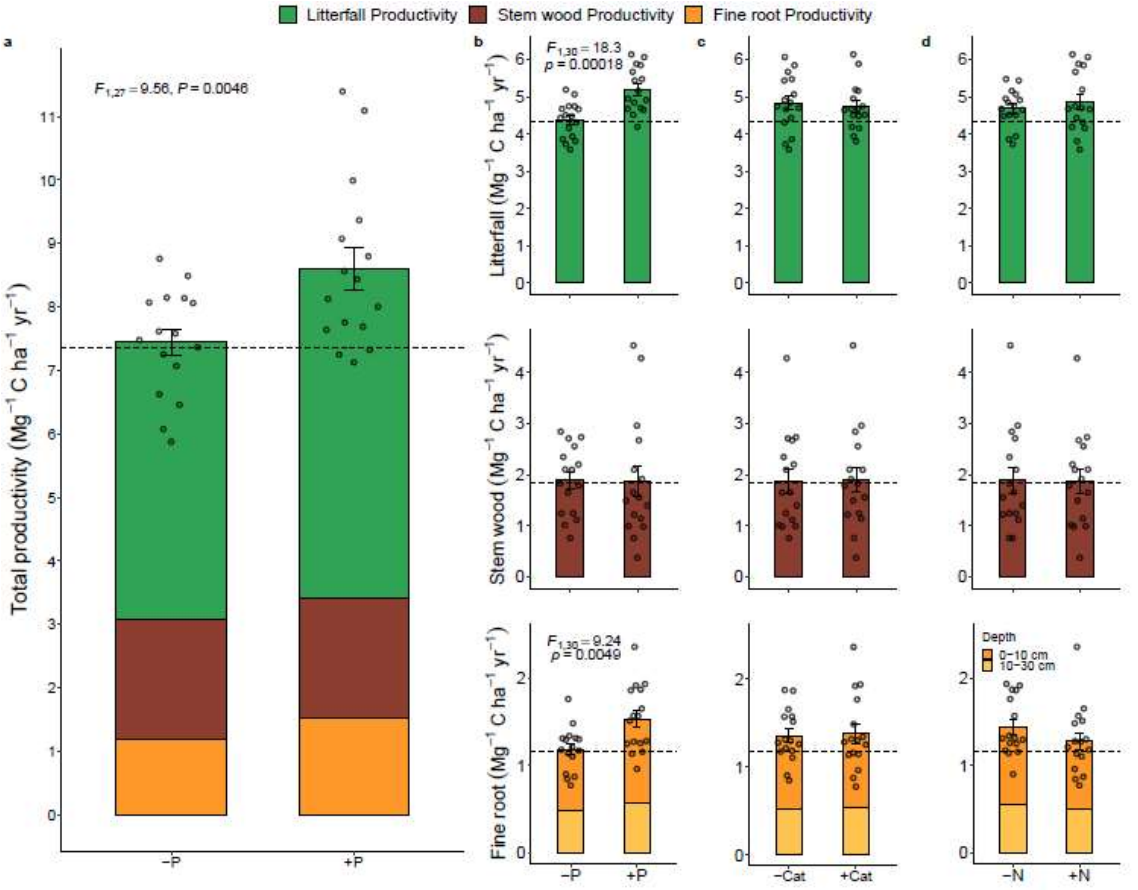

Figure 2 | The effect of N, P and base cation availability on total net primary productivity and its components. a, The responses of total net primary productivity (NPP), representing the sum of NPP components. Only the statistically significant P effects are shown for total NPP, as N, base cation and all interactions had no effect (Table S2-4). **b-d,** The individual components of NPP where litterfall, stem wood and fine root productivity are shown in green, brown and orange bars, respectively. **b,** Litterfall productivity showed an increase with P addition only, and base cation (**c**) and N (**d**) are shown for comparison (Table S6-8). **b,** In stem wood productivity there was no effect of any nutrient addition (Table S32-33). **b,** Fine root productivity (0-30 cm) showed an increase with P addition only, and base cation (**c**) and N (**d**) are shown for comparison (Table S21-23). Both 0-10 and 10-30 cm had higher fine root productivity with P addition, but only the 0-10 cm layer had significantly different means. Means ± 1SE are presented, n=16 plots. The dotted lines represent the mean values for the control plots (no nutrients added; n=4 plots) for comparison purposes. Linear mixed models were performed 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 ~ N * P * Cations + (1|Block) . Only P 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 (**c**) and nitrogen (**d**) panels for NPP components are added for comparison only.

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Author contribution H.F.V.C., C.A.Q., I.P.H. and K.M.A. planned the study. H.F.V.C., R.P., A.M., M.P., J.S.R., B.B., A.L., S.D.C., S.T., F.A., L.S.S., G.R., R.L.A., A.C.S., B.T., C.M., L.F.L., E.O. and J.L.C collected the data and/or helped with project logistics. I.P.H., L.M., L.A., T.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 organised the datasets. H.F.V.C., K.M.A., I.A. and A.M. conducted the statistical analyses. H.F.V.C., L.F.L., I.P.H., C.A.Q., L.M., S.G., I.A., K.M.A., F.D.S., T.D., A.L., P.M., R.P., R.L.A., L.A. and L.N discussed the results and the structure of the paper and improved the manuscript.

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METHODS

Site. This 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, Brazil, 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 (BDFFP)[51]. The experimental site is located in *terra firme* forest and has a high-species diversity, with about 280 plant species (≥10 cm DBH) per hectare[52]. 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 is *c.* 26 °C[53], 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[54]. Above ground biomass (AGB) was estimated to be 322 ± 54 Mg ha^{-1} (tree individuals ≥ 10 diameter at breast height - DBH) with mean wood density of 0.67 g cm^{-3} [55]. Local soils are geric Ferrasols (WRB Soil Classification) also known as Oxisols (USDA Soil Taxonomy) [56,57]. The soils are deep (≥ 400 cm) with good particle aggregation, friable and with low subsoil bulk density (0.8 – 1.2 g cm^{-3}) [58], typically acidic (pH ~ 4.1), with low concentrations of nutrients such as P (total P = 87.5 mg kg^{-1}), calcium (Ca) (0.034 cmolc kg^{-1}), and 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 is composed of thirty-two 50 m x 50 m plots distributed across four blocks separated by at least 200 m[30]. Each of the four blocks comprises eight plots, which are separated by at least 50 m, representing eight 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.

Fertilisation consists of 125 kg ha^{-1} $year^{-1}$ of N as urea ($CO(NH_2)_2$), 50 kg ha^{-1} $year^{-1}$ of P as triple superphosphate ($Ca(H_2PO_4)_2$) and base cations with 160 kg ha^{-1} $year^{-1}$ as dolomitic limestone ($CaMg(CO_3)_2$ for Ca and Mg plus 50 kg ha^{-1} $year^{-1}$ as potassium chloride (KCl) for K. Annual doses of N, P and K are similar to the Panama fertilisation experiment, in order to facilitate comparisons[59], while 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 2 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. (2021). 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 minutes, which was split into 15 minutes 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 minutes of manual root extraction and used to predict the pattern of extraction up to 180 minutes[30,60].

We used the census from November 2017 to September 2019, comprising two years of data collection (Year 1: November 2017 to Sept 2018 and Year 2: Dec 2018 to Sep 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 two. 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} $year^{-1}$.

Stem wood productivity. To calculate stem wood productivity, the stem diameter of all identified trees with a diameter at breast height (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[61] was applied to convert tree DBH (cm), species wood density (g cm^{-3}) and a bioclimatic parameter (*E*) in woody biomass. The equation has the following expression:

$$AGB = \exp(-2.024 - 0.896E + 0.920 \ln(WD) + 2.795 \ln(D) - 0.0461 [\ln(D)^2])$$

This is the slightly modified Eq 7 of Chave et al. 2014 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[61] related to climatic water deficit, temperature seasonality and precipitation seasonality, inferred when the site coordinates were given (lat 2° 40`S; long 60° W).

Wood density was estimated for each species from the *getWoodDensity* function from R *biomass* package using the global wood density database as a reference[62,63], 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 4600 tree individuals, we selected a census length of two years (2017-2019) and for 249 trees where one census was missing (*e.g.*: tree not measured in 2017, recruited in 2018 census, 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 two 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 ≥ 10 cm DBH in each plot (2500 m^2) and extrapolated to estimate the change in biomass per hectare. To convert biomass values into C, we assumed that dry stem biomass corresponds to 50% C[64] and stem wood productivity was expressed in Mg C ha^{-1} $year^{-1}$. To avoid or minimise 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 due to hydrostatic effects in times of drought[65].

Litterfall Productivity. Litterfall production was estimated by sampling litterfall every fifteen days in five litter traps (0.25 m^2) placed 1 m above the ground within the central area of each plot (30 x 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 were 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 two years. Litterfall productivity in g m^{-2} day^{-1} was extrapolated to Mg ha^{-1} $year^{-1}$ and the average was obtained considering two years of collection (Moraes et al, in prep; 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} $year^{-1}$.

Leaf area index (LAI). A LAI-2200C (LI-COR Biotechnology, Lincoln, Nebraska USA) was used to measure LAI inside the central 30 m x 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 6 am to 5 pm, avoiding recording data between 12:00 and 2:00 pm, 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/ m^2 ground area) and computed with 4 rings. These four rings read radiation in 4 angles, which are 7°, 23°, 38° and 53°. The data were collected during 10 to 13 October 2017, 22 to 25 March 2018, 07 to 10 August 2018 and between 29 October and 02 November 2018. LAI was based on these 4 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:

$$NPP_{total} = NPP_{fineroots} + NPP_{stem} + NPP_{litterfall}$$

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

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 ([66]). Leaf biomass was calculated by dividing the mean LAI of four campaigns (10 to 13 October 2017, 22 to 25 March 2018, 07 to 10 August 2018 and between 29 October and 02 November 2018) by specific leaf area (SLA). The SLA was included in two 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 et al, unpublished) 2) Obtained from sampling in litter traps (-P: 162.50 ± 26 g m^{-2} and +P: 128.75 ± 11 g m^{-2}). Transformations from 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 one 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 *lmerTest* package, based on the drop1 function which systematically drops fixed factors in order of the model hierarchy[67]. 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* were 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, leaf area index and leaf residence time. All models were run using *lme4* and *lmerTest* R packages[68]. 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 (i.e – P, n = 16) are compared to all plots where that nutrient was added (i.e., +P, n = 16) [22,30]. Original datasets from this study are publically available (Moraes et al. 2020[69], Cunha et al. 2021a[70], Cunha et al. 2021b[71], Cunha et al. 2021c[72]). Compiled datasets and R scripts used for statistical analyses, figures and tables are available at https://github.com/kmander7/Paper-AFEX-NPP.

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, 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 and at (https://doi.org/10.5285/6e70665f-b558-4949-b42a-49fbaec7e7cc) for leaf area index. Global Wood Density Database can be requested from http://datadrayad.org/handle/10255/dryad.235. 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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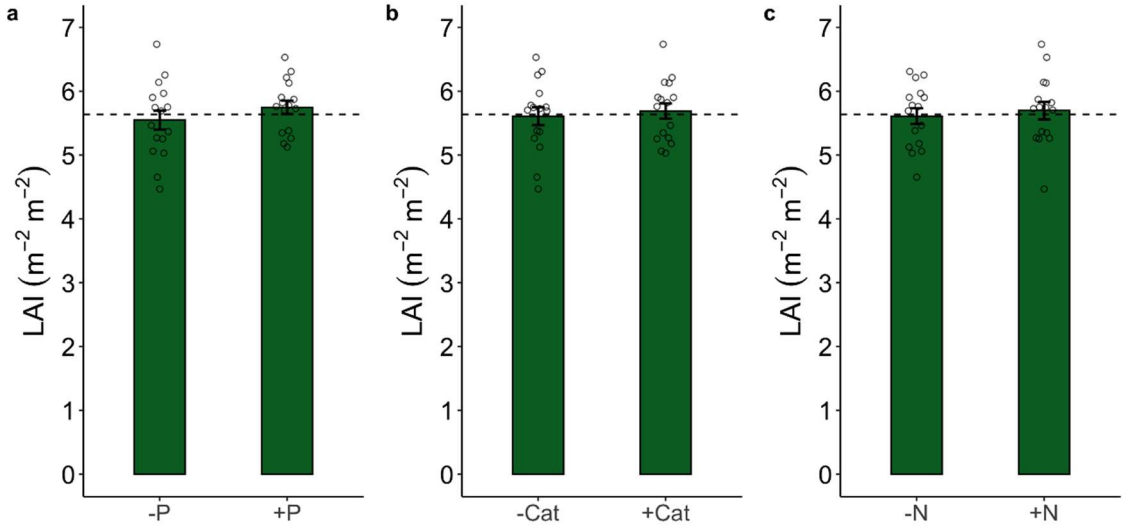
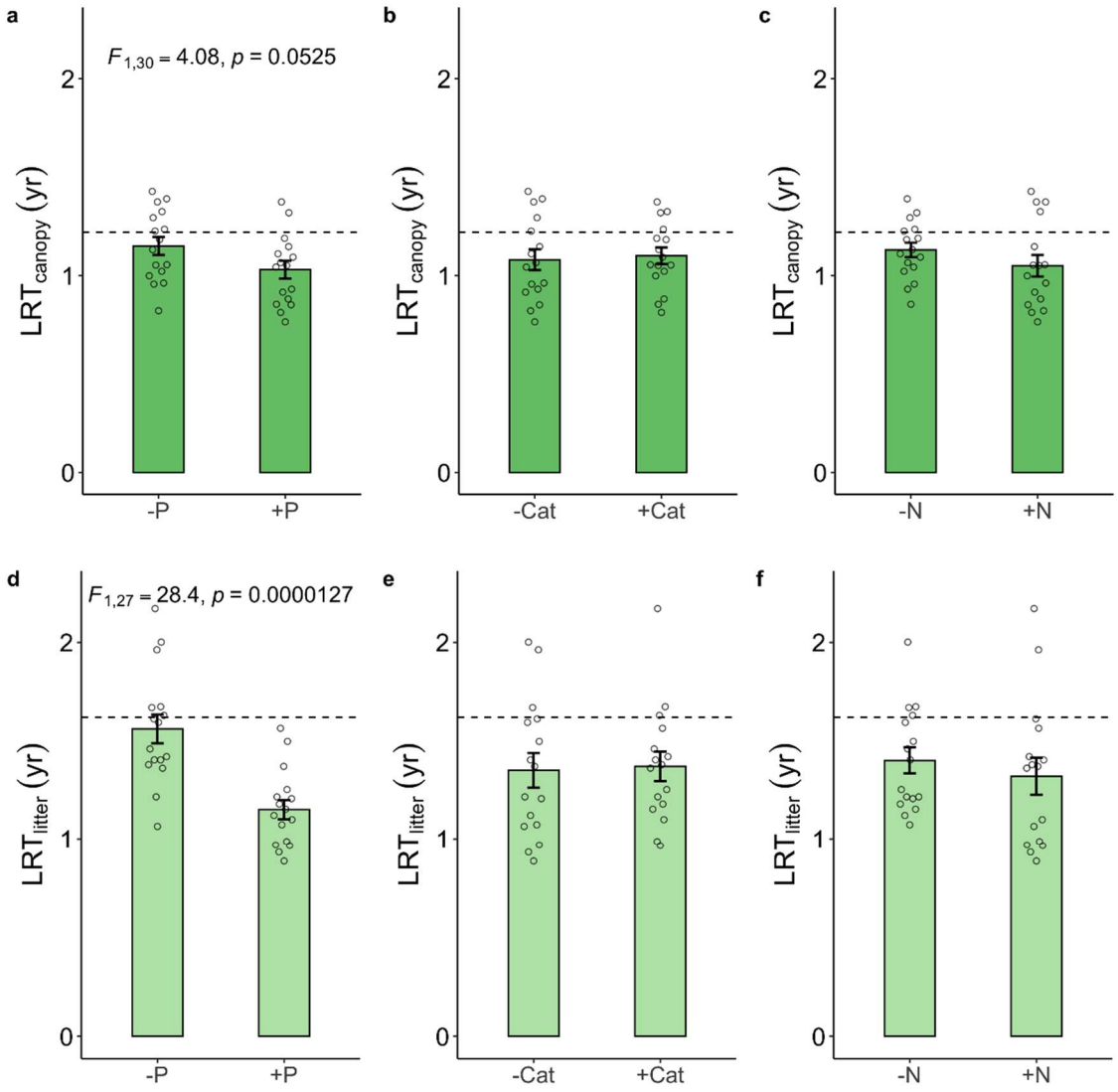


Extended Data Figure 1| Nutrient addition effects on Leaf area index. LAI was measured over four field campaigns across treatments in a lowland forest in Central Amazon. Each panel represents mean ± 1SE LAI with (+) or without (-) the addition of specific nutrients (phosphorus addition (a); base cation 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 Figure 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.

	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].

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

CAPÍTULO 2

Cunha, H.F.VC; Andersen, K.M; Quesada, C. A et al. Experimental evidence for nutrient limitation on Autotrophic and Heterotrophic respiration in a Central Amazon Forest. Manuscrito em preparação para *Ecology Letters*.

Experimental evidence for nutrient limitation on Autotrophic and Heterotrophic respiration in a Central Amazon Forest

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Abstract

Tropical forests are more productive terrestrial ecosystems compared to other biomes on Earth. However, to fully understand their contribution to global carbon (C) balance, it is necessary to determine respiratory fluxes in these ecosystems. Autotrophic (leaf, stem and roots) and heterotrophic respiration (soil microbes) return CO_2 from the ecosystem to the atmosphere and is affected by soil nutrient availability. However, such carbon-nutrient interactions remain a major uncertainty in tropical forest ecology. Since the majority of forests in Amazonia grow in soils with very low availability of rock-derived nutrients (e.g: phosphorus (P), cations), we hypothesized that both autotrophic and heterotrophic respiration would be affected by soil nutrient manipulation in a Central Amazonia Forest. To explore how ecosystem respiration changed with soil fertilisation, we used a randomly blocked, fully factorial nitrogen (N), P, and cation addition experiment (the Amazon Fertilisation Experiment (AFEX) installed in May 2017, with 8 treatments x 4 blocks in 50 x 50 m plots) in an old growth forest near Manaus, Brazil. We measured canopy leaf and stem respiration in October 2018 and October 2019, respectively. Soil autotrophic and heterotrophic respiration were measured in monthly surveys from September 2017 to August 2018. The addition of base cation in the absence of phosphorus and vice versa had a trend to enhance leaf respiration. Stem respiration (without the extrapolations) decreased by 12% with P addition (+P) compared to without P addition (-P) (- P: 1.26 ± 0.05 versus +P: 1.11 ± 0.05 μmol m^{-2} s^{-1}), and was driven by trees within the 35-45 cm diameter size class. Soil heterotrophic respiration increased 13% with added P (-P: 4.51 ± 0.18 versus +P: 5.10 ± 0.23 μmol m^{-2} s^{-1}). However, soil autotrophic respiration decreased when N was added without cations compared to other N * cation combinations. Overall, we found stronger soil than aboveground respiration responses to nutrient additions. However, a previous study at our site showed stronger above and below ground productivity responses to added nutrients compared to the respiration responses reported here. Therefore, it appears that the rapid productivity responses did not come with a high respiration cost, at least in the short-term. Together, initial responses from the AFEX project indicate that nutrient limitation needs to be accounted for to better understand ecosystem level carbon dynamics in Amazonian tropical forests.

Keywords

CO_2 efflux, multiple nutrients, tropical forest.

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INTRODUCTION

Respiration is one of the major components of the carbon balance in forests. The difference between Gross Primary Productivity (GPP) and ecosystem respiration (which combines both autotrophic and heterotrophic respiration) results in the Net Ecosystem Productivity (NEP) (Gifford, 2003). The NEP of Tropical Forests is estimated to be 410 g C m^{-2} yr^{-1} and is predicted that *c.* 10% of CO_2 influx by photosynthesis remains in the ecosystem (Luyssaert *et al*. 2007). The factors that increase respiration and, therefore, carbon (C) release can weaken or potentially reverse the carbon sink service in tropical forests (Rowland *et al*. 2018; Ogle, 2018). Understanding the drivers and the influence of nutrient limitation on respiratory regulation is necessary to improve model predictions of the carbon balance in tropical forests in the face of climate change. It has been shown that, across different ecosystems around the world, higher nutrient availability seems to channel C fixed by GPP towards the production of biomass, rather than being respired back to the atmosphere, increasing the potential of carbon sink (Fernandez Martinez *et al*. 2014). However, of all analyzed data, only 12% were from tropical forests, with the remaining 88% being from temperate, boreal and Mediterranean forests. Therefore, it is critical to examine nutrient limitation effects on respiration rates in tropical forests.

Combining respiration and productivity data, is possible to estimate the carbon use efficiency (CUE), the ratio of net primary productivity (NPP) to GPP, where a value of 0.3 indicates that 30% of the assimilated C is allocated into new tissues and 70% is released by respiration (Chambers *et al*. 2004). Based on this, CUE should be higher when respiration is reduced and when total NPP is increased. There is some evidence based on natural fertility gradients across Amazonian tropical forests, that CUE is positively correlated to soil fertility (Malhi *et al*. 2009; Doughty *et al*. 2017), but few direct evidence coming from fertilisation experiments. Indeed, if both total NPP and Autotrophic Respiration increases in the same proportion, no change in carbon use efficiency is expected with fertilisation (Maier *et al*. 2004).

Although tree growth is one of the most measured parameters in nutrient addition experiments (Santiago, 2015), there are limited studies about the effects of nutrient limitation on leaf (Rowland *et al*. 2016) and stem respiration in tropical forests. Leaf

respiration can be predicted from other leaf traits and was found to be positively correlated with leaf nitrogen, phosphorus, and photosynthetic capacity (Rowland *et al*. 2016; Atkin *et al*. 2015; Souza *et al*. 2021). However, the mechanisms that regulate the interaction between C balance and soil nutrients, and their effect on leaf respiration are not fully understood. The emission of CO_2 via respiration is a primary and vital plant process where carbon skeleton intermediates and energy (ATP) required for most maintenance and growth processes are synthesized (Gifford, 2003). Much of respiration involves maintenance, repair and replacement of proteins as well as the maintenance of photosynthetic activity (Amthor, 2000).

In stems, most of the respired CO_2 is a product of the respiration of living cells found in the cambium, inner bark (phloem) and xylem (Teskey *et al*. 2008; Meir *et al*. 2017). Stem respiration has been positively correlated with woody increment in tropical forests (Rowland *et al*. 2018; Katayama *et al*. 2016). In a *Eucalyptus saligna* plantation in the Island of Hawai'i, the addition of macro and micronutrients increased wood growth rates resulting in increased wood respiration relative to control plots (Giardina *et al*. 2003). However, no signs of wood growth (Diameter at Breast Height - DBH > 10 cm) were detected at the community level in large-scale nutrient addition experiments in species-rich tropical forests (Wright *et al*. 2018).

Growth limitation of microbial communities has been measured by changes in soil respiration in response to added nutrients, as a proxy for their growth or interpreted as microbial activity (Soong *et al*. 2019). It was observed that P addition stimulated soil respiration in a lowland tropical forest in Costa Rica, with data suggesting that this was mainly a heterotrophic response (Cleveland & Townsend 2006). It has been concluded in a review of 34 tropical sites that soil microbes are P-limited in tropical forests (Camenzind *et al*. 2018), with microbial biomass also found to be constrained by P availability (Turner & Wright 2014). Soil CO_2 fluxes also represent live root respiration, and although, root respiration rates are highly correlated with root N concentration (Burton *et al*. 2002), this parameter can depend on root biomass, with no changes in root biomass translating into no influence on total soil respiration (Clevelend & Towsend 2006). In addition, it is poorly known how the interaction between other nutrients affect root respiration.

The objective of this study was to investigate the responses of all large fluxes of autotrophic and heterotrophic respiration to the addition of P, N and base cations in a Central Amazon forest. For our knowledge, this is the first time that a large-scale fertilization experiment has measured all main ecosystem CO_2 fluxes in a tropical forest. Our results have great implications for improving dynamic vegetation models providing information on the relationships between soil nutrients and ecosystem respiration in tropical forests. To that aim, we tested the following hypothesis:

Hypothesis

1) *Leaf respiration will increase with P additions, triggered by the higher photosynthesis we detected in the first two years of the experiment, since the higher metabolism will imply a higher maintenance cost.*

2) *Since no growth responses on stem wood productivity were detected in our experiment, stem respiration will have muted responses to the addition of nutrients.*

3) *Root respiration (autotrophic) will be higher with P addition, triggered by the strong positive response of P addition in fine root productivity.*

4) *Heterotrophic respiration will be higher with P addition due to larger microbial biomass with P addition.*

5) *Carbon use efficiency will not change with P addition, because the higher total NPP will result in higher respiration of each compartment.*

METHODS

Study Site

The study site is located at one of the continuous old growth evergreen forests of the Biological Dynamics of Forest Fragments (BDFF), 80 km north of Manaus, Brazil, in Central Amazonia (2° 30´S, 60° W) (Laurance *et al*. 2018). The experimental area is located in a *terra firme* forest and has a high-species diversity, with about 280 plant species (≥ 10 cm DBH) per hectare (Oliveira & Mori 1999). The dominant tree families in the 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 temperature is *c.* 26 °C (Ferreira *et al*. 2005), and mean annual precipitation is 2400 mm with a dry season from June to October, when monthly precipitation can reach less than 100 mm (Tanaka *et al*. 2014). There is little variation in soil temperature throughout the year, with an average of 25,5 °C during the dry season and 25,3 °C during the rainy season (AFEX data). Above ground biomass (AGB) is estimated to be 322 ± 54 Mg ha^{-1} (tree individuals ≥ 10 DBH) and mean wood density is 0.67 g cm^{-3} (Duque *et al*. 2017). For a summary about vegetation status at the start of the experiment (June, 2017), see the Supplementary information (Extended Data Table 1).

Local soil is classified as geric Ferrasols (World Reference Base Soil Classification) also known as Oxisols (US Department of Agriculture Soil Taxonomy) (Quesada *et al*. 2011; Quesada *et al*. 2010). The soils are deep (≥400 cm) with good particle aggregation, friable and low subsoil bulk density (0.8 – 1.2 g cm^{-3}) (Martins *et al*. 2014; Quesada *et al*. 2011), typically acidic (pH ~ 4.1) and poor in nutrients such as P, calcium (Ca) and K. In the control plot for the 0-30 cm soil depth there was a sum of bases in the soil (Ca, Mg, K

and Na) of 0.155 ± 0.021 $cmol_c\ kg^{-1}$ and a total phosphorus of 87.46 ± 1.84 mg kg^{-1}. The soil is also very clayey, with clay *c*. 77% (AFEX data).

The study was carried out at the experimental site of the Amazon Fertilisation Experiment – AFEX project, which aims to evaluate the effects of soil fertility on the carbon cycle of the Amazon Forest through a soil fertility manipulation experiment.

Experimental design

The AFEX experimental design consists of thirty-two 50 m x 50 m plots distributed across four blocks separated by at least 200 m (see complete description in Lugli *et al*. 2021). Each of the four blocks comprises eight plots, which are separated by at least 50 m, representing eight 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.

Fertilisation consists of 125 kg ha^{-1} $year^{-1}$ of N as urea ($CO(NH_2)_2$), 50 kg ha^{-1} $year^{-1}$ of P as triple superphosphate ($Ca(H_2PO_4)_2$) and base cations with 160 kg ha^{-1} $year^{-1}$ as dolomitic limestone ($CaMg(CO_3)_2$ for Ca and Mg plus 50 kg ha^{-1} $year^{-1}$ as potassium chloride (KCl) for K. Annual doses of N, P and K are similar to the Panama fertilisation experiment, in order to facilitate comparison (Wright *et al*. 2011), while 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. The annual input of nutrients through the fine litter in the site is 151 kg ha^{-1} $year^{-1}$ of N, 3.1 kg ha^{-1} $year^{-1}$ of P, 15 kg ha^{-1} $year^{-1}$ of K, 36.7 kg ha^{-1} $year^{-1}$ of Ca and 13.8 kg ha^{-1} $year^{-1}$ of Mg (Luizão, 1989). The addition of P by fertilisation is ~ 16 x the annual input by litterfall, the addition of K is ~ 3 x the annual input by litterfall and N addition is ~ 83% of the annual input by litterfall.

Nutrient additions are split into three equal applications throughout each wet season, with nutrients added every year since May 2017. The results presented here correspond to leaf, stem and soil respiration measured between 2017 and 2019.

Species selection for leaf and stem respiration

To avoid confounding factors of phylogenetically conserved traits, we selected the trees from five hyperdominant families occurring in each plot (Ter steege *et al*. 2013; Fauset *et al*. 2015). For leaf respiration measurements, we selected eight individuals per plot (ranging from 5 to 12 trees per plot), totaling 238 trees. We focused most of the measurements in tree families and genera that were repeated between plots. The selection also considered practical aspects, which included the access of as many branches as possible of different tree species in a single climb on the tree. The most frequent families sampled were: Fabaceae (22,10%), Sapotaceae (15,22%), Lecythidaceae (14,85%), Burseraceae (13,41%) and Malvaceae (13,04%) and the most frequent genera measured were: *Eschweilera* (13,41%), *Protium* (12,68%), *Scleronema* (12.32%), *Pouteria* (10,51%), *Swartzia* (9,42%), *Tachigali* (6,88%) and *Ocotea* (6,52%). For stem

respiration, we selected trees > 25 cm DBH, for logistical reasons (Marthews *et al*. 2014), with dendrometry bands. However, although large trees have a major contribution to biomass, their influence on total stem ecosystem respiration may be less than smaller size classes (Katayama *et al*. 2016). The most frequent families sampled were Lecythidaceae (21,5%), Fabaceae (15,63%), Sapotaceae (14,06%) and Euphorbiaceae (9,375%) and the most frequent genera collected were *Eschweilera* (15%), *Scleronema* (8,4%), *Pouteria* (7,2%) and *Micrandropsis* (6,56%). There was an overlap of 89 trees between stem and leaf respiration measurements due to similarities in tree selection criteria. The trees are not more similar between the leaf respiration and stem respiration campaigns, because the leaf respiration focused on the canopy trees, while in the stem respiration, the focus was on trees with DBH > 26 cm, regardless whether they were in the canopy or not.

Leaf Respiration

Leaf dark respiration (R_{dark}) was measured in the dry season between 02 and 18 October 2018, ~ 15 months after the first fertilisation. To prioritize the sampling of as many trees as possible, we measured one mature, healthy and fully expanded leaf per tree (Atkin *et al*. 2015; Souza *et al*. 2021). Fully exposed canopy branches were cut from the tree crown by tree climbers and immediately placed in a bucket with water to restore hydraulic conductivity (Rowland *et al*. 2016). The selected leaves were wrapped in aluminum foil for 30 min before the measurements to acclimate to the dark and ensure that steady-state conditions had been achieved (Atkin *et al*. 1998). Henceforth, R_{dark} will be called only leaf respiration.

Leaf respiration was measured between 8:00 and 15:00 h using a portable infrared gas analyser (IRGA – LI – 6400XT, LiCor, BioSciences, Lincoln, NE, USA). The IRGA chamber was set to a photosynthetic photon flux density (PPFD) of 0 μmol m^{-2} s^{-1}, 400 ppm CO_2 concentration, 32 °C chamber temperature, 60 to 75 % relative humidity, and 300 μmol s^{-1} air flow rate. The IRGA was configurated to match the water and CO_2 concentrations of the sample and reference before each measurement to improve the accuracy of the measurements.

To scale up the values, the average leaf respiration per unit leaf area per plot was multiplied by the average leaf area index (LAI) per plot, using LAI 2200-C measured in 16 points on a grid with an even spacing of 10 m in a plot central area representing 30 x 30 m. The LAI was collected from 6 am to 5 pm, avoiding recording data between 12:00 and 2:00 pm to avoid direct sun, in late October 2018.

Stem Respiration

Stem respiration (R_{stem}) measurements were performed on 320 trees with DBH > 26 cm, in 10 trees per plot, from 20 to 27 October 2019. To obtain a mean R_{stem} value per plot, we calculate the average of the 10 trees from each plot. The measurements were made between 8:00 and 16:00 h, based on Rowland *et al*. 2018.

R_{stem} was measured with an infrared gas analyser (IRGA-EGM-4, PP systems) and a chamber temporarily placed onto a collar installed *c*. 10 cm above the ink mark of the DBH measurement. The collar was used to detect an increase in CO_2 concentration for 120 s inside the chamber. The mosses along the stems were removed with a soft cloth before attaching the collar to the tree (Robertson *et al*. 2010). The collar was fixed with a special glue (Siloc PU 36) to eliminate any gaps between the collar and the stem.

We calculate stem respiration efflux from the raw data (ppm of CO_2) based on the manual developed by the RAINFOR (www.rainfor.org/pt/manuais/em-campo), using the following equation:

$$R_{uc} = \frac{\Delta CO2}{\Delta T} * \frac{ATMP}{1000} * \frac{273}{273} * \frac{44.01}{22.41} * \frac{Vd}{A} * 3600$$

Where R_{uc} is the uncorrected CO_2 efflux (g CO_2 m^{-2} h^{-1}), Δ CO_2 is the difference between final and initial CO_2 concentrations (ppm), Δ T is the difference between final and initial time (s), ATMP is the atmospheric pressure *c*. 968 milibars, T is the air temperature 26 °C, Vd is the chamber volume 0.0012287 m^3 and A is the area enclosed within the chamber 0.00882 m^2.

After that, the following equation was applied, using the R_{uc} previously calculated.

$$R_c = R_{uc} * ((A/Vd) * ((A * (H/100)) + Vd)/A)$$

Where R_c is the CO_2 efflux corrected by the chamber volume (g CO_2 m^{-2} h^{-1}), R_{uc} is the uncorrected CO_2 efflux in (g CO_2 m^{-2} h^{-1}), A is the area enclosed within the chamber 0.00882 m^2, Vd is the chamber volume 0.0012287 m^3, H is the height of the collar connected to the chamber (3 cm). The value in g CO_2 m^2 h^{-1} was transformed in µmol CO_2 m^{-2} s^{-1}.

For scaling up stem CO_2 efflux, we used a similar approach that we used for the R_{dark}. First, we calculated the stem surface area (A_{stem}) following the equation of Chambers *et al*. 2004, and we applied the equation to all trees with DBH > 10 cm of each plot using data from the 2019 inventory. A_{stem} in m^2 is the surface of all above ground wood except small twigs. Then, the stem area index (SAI) value per plot was the mean value of all stem surface area (A_{stem}) in the plot (no unit/dimensionless).

$$A_{stem} = -0.105 - 0.686 * \log(\text{DBH}) + 2.208 * \log(\text{DBH})^2 - 0.627 * \log(\text{DBH})^3$$

Where DBH is the diameter at breast height.

Although R_{stem} measurements were taken from trees > 26 cm, we extrapolated the ecosystem stem respiration to all trees > 10 cm, in the same way as leaf respiration of dominant sun trees was extrapolated to the whole plot scale leaf cover. Also, simulating the use of SAI only on trees > 26 cm, tended to increase the estimates of ecosystem stem respiration (data not shown).

To explore how stem respiration varies with DBH, trees were classified into four diameter classes: 1) 26-35 cm (112 trees) 2) 35-45 cm (123 trees) 3) 45-55 cm (50 trees) and 4) > 55 cm (35 trees). In a previous study, stem respiration in the wet season was driven by a particular class of tree size (<40 cm DBH), and for this reason, we used a similar approach (Rowland *et al*. 2018).

Soil Respiration

In June 2017, we established a soil respiration partitioning experiment consisting of two pairs of collars (20 cm diameter polyvinyl chloride plastic – PVC – rings) installed at two random points of the central plot of 30 x 30 m. The 5 cm surface collars integrated all components of total respiration ($R_{microbes} + R_{autotrophic}$). To avoid CO_2 leakage in the surface collars during measurements, slightly moist clay from each plot was placed around the base of the superficial collars before the measurements. The ($R_{microbes}$) collars had1 µm mesh size window and were inserted to 25 cm depth to exclude roots and mycorrhizae hyphae. The hyphal diameter of mycorrhizal fungi is reported to be between 2 and 20 µm (Friese & Allen 1991). The contribution of autotrophic respiration in the soil was calculated from difference between the collars ($R_{autotrophic}$= ($R_{microbes}$ + $R_{autotrophic}$) - ($R_{microbes}$). (Heinemeyer *et al*. 2007). The first measurement of July 2017 was not included in the analysis to avoid the effect of soil disturbance associated with the installation of the deep collar. Thus, the collars were allowed to stabilize for 81 days before the data collection started, which was time enough for the CO_2 effluxes to return to baseline values (Supplementary Note 1, Fisher *et al*. 2013).

We measured soil respiration (R_{soil}) rates using an automated soil CO_2 flux system (LI – 8100A, Li-Cor, Lincoln, NE, USA), equipped with a 20 cm survey chamber (Model:8100-101). The observation length was 90 seconds, with pre and post purge of 10 seconds to allow flushing the system between each measurement, with duplicate measures on each collar. As soil respiration activity is sustained by organic matter inputs to the soil from aboveground and from roots (Raich, 1992) we standardized the number of leaves in all collars for each plot prior to each measurement.

The linear flux with a coefficient of variation (CV) ≤ 1.2 was estimated using Soil FluxPro version 4.2.1 software (Li-Cor, Lincoln, NE, USA). To estimate the mean efflux by plot for each type of collar, mean soil respiration values per collar type were averaged to represent plot means for each sampling period. We estimated soil respiration and its components for one year, from September 2017 until August 2018. No measurements were taken in December 2017, so the annual mean was calculated with data from 11 censuses and transformed to a single representative value of soil respiration over one year.

Ecosystem fluxes

We calculated total autotrophic respiration (R_A), using the following equation:

$R_A = R_{leaf} + R_{stem} + R_{aut\_soil}$

All terms are expressed in Mg C ha^{-1} $year^{-1}$ and µmol m^{-2} s^{-1}

All the major components of respiration were summed to R_A and R_{hetero}, resulting in the Ecosystem Respiration (R_{eco}) (Metcalfe *et al*. 2010). We estimated the carbon use efficiency (CUE) as the ratio between total net primary productivity (NPP) and gross primary productivity (GPP) (DeLucia *et al.* 2007). The GPP was calculated as the sum of total NPP and $R_{A,}$ using the total NPP previously calculated for the site (Cunha *et al*. 2022).

Data analyses

Linear mixed models were used to test the effect of nutrients and their interaction in the factorial design N x P x base cations with the four blocks as random factors and the response variables were leaf, stem, autotrophic respiration in the soil, total autotrophic respiration, GPP, ecosystem respiration, carbon use efficiency. All dependent variable were based on their mean values per plot before inclusion in the model. The models were run using *lme4* and *lmerTest* R packages (Bates *et al*. 2015). We started with the full model including all nutrients and their interactions, and followed a stepwise backward elimination of non-significant effects based on chi square test comparing two consecutive models using the *step*() function in 'lmerTest' package (Kuznetsova *et al*. 2017). Results are reported for the best fit model in the text and figures. Results are shown for single nutrient addition only if no significant effects of nutrient interaction were found between the different nutrients added. For the main effect of specific nutrient, all plots where the nutrient was not added (i.e. – P, n=16) were compared to all plots where that nutrient was added (i.e. + P, n=16) (Wright *et al*. 2011; Lugli *et al*. 2021)

We checked the assumptions for normality and homogeneity of variance to meet assumptions for linear models, using the Shapiro-Wilk and Leven tests. A probability < 0.05 was adopted to determine significance. Fluxes are given in Mg C ha^{-1} yr^{-1} (1 Mg C ha^{-1} yr^{-1} = 0.264 µmol m^{-2} s^{-1}). Original datasets for stem and soil respiration from this study are publicly available (Cunha *et al*. 2021).

RESULTS

Leaf respiration

After 16 months of nutrient addition, mean R_{leaf} across all control plots (n = 4 plots) was 1.42 ± 0.09 µmol m^{-2} s^{-1}. At the leaf scale, there was an interaction between P and base cations affecting R_{leaf} (P:Cations: $F_{1,25} = 4.09$, $p = 0.054$; Fig. 1). R_{leaf} was similar in plots with neither and both phosphorus and cations, but the addition of base cation in the absence of phosphorus and vice versa had a trend to enhance leaf respiration.

The mean upscaled R_{leaf} across all control plots (n = 4 plots) was 8.17 ± 1.30 Mg C ha^{-1} $year^{-1}$. We observed no effect of P, N, or base cation nor their interactions on mean upscaled R_{leaf}.

Stem Respiration

The mean R_{stem} across control plots (n = 4 plots) was 1.28 µmol m^{-2} s^{-1} ± 0.12. Phosphorus addition significantly decreased R_{stem} by 12% without the extrapolations (- P: 1.26 ± 0.05 *versus* + P: 1.11 ± 0.05 µmol m^{-2} s^{-1}; $F_{1,30}$= 4.1, p= 0.05; Table 1) compared to plots without P addition. This decrease in R_{stem} with P addition was mainly driven by trees in the 35 - 45 cm DBH class (17% decrease; - P: 1.28 ± 0.08 *versus* + P:1.06 ± 0.08; $F_{1,25}$= 4.8, p = 0.038; Extended Data Figure 2). In this same diameter class of 35 - 45 cm DBH, we also observed significantly lower values of R_{stem} with base cations addition (19% decrease; - CAT: 1.29 ± 0.09 *versus* + CAT: 1.04 ± 0.08; $F_{1,25}$= 6.13, p = 0.02) compared to plots without cations. However, there were no interactive effects of any nutrient, nor was there an effect of added N.
The mean upscaled R_{stem} across control plots (n = 4 plots) was 6.21 ± 0.67 Mg C ha^{-1} $year^{-1}$. The mean stem area index (SAI) considering the 32 plots was 1.3 (no units/dimensionless). Compared to plots without P, the addition of P showed a marginal nonsignificant trend towards decreasing stem respiration (12% decrease; - P: 6.13 ± 0.26 *versus* + P 5.43 ± 0.25 Mg C ha^{-1} $year^{-1}$; $F_{1,30}$ = 3.65, p = 0.065). There was also a tendency to decrease stem respiration with cation addition, although non-significant (-CAT: 6.09 ± 0.32 *versus* +CAT: 5.48 ± 0.19 Mg C ha^{-1} $year^{-1}$; $F_{1,29}$ = 2.95, p =0.09). We did not observe any influence of N addition on R_{stem}.

Soil Respiration

The mean total R_{soil} across control plots (n = 4 plots) was 5.55 ± 0.80 µmol m^2 s^{-1}. We observed no effect of N, P or Cations on total R_{soil} (N: $F_{1,26}$ = 0.06, p = 0.8; P: $F_{1,27}$ = 2.54, p = 0.13; Cations: $F_{1,24}$ = 1.47, p = 0.24), and no interaction between nutrients was found.

The mean plot-scale total R_{soil} across control plots (n = 4 plots) was 21.04 ± 3.03 Mg C ha^{-1} $year^{-1}$. After one year of nutrient addition, no significant changes in total R_{soil} were detected with the addition of N, P or Cations (N: $F_{1,26}$ = 0.06, p = 0.8; P: $F_{1,27}$ = 2.54, p = 0.13; Cations: $F_{1,24}$ = 1.47, p = 0.24). Of the total CO_2 soil flux *c*. 73% was from heterotrophic respiration and *c.* 27% from autotrophic respiration including roots and mycorrhizal.

The mean autotrophic soil respiration (roots + mycorrhiza) across control plots (n = 4 plots) was 1.48 ± 0.66 µmol m^{-2} s^{-1}. Autotrophic soil respiration showed an interaction between N and Cations (N X Cation; $F_{1,28}$ = 6.05, p=0.02;) with a decrease in respiration rates with the addition of N in the absence of cation. The same pattern and magnitude of response were observed for plot-scale autotrophic respiration (N X Cation; $F_{1,28}$ = 6.05, p = 0.02; Fig. 02a).

The mean heterotrophic respiration (soil microbes) across control plots (n = 4 plots) was 4.07 ± 0.17 µmol m^{-2} s^{-1}. Phosphorus addition significantly increased the mean heterotrophic respiration by 13 % compared to plots without added P (- P: 4.51 ± 0.18 *versus* + P: 5.10 ± 0.23 µmol m^{-2} s^{-1}; $F_{1,24}$ = 5.2, p = 0.03; Table 01). There was also an interaction between N and Cations addition (N X Cation; $F_{1,24}$ = 5.66, p= 0.02), where the addition of N in the absence of Cations increased heterotrophic respiration. As expected, the same pattern and magnitude of response were observed for plot-scale heterotrophic respiration (- P: 17.09 ± 0.7 *versus* + P: 19.33 ± 0.86 Mg C ha^{-1} yr^{-1}; $F_{1,24}$ = 5.21, p = 0.03 and N X Cation; $F_{1,24}$ = 5.66, p= 0.02; Fig. 2c and Fig. 2b).

Gross Primary Productivity and Carbon Use efficiency

The mean GPP across control plots (n = 4 plots) was 27.4 ± 3.98 Mg C ha^{-1} ano^{-1} and the mean CUE was 0.30 ± 0.078. We did not observe any influence of N, P, or Cations addition on CUE (Fig. 03), GPP, total R_A or R_{eco}.

Table 1 The effect of N, P and Cations on different compartments of forest respiration. Summary of autotrophic and heterotrophic respiration compartments (µmol m^{-2} s^{-1}) in control plots (n = 4 plots; grey shaded column) and with (+) and without (-) N, P and Cations addition (n=16 plots) in a Central Amazon forest. The control plot is only a parameter for comparison. Values are presented as mean ± 1SE. Significant effects are indicated in bold by *, **, and ***, representing probability at the 0.05, 0.01, and 0.001 levels respectively.

	Control	-N	+N	-P	+P	-Cat	+Cat
R_{leaf}	1.42± 0.09	1.53 ± 0.07	1.53 ± 0.06	1.56 ± 0.08	1.50 ± 0.06	1.53 ± 0.06	1.53 ± 0.08
R_{stem}	1.28 ± 0.12	1.16 ± 0.05	1.21 ± 0.05	**1.26* ± 0.05**	**1.11* ± 0.05**	1.25 ± 0.06	1.12 ± 0.04
R_{soil}	5.55 ± 0.80	5.76 ± 0.27	5.67 ± 0.26	5.44 ± 0.23	6.00 ± 0.28	5.51 ± 0.26	5.93 ± 0.27
R_{het}	4.07 ± 0.17	4.68 ± 0.20	4.94 ± 0.23	**4.51* ± 0.18**	**5.10* ± 0.23**	4.75 ± 0.22	4.86 ± 0.22
R_{aut_soil}	1.48 ± 0.66	1.08 ± 0.22	0.74 ± 0.23	0.93 ± 0.23	0.90 ± 0.23	0.76 ± 0.25	1.07 ± 0.19

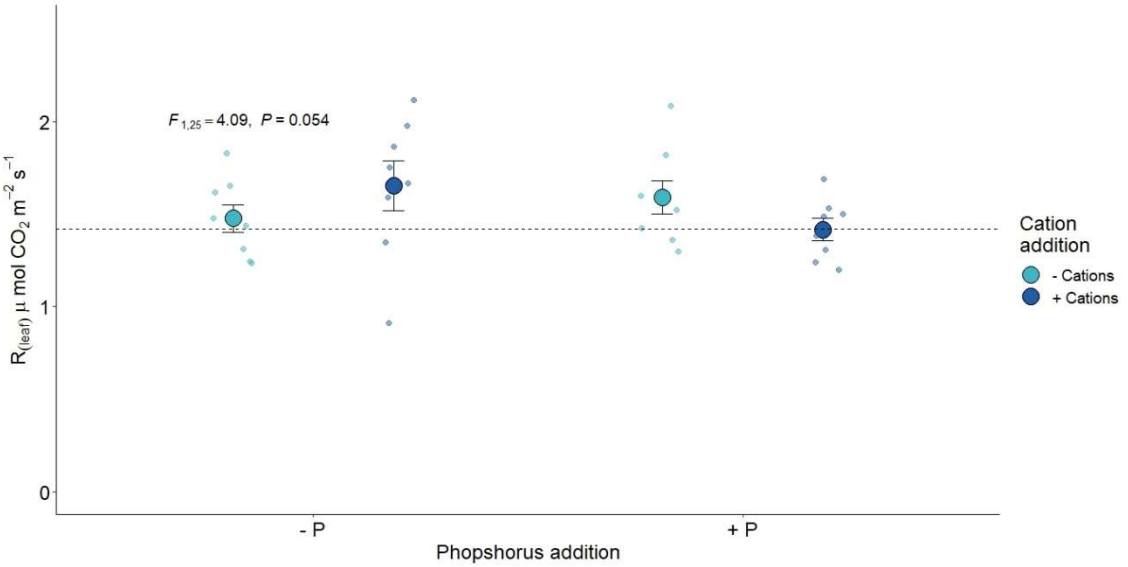

Figure 01 Nutrient addition effects on Leaf Respiration. Interaction between P and cations addition (n = 8 plots) on leaf respiration (µmol CO_2 m^{-2} s^{-1}), presented as mean ± 1 SE, in a Central Amazon Forest. The light blue circles represent the plots without cations addition, and dark blue circles represent the plots with cation addition. The dashed line represents the control plots (n = 4 plots, no nutrient added). Linear mixed models were performed to evaluate responses in leaf respiration to added nutrients.

Table 02 The effect of N, P and Cations on different ecosystem CO_2 fluxes. Summary of Total NPP, Autotrophic Respiration and Gross Primary Productivity (Mg C ha^{-1} $year^{-1}$) in control plots (n = 4 plots; grey shaded column) and with (+) and without (-) N, P and Cations addition (n = 16 plots) in a Central Amazon forest. The control plot is only a parameter for comparison. Statistical analyses compare plots with presence and absence of a specific nutrient. No significant differences among the means were detected in linear mixed models for Total R_A and GPP.

	Control	-N	+N	-P	+P	-Cat	+Cat
Total NPP	7.37 ± 0.59	8.01 ± 0.30	8.02 ± 0.33	**7.44± 0.21**	**8.60± 0.33**	8.04± 0.35	8.00± 0.28
Total R_A	19.99± 4.34	19.10± 1.35	17.91± 1.18	18.70 ±1.41	18.32 ± 1.13	17.83 ± 1.49	19.19 ± 0.99
GPP	27.36± 3.98	27.12 ± 1.22	25.94 ± 1.28	26.14 ±1.36	26.92 ± 1.4	25.87 ± 1.53	27.19 ± 0.88

Note: The available data are not still adequate to make a carbon balance, to know if the system is a source or sink of carbon.

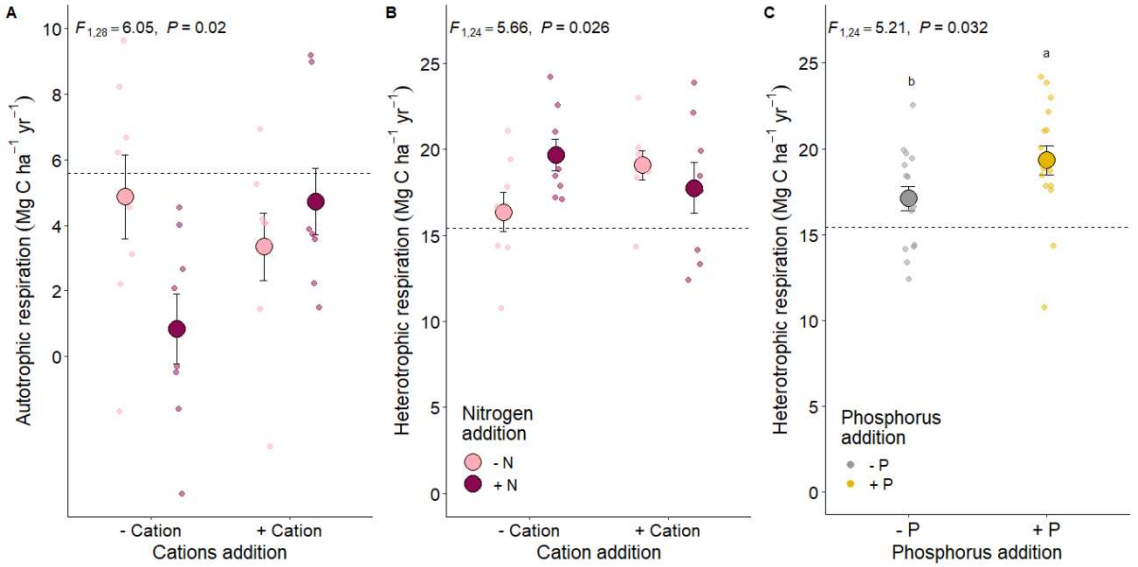

Figure 02 Influence of nutrient addition in soil respiration. A and B Interaction between N and Cations addition (n = 8 plots) on autotrophic and heterotrophic soil respiration. The light pink circles represent the plots without N addition, and the dark pink circles represent the plots with N addition. The legend of panel B is the same of the panel A. **C** Heterotrophic respiration (Mg C ha^{-1} year^{-1}) with (+) and without (-) P addition (n = 16 plots) in a Central Amazon Forest. The values are presented as means ± 1SE. The dashed line represents the control plots (n = 4 plots, no nutrient added).

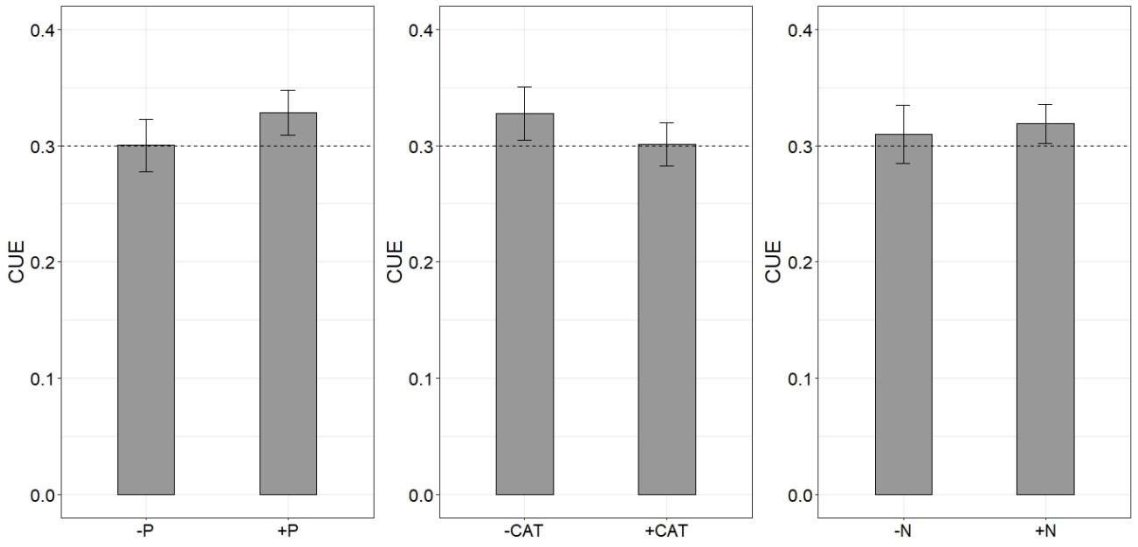

Figure 03 Nutrient addition effects on Carbon Use Efficiency. Carbon use efficiency (NPP/GPP) in control plots (n = 4 plots, dashed line) and with (+) and without (-) N, P,

and Cations addition (n = 16 plots) in a Central Amazon forest. We found no significant differences within treatments.

DISCUSSION

We examined the effect of nutrient addition on the respiration of leaf, stem wood and soil compartments in a nutrient manipulation experiment at an Amazon forest. Assuming a positive relation between productivity and respiration, we expected a phosphorus addition main response for respiration processes. In contrast, the components of respiration responded to various combinations of added nutrients. The lack of a main response of phosphorus addition in leaves and autotrophic respiration in the soil, and the decrease in stem respiration may indicate that the strong responses to phosphorus addition in root and canopy growth were occurring with little respiratory cost. Furthermore, phosphorus addition decreased stem respiration. However, these plant responses to added nutrients did not result in changes in GPP or CUE. Multiple nutrients were limiting heterotrophic respiration, including phosphorus and interactions between nitrogen and cations. These results contribute to a better understanding of the impact of nutrients in respiration processes and have relevance to the modelling of carbon cycling in tropical forests.

Leaf Respiration

We hypothesized a main effect of phosphorus in leaf respiration, to maintain higher leaf level photosynthesis with added phosphorus at our site (Silva, 2020). However, we found an interaction between phosphorus and cations where the addition of phosphorus in the absence of base cations and vice versa had a trend to enhance leaf respiration. In contrast, when both phosphorus and cation are added together, leaf respiration rates were similar to control plots. Together, this indicates less favorable foliar carbon processing capacities with the addition of single nutrients suggesting the balance of carbon in leaves more favorable with phosphorus and cation alleviation. This response was marginally significant, and may change over time. This response can suggest that the plant's strategy is to produce more leaves, since we observed a higher production of leaves with phosphorus addition (Cunha *et al*. 2002), and not in boost per leaf metabolism. In addition, there are some methodological issues, an effect in photosynthesis is easier to detect when compared to respiration, because the fluxes are larger in the former, while in respiration the fluxes are small. Further investigation is necessary to determine the mechanistic processes responsible for these changes in leaf respiration rates.

The leaf respiration in the control plot of 1.42 ± 0.09 μmol m^{-2} s^{-1} was similar to an eastern Amazonian lowland tropical forest, with a mean of 1.42 μmol m^{-2} s^{-1} (which ranged from 0.93 to 2.42 μmol m^{-2} s^{-1} for five species classified as full sunlit-canopy trees (Domingues *et al*. 2014). In a study across three tropical forests representing a soil P gradient from French Guiana (222.5 mg kg^{-1}) to Australia (370.4 mg kg^{-1}) to Peru (392.6 mg kg^{-1}) found that R_{dark} is greater at the site with the lowest available soil and leaf P, indicating that the

demand for respiratory products was greater at the most nutrient-limited site (Rowland *et al*. 2016).

Contrary to our expectation, leaf respiration at plot scale did not change with nutrient addition. Possible reasons include i) short-time nature of the experiment (~ 1.6 years after the beginning of fertilisation or ii) the use of LAI as parameter to scale up, since there was no significant effect of nutrient yet for this variable (Cunha *et al*. 2022).

Stem Respiration

We hypothesized muted responses to the addition of nutrients on stem respiration. In contrast, stem respiration was reduced by P addition. This response can suggest that carbon is being sent to tissues with greater demand for phosphorus. Respiration could be lower in wood tissues with phosphorus addition, because living tissue in the trunk contains more structural material (lignocellulosic cell walls) with slow turnover (Gifford, 2003), and possibly demands less respiratory products when compared with leaves for example. Alternatively, there is evidence that reduced stem respiration rates are correlated with high sap flux rates, at least for a tropical forest in Central Amazonia (Kunert and Cárdenas, 2012). In this sense, with the higher sap flux rates, a portion of the CO_2 released by respiration of local cells can be dissolved in the xylem sap and transported away in the transpiration stream (Teskey, 2008), with less time to diffuse for the atmosphere in the point of measurement, underestimating the measures of stem respiration.

When we compared the upscaled values of stem respiration (6.21 Mg C ha-1 yr^{-1}) with three other sites along the Basin (Caxiuanã National Forest Reserve-Brazil, Cuieiras Biological Reserve - Brazil and Tambopata Biological Reserve- Peru), we did not observe a relationship between the total soil phosphorus and stem respiration across the sites (Table 3). The highest stem respiration value was observed at the Caxiuanã (7.07 Mg C ha^{-1} yr^{-1}), in a sandy soil with low total phosphorus content (37.4 mg kg^{-1}), which is close to the value observed at the Tambopata site (6.44 Mg C ha^{-1} yr^{-1}) in a soil with high concentration of total phosphorus (528 mg kg^{-1}). The mean flux of stem respiration on the control plots (6.21 Mg C ha^{-1} yr^{-1}) was higher than found by a previous study in the nearby Cuieiras reserve (4.2 Mg C ha^{-1} yr^{-1}) (Chambers *et al*. 2004) (Table 3). The difference may result from differences in the selection of trees, since our experiment comprises 320 trees > 26 cm DBH (trees with higher biomass), while Chambers *et al* 2004 reported results from 50 trees across five growth classes, including a class that experienced no measurable growth.

Notably, it has been found in a previous study that ~35% of CO_2 respired in the stem is not emitted locally in the tissue, and is probably transported upward in the transpiration stream, theoretically reaching the canopy (Angert *et al*. 2012). This can cause an underestimation of the stem respiration, but this is happening for all treatments in our experiment, and differences between treatments that real matter can be detected.

Soil Respiration

We hypothesized that phosphorus addition would increase root respiration. Contrary to hypothesis 3, P addition did not stimulate autotrophic soil respiration. In a previous study, root biomass did not change with phosphorus addition in the first two years of experiment (Schmeisk Rosa, not published), which suggests that root respiratory costs also remained stable. By contrast, the addition of N without cations decreased autotrophic respiration as well as the turnover of roots in the second year (2018-2019; Schmeisk unpublished). Together, this suggests that roots are becoming more conservative, with a less active metabolism and longer life span with the addition of nitrogen without cations. This can indicate an inadequate ratio of N/Ca in tissues, when roots are not well supplied with mineral nutrients, their rates of growth and ion uptake are greatly reduced (Lambers *et al*. 2008).

We hypothesized that phosphorus addition would increase heterotrophic respiration. As expected, P addition increased heterotrophic respiration by 13%. The P addition can decrease microbial P limitation and stimulate microbial activity in tropical forests increasing heterotrophic respiration rates (Cleveland *et al*. 2002; Cleveland & Townsend 2016). In a meta-analysis across different tropical fertilisation experiments, soil microbial biomass was stimulated with P addition, resulting in increased heterotrophic respiration (Camenzind *et al*. 2018). With the alleviation of P limitation, soil microorganisms could shift towards more acquisitive strategies, maximizing their growth and therefore requiring high respiratory rates and accumulating higher microbial biomass. Further examination of the microbial community is required to determine the mechanisms behind the increased heterotrophic respiration rates found in our experiment.

The greatest magnitude of heterotrophic respiration with P addition were observed between January and March 2018 (Extended Data Figure 01). These values may be associated with the highest precipitation during this period and with the fertilisation that occurred in February and March 2018. Indeed, microbial biomass can vary seasonally, with a higher concentration in the rainy season (Turner & Wright 2014), which highlights how fast the microorganisms can respond to short-term changes in soil resource availability, reflecting the microbial capacity for immediate growth (Chen *et al*. 2019). Once an unexpected peak of response to phosphorus addition occurred in July 2018 (in the early of dry season), we suggest that during this time, an amount of the month's precipitation concentrated in the week of collection, associated with a higher input of litterfall commonly observed during this period (Luizão, 1989).

Alternatively, added P could have increased microbial respiration because added inorganic P exchanges with sorbed organic compounds in soil, which makes them available for microbial uptake (Spohn & Schleuss 2018). This occurs because inorganic P is an anion that competes more successfully for binding sites than many organic compounds (Mori *et al*. 2017). Hence, the desorption of organic compounds might alleviate microbial C limitation and thereby would explain higher respiration rates in soil after inorganic P addition (Spohn & Schleuss 2018). Future research should investigate whether these microbes are primarily limited by carbon in this first layer of forest floor (Soong *et al*. 2019).

In addition to the strong heterotrophic response to added P, we also found an interaction between N and cations on heterotrophic respiration, where added N, cations and their interaction had a trend to increase soil respiration rates. Soil microbes are strong candidates for multiple nutrient limitation (Kaspari *et al*. 2008), since microorganisms need to maintain a balanced composition of C and nutrients in their bodies (Manzoni *et al*. 2012; Soong *et al*. 2019). The addition of N in the absence of cations had a pattern to increase heterotrophic respiration in our study, and this can show a possible importance of nitrogen for microbes in the short term. Although there was no tropical experiment examining nutrient interactions to directly compare with our results, N enrichment reduced microbial biomass in many ecosystems in a meta-analysis of 82 published studies, with corresponding declines in soil CO_2 emissions (Treseder, 2008). However, it is important to highlight that the decline in abundance of microbes and fungi were more evident in studies of longer duration (longer than 5 years) and with higher total amounts of N added (Treseder, 2008). In the short term, nitrogen can be important in composing microbial cell walls that frequently contain nitrogen, such as chitin, for example, which is a common constituent of fungal cell walls (Kallenbach *et al*. 2016). Additionally, nitrogen can be important in composing N-rich enzymes to get carbon and phosphorus (phosphatases) for example (Nasto *et al*. 2014; Martins *et al*. 2021).

There was also an increase in heterotrophic respiration with added cations – with or without added N, and this can suggest a possible importance of cations, at least for a portion of the microbial population. It appears that these microbes, in particular, tend to fit more to the multiple nutrient limitation (Kaspari & Powers, 2016).

In general, our results indicate that nutrient addition increases heterotrophic respiration. The increase in heterotrophic respiration could be driven by higher microbial biomass or shifts in species composition leading to higher metabolic activity. Further work is needed to find out whether the increase in heterotrophic microorganisms was driven by different dominant groups of microorganisms in the soil (e.g: fungi or bacteria). Nutrient addition could shift microbial communities towards more copiotrophic (an organism found in environments rich in nutrients) organisms, which can have lower microbial rates (Mori *et al*. 2017). Thus, in the long term, different patterns of soil microbial functioning could be captured in our study site.

Although the mean upscaled flux of total soil respiration that we observed across the control plots was 1. 6 fold that observed in a tropical forest in Borneo (21 ± 3.03 Mg C ha^{-1} $year^{-1}$ vs. 12.7 Mg C ha^{-1} $year^{-1}$). The partitioning between the two sites was similar, with the heterotrophic respiration dominating over autotrophic respiration (Table 03), and in this study heterotrophic respiration accounted for 76% of total respiration (Riuta *et al*. 2021), which is comparable to the heterotrophic respiration in our site that accounted for 73% of total respiration.

Ecosystem level fluxes

Although NPP increased 15% with phosphorus addition, the GPP did not change, because autotrophic respiration had a non significant trend to reduce with phosphorus addition. This caused the GPP estimates to be very similar (-P: 26.14 ± 1.36 and +P: 26.92 ± 1.4 Mg C ha^{-1} yr^{-1}) in the plots without and with added P. The GPP found in the control plot was 27.3 Mg C ha^{-1} yr^{-1}, and is in the range found for mature tropical forest from 19.6 to 33 Mg C ha^{-1} yr^{-1} in bottom up approach through biometric studies (Malhi, 2012). GPP did not increase with fertilisation, so we suggest instead that GPP can be more related to climatic conditions (Luyssaert *et al*. 2007; Malhi, 2012).

Although we arrived at the same value of carbon use efficiency (0.3) as found previously (Malhi *et al*. 2009; Chambers *et al*. 2004), our results add confidence, since all fluxes were directly measured at the same site (Dought *et al*. 2017). We hypothesized that carbon use efficiency would not change with P addition, because the higher total NPP would result in higher respiration of each compartment. However, CUE did not change, because the increase in total NPP with phosphorus addition was offset by the total respiration that did not change with phosphorus addition. Nutrient rich forests promote greater ecosystem carbon use efficiency when compared to nutrient poor forests, but the data are biased towards temperate forest (Fernandez Martinez *et al*. 2014). It may be relevant to note that in temperate forest, nitrogen availability tends to increase wood increment (Norby *et al*. 2010) and reduce soil respiration (Janssens *et al*. 2010), which favors greater carbon use efficiency. The greater carbon use efficiency found in more fertile sites when compared to less fertile sites across the Amazon Basin may be related not only to fertility, but also to confounding factors, like differences in life stages in trees or wood residence time (Doughty *et al*. 2017). The fast growing species in the western Amazon should allocate more carbon to NPP as they compete spatially for light and nutrients, but long lived trees in eastern Amazon can invest more in maintenance of their biomass and also allocate carbon in defense costs that raise respiration rates (Malhi, 2012). In addition, there is evidence for an increase in carbon allocated below ground in lower fertility sites, which was associated with lower carbon use efficiency (Doughty *et al*. 2017).

Limitation of the study

The mean upscaled R_{leaf} of the control plot was 8.17 Mg C ha^{-1} yr^{-1}, but this estimate may be overestimated since the fluxes were measured in sun exposed leaves over a one time period (October, dry season) and LAI is a measurement of the whole plot scale leaf cover. There is evidence that leaf respiration is larger at the upper canopy and then tend to decrease through the vertical profile (Carswell *et al*. 2000; Souza *et al*. 2021). And although there is a possibility for overestimation, differences between treatments, which are the focus of this study, are real. The sampling in the more shaded vertical profile should be the focus in future studies in our site. Our value of 8.17 Mg C ha^{-1} y^{-1} is higher than the control plot in the Caxiuanã Reserve, with 5.69 Mg C ha^{-1} yr^{-1} (Table 03), but the leaves were sampled in different canopy layers. Another factor that would improve scaling values would be to consider variations in leaf age.

The decrease in stem respiration with phosphorus addition, was driven by the medium size class (trees with 35-45 cm DBH, and this could possibly be a sample size effect. This class was the class with more trees sampled (123 trees) related to other classes. This class also had a good representation in basal area, where of the total basal area calculated in the collection, this class (35-45 cm DBH) alone represented 33% of the total basal area, followed by the class > 55 cm (26%), 45-55 cm (21%) and 26-35 cm (20%).

There are also some limitations in the soil respiration partitioning method. The installation of the deep collar may affect the activity of microorganisms. This happens because, despite maintaining the food source for the microorganisms with the input of litterfall, the deep collar cuts out a food source for the microorganisms, which is the input of roots, which occurs in a natural system. However, it is happening for all treatments and differences between treatments, which are the focus of this study, are real.

CONCLUSION

Phosphorus strongly affected microbial carbon dynamics along with a combination of nitrogen and cation, which stimulated heterotrophic respiration in a Central Amazonian Forest. Phosphorus additions reduced stem respiration rates. The two rock derived nutrients (P and base cations) affected leaf respiration. These results can help improve model predictions of CO_2 release in face of climate change, and we recommend that interactions between nutrients should be account for some processes, mainly leaf and soil respiration. However, further research is necessary to gain a better understanding of the mechanisms that explain these responses and nutrient interactions.

Soil respiration represents the largest fraction of ecosystem respiration, and can therefore determine whether the forest acts as a source or sink of CO_2 (Ogle, 2018). With the climate change, an increase in temperature is expected, which has been linked to an increase in the microbial activity (Ogle, 2018). However, we suggest that the microbial community is limited by P and may not respond as predicted to warming, with consequences for ecosystem C fluxes. In addition, human activities associated with nitrogen deposition may have the potential to change soil heterotrophic respiration fluxes, as seen by the interaction between N and cation.

In contrast to NPP, the components of respiration responded to various combinations of nutrients. This means that different process (growth or respiration) can be constrained by different nutrients. The magnitude of responses in tissue production with phosphorus addition were in the order of 15%, 19% or 29.5 % in total productivity, canopy production and fine root production respectively. These results may suggest that tissue production is demanding little cost and products from respiration, since stem respiration decreased with phosphorus addition. The tendence of greater GPP with phosphorus addition were driven by total NPP alone and not by autotrophic respiration. At ecosystem levels, P had only a tendency to increase carbon use efficiency over the short term, and perhaps it may be useful to test another extrapolation methods. Continuous monitoring of the effect of

nutrient additions would add vital information for understanding the long-term consequences of carbon gains and losses due to alleviation of nutrient limitation.

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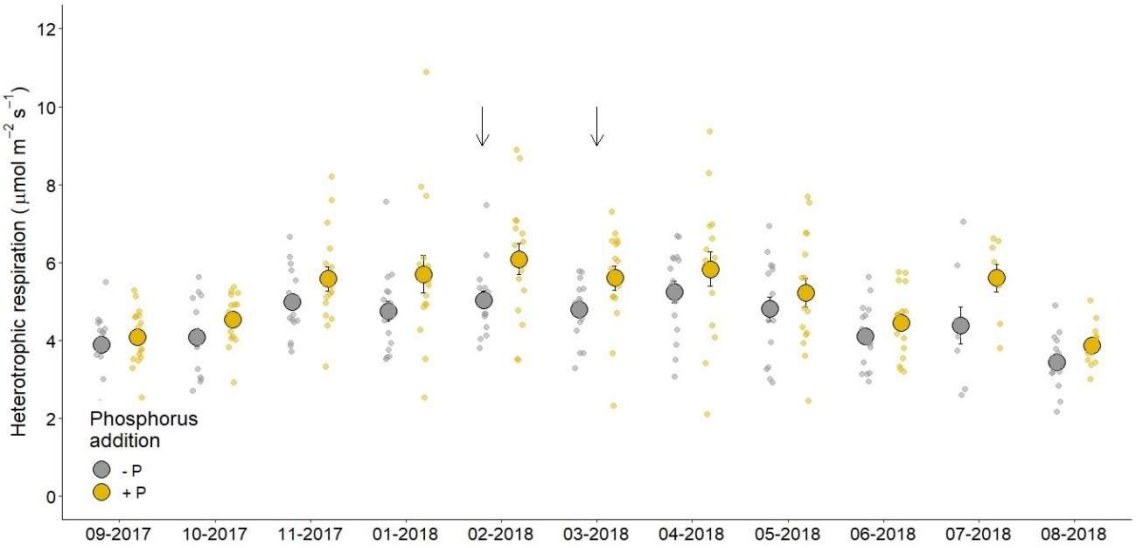
Supplementary information

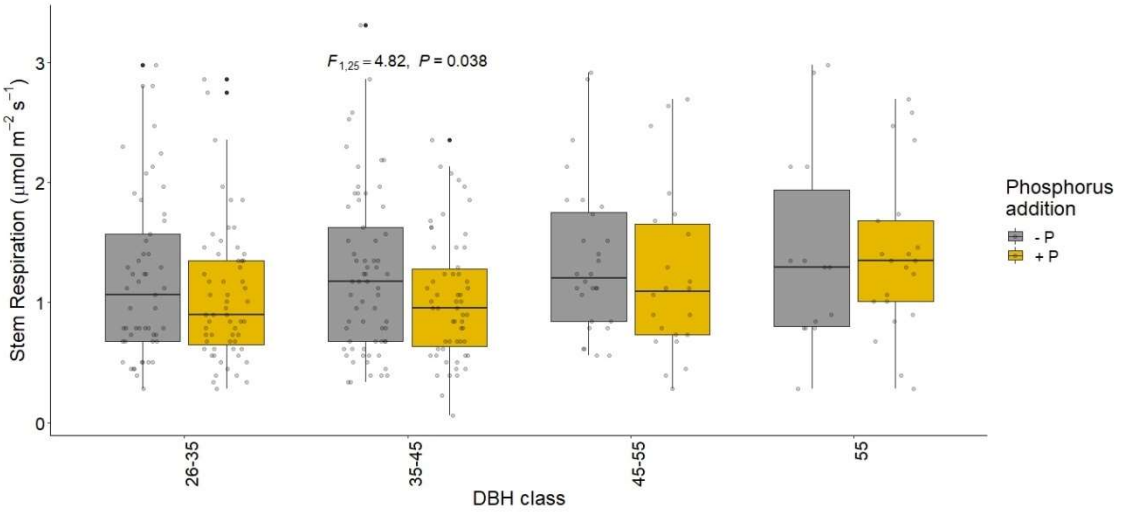
Extended Data Table 1| Characteristics of the vegetation from trees > 10 cm diameter at breast height (DBH) at the start of the experiment (May, 2017) in the control plots (n = 4; no nutrient addition) and in the plots fertilised with N, P and Cations (n=16). For these variables, there was no significant differences between plots, indicating that there was similar forest structure between the assigned treatments. It is important to highlight that the first fertilisation occurred almost at the same time as the tree inventory, and was already expected the lack of significant values.

	Control	**-N**	**+N**	**-P**	**+P**	**-CAT**	**+CAT**
Aboveground Biomass (Mg ha^{-1})	357.7 ± 68.7	367.2 ±27.9	342.4 ± 24.1	337.7 ± 24.1	371.9 ±27.6	350.3 ± 22.9	359.3 ± 29.3
Number of individuals (ha^{-1})	602 ± 42	595 ± 16	574 ± 16	594 ± 15	575 ± 18	575 ±16	594 ± 16
Basal area (m^2 ha^{-1})	27.53 ± 4.40	27.66 ± 1.50	26.13 ± 1.34	26.33 ± 1.39	27.46 ± 1.47	26.68 ± 1.31	27.11 ± 1.55



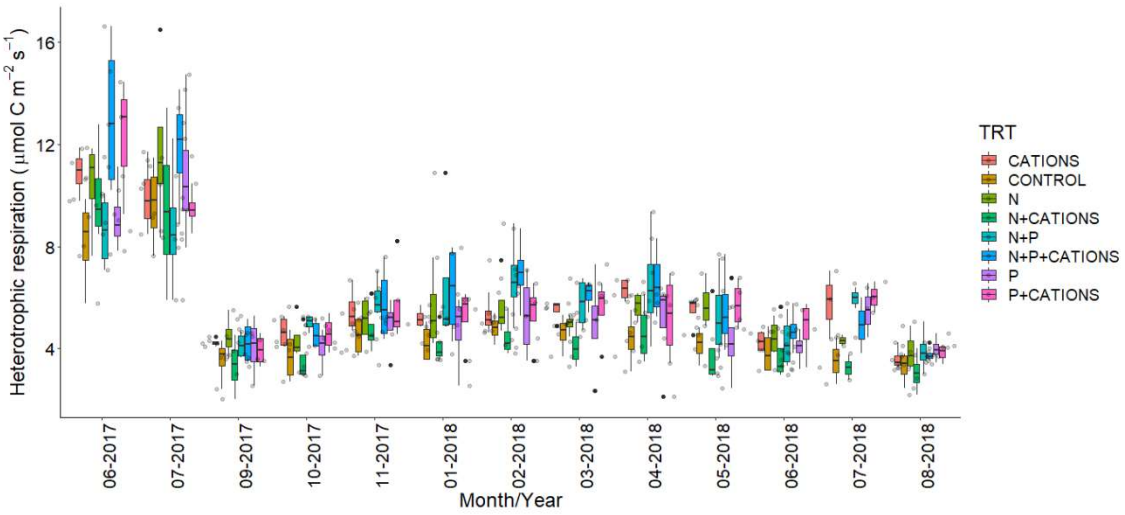
Extended Data Figure 01| Heterotrophic respiration across time. Heterotrophic respi ration (µmol m^{-2} s^{-1}) in response to with (+) and without (-) P addition across one year o

f collection (September 2017 to August 2018) in an old growth tropical forest. Arrows in dicate the month of fertilisation. Means ± 1SE are presented.



Extended data Figure 2| P addition effects across DBH classes. Stem CO_2 respiration (µmol m^{-2} s^{-1}) in response to with (+P) and without P addition (-P) across four size class es 26-35, 35-45, 45-55 and > 55 cm DBH in an old growth tropical forest. Data for indi vidual 320 trees are shown by gray dots.

Supplemental Note 1|: Recover from disturbance



In the first two months after installation was possible to check anormal values of heterot rophic respiration due to the disturbance in soil structure and/or decomposition of roots. This was normalized in September, the month where we started to use the data.

GENERAL CONCLUSION

The nutritional limitation for a given nutrient depends on the process studied (net primary productivity or respiration). Phosphorus was the only major community level nutrient limiting net primary productivity, which was driven by litterfall productivity and fine roots. Otherwise, various combinations of nutrients drove both autotrophic and heterotrophic respiration.

Ecosystem respiration does not change with nutrient addition, because the flows compensate each other, while stem respiration decreases with phosphorus addition, heterotrophic respiration in the opposite direction, increases the fluxes with the addition of the same nutrient.

Nutrients affect the carbon cycle mainly by productivity rather than respiration. The responses of phosphorus addition in total productivity and in the leaf and root compartments, showed highly significant results, which shows that these responses tend to remain with time. Conversely, respiration responses to nutrient addition were marginally significant, showing that these responses can change or weaken further with time.

Future research is needed to understand how the nutritional limitation on productivity behaves on a smaller scale than the community level, investigating the limitation by tree size classes, species or functional groups.

Table 3: Mean respiration efflux (Mg C ha^{-1} $year^{-1}$) in different tropical forests around the world.

The authors, length of the study period and the method are also indicated. In ([a]) the soil data are derived from Quesada et al. 2010.

Study site	Methods	Plot/ subplot size	Authors	Study length [a]	Parameter analysed	Results	P total
Borneo (old growth forest)	Partitioned respiration (GEM protocol)	25 points per plot	Riutta et al. 2021	Between 2 and 6 years	Root respiration	0.89 ± 0.32 Mg C ha^{-1} $year^{-1}$	199 ± 51.1 mg kg^{-1}
Borneo (old growth forest)	Partitioned respiration (GEM protocol)	25 points per plot	Riutta et al. 2021	Between 2 and 6 years	Mycorrhizal respiration	1.41 ± 0.40 Mg C ha^{-1} $year^{-1}$	199 ± 51.1 mg kg^{-1}
Borneo (old growth)	Partitioned respiration (GEM protocol)	25 points per plot	Riutta et al. 2021		Heterotrophic Respiration	9.54 ± 0.57 Mg C ha^{-1} $year^{-1}$	199 ± 51.1 mg kg^{-1}
Caxiuan Reserve	Partitioned respiration	9 points per plot	Metcalfe et al. 2010	2 times (Nov 2004 and June 2005)	Heterotrophic respiration	10.2 Mg C ha^{-1} $year^{-1}$	NA
TAM - 06 (Peru)	Extrapolation with stem surface area	1 ha (50 trees selected)	Robertson et al. 2010	1 time (May, 2007)	Stem respiration	6.44 ± 1.12 Mg C ha^{-1} $year^{-1}$	528.8 mg kg^{-1} [a]
Cuieras Reserve	Extrapolation with stem surface area	50 trees selected	Chambers et al. 2004	8 times Aug 2000 and Jun 2001	Stem respiration	4.2 Mg C ha^{-1} $year^{-1}$	148.4 mg kg^{-1}
CAX - control	Surface area		Rowland et al. 2018		Stem respiration	7.07 Mg C ha^{-1} $year^{-1}$	37.4 mg kg^{-1}
Borneo	Extrapolatio	52 trees	Katayama et	5 times	Stem respiration	7.06 ± 2.09 Mg C ha^{-1}	NA

	n with stem surface area	selected	al. 2016	Jan 2012-July 2014		$year^{-1}$	
CAX - Control	Extrapolation with LAI	15 trees per plot	Da Costa et al.2014	1 time 2005	Leaf respiration	5.69 Mg C ha^{-1} $year^{-1}$	37.4 mg kg^{-1}