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In situ short-term responses of Amazonian understory plants to elevated CO₂

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Abstract

The response of plants to increasing atmospheric CO₂ depends on the ecological context where the plants are found. Several experiments with elevated CO₂ (eCO₂) have been done worldwide, but the Amazonian forest understory has been neglected. As the central Amazon is limited by light and phosphorus, understanding how understory responds to eCO₂ is important for foreseeing how the forest will function in the future. In the understory of a natural forest in the Central Amazon, we installed four open-top chambers as control replicates and another four under eCO₂ (+250 ppm above

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ambient levels). Under eCO₂, we observed increases in carbon assimilation rate (67%), maximum electron transport rate (19%), quantum yield (56%), and water use efficiency (78%). We also detected an increase in leaf area (51%) and stem diameter increment (65%). Central Amazon understory responded positively to eCO₂ by increasing their ability to capture and use light and the extra primary productivity was allocated to supporting more leaf and conducting tissues. The increment in leaf area while maintaining transpiration rates suggests that the understory will increase its contribution to evapotranspiration. Therefore, this forest might be less resistant in the future to extreme drought, as no reduction in transpiration rates were detected.

KEY WORDS

apparent photosynthetic quantum yield, CO₂ enrichment, leaf area, open-top chambers, photosynthesis, tropical forest, water-use efficiency

1 | INTRODUCTION

The Amazon forests store around 100 petagrams of carbon (Pg C) in their aboveground live biomass (Feldpausch et al., 2012; Malhi et al., 2006), which represents c. 20% of the carbon (C) stored in the world's forest vegetation (Baccini et al., 2012; Houghton, 2007; Saatchi et al., 2011). Intact Amazonian forests have acted as a large C sink over the past decades (Brienen et al., 2015; Hubau et al., 2020), accounting for c. 25% of the terrestrial C sink (Pan et al., 2011; Phillips et al., 2009). However, the response of Amazon forests to increasing atmospheric carbon dioxide concentration ([CO₂]) is highly uncertain (Cernusak et al., 2013) since the increase in [CO₂] does not necessarily translate directly into an increase in CO₂ uptake by the forests (Terrer et al., 2019). Given that nutrient availability, rainfall variability and light environment constrain plant C assimilation, these factors impose limitations to the forests' responses to elevated CO₂ (eCO₂) (Chazdon, 1988; Ellsworth et al., 2017; Fleischer et al., 2019; McCarthy et al., 2010; Walker et al., 2021).

The Amazonian *terra-firme* (upland) forests are characterized by high species diversity (Fauset et al., 2015; ter Steege et al., 2013) that forms a dense and continuous canopy, resulting in vertical gradients of irradiance, decreasing from the top of the canopy to the forest understory (Chazdon, 1988; Wright & Van Schaik, 1994). The irradiance that reaches the lower layer of the Amazon Forest is usually less than 5% of that reaching the top canopy (Dos Santos et al., 2019), potentially making light the strongest limiting factor for understory plants (Baldocchi & Collineau, 1994; Chazdon, 1988). The vertical gradient in incident light in the Amazon is closely reflected in leaf CO₂ assimilation rates throughout the vertical forest strata (Domingues et al., 2005), with plants inhabiting the understory being dependent on brief and unstable periods of high light density (sunflecks) to maintain a positive carbon balance in the long term (Chazdon, 1988; Chazdon & Pearcy, 1991; Neufeld & Young, 2003). Limiting light level conditions, close to the plant compensation point, is thought to trigger the largest responses of plants to eCO₂ (Kimball, 1986;

Poorter and Pérez-Soba, 2001). Therefore, forest understories are a highly interesting component of eCO₂ research.

The understory of tropical forests is inhabited not only by species that are restricted to completing their life cycles under shaded conditions (typical understory species), but also by juveniles of trees and lianas that will eventually reach the full sunlight environment at the top of the canopy (Valladares & Niinemets, 2008). Despite the relatively lower biomass stored in the midcanopy and understory compartments (30 vs. 70% from canopy trees for a Central Amazonia site), it is nonetheless a relevant component of the forest, contributing up to 32% of the wood productivity (Araujo et al., 2020). Moreover, it has been suggested that understory plants may be particularly responsive to eCO₂ as they often operate near-neutral carbon balance due to limiting light availability, that is, close to their physiological light compensation point (LCP) (Curtis & Wang, 1998; Kubiske & Pregitzer, 1996; Lloyd & Farquhar, 2008; Würth et al., 1998). If understory plants are indeed particularly responsive to eCO₂, this may not only have consequences on C sequestration but may also result in changes in community structure and the future composition of tropical forests, as less responsive plants might be excluded from that community by competition (Hubau et al., 2019; Lapola et al., 2009). Therefore, there is an urgent need to better understand the response of the understory plant community to ongoing increase in [CO₂].

As eCO₂ increases photosynthetic efficiency, plants are expected to acclimate to the decreased demand for investments in ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), often represented by the leaf maximum carboxylation capacity (V_{cmax}) (Rogers & Humphries, 2000). On the other hand, eCO₂ can also increase the demand for cofactors produced by photosynthetic light reactions (ATP and NADPH), which can shift photosynthesis from being limited by Rubisco to being limited by the regeneration capacity of ribulose 1,5-bisphosphate (RUBP) (Drake et al., 1997), often represented by the leaf maximum electron transport rate (J_{max}). This second possibility seems more likely in shaded conditions (Sharkey, 1985), but might be true also

for high-light environments. In addition, to offset the low light availability, understory plants tend to invest resources in strategies to optimize light capture, such as greater leaf area (Gommers et al., 2013; Valladares & Niinemets, 2008).

In that sense, an increase in understory leaf area could enhance the contribution of this forest stratum to the leaf-to-atmosphere moisture flux, offsetting a fraction of the possible decline in leaf transpiration (E) due to reduced stomatal conductance (g_s) under eCO₂ (Xu et al., 2016). The negative effect of eCO₂ on E has the potential to cascade into basin-wide changes in rainfall, which, particularly in the Amazon, is highly dependent on water vapour transferred back to the atmosphere via forest evapotranspiration (Sampaio et al., 2021; Zemp et al., 2017; but see Yang et al., 2016).

In situ field experiments are crucial for reducing uncertainties surrounding the response of tropical forests to eCO₂ and climate change. Through these experiments, it is possible to enhance the understanding of ecosystem processes to improve terrestrial biosphere models (Norby et al., 2016). While numerous experiments have been conducted with elevated CO₂ levels, in situ experiments, specifically in tropical forests, remain rare (for a review see Ainsworth & Long, 2004; Norby & Zak, 2011; Walker et al., 2021). As a result, the current knowledge about tropical rain forest plants response to eCO₂ is primarily based on experiments with seedlings growing in pots (e.g., Cernusak et al., 2011a; Reekie & Bazzaz, 1989; Slot et al., 2021; Ziska et al., 1991) and planted species (Arnone & Körner, 1995; Körner & Arnone, 1992; Würth et al., 1998).

Here we employed in situ open-top chambers (OTCs) to investigate the short-term (120–354 days) effect of eCO₂ on the photosynthetic parameters related to carbon assimilation and water use, and carbon allocation for the naturally occurring understory community of an old-growth forest in the Central Amazon. We tested the following hypotheses:

- (i) Plants in the tropical forest understory are responsive to eCO₂, increasing their net CO₂ assimilation at saturating light (A_{sat}), intrinsic water-use efficiency ($iWUE$), J_{max} , apparent quantum yield (Φ), relative growth rate (RGR) and leaf production (Lf_p), while decreasing V_{cmax} , g_s , E and LCP.
- (ii) Due to their adaptation to low-light environments understory plants under eCO₂ prioritize allocation of carbon to leaf area (Lf_{area}).

2 | MATERIALS AND METHODS

2.1 | Site description

The study site is located at the Experimental Station of Tropical Forestry (EEST/ZF-2), in Central Amazon (2°35'39"S, 60°12'29"W) at the experimental site of the AmazonFACE program (Free-Air CO₂ Enrichment—<https://amazonface.inpa.gov.br>). The site is situated on a low fertility and highly weathered deep soil, classified as geric Ferralsol, rich in clay (76%), well drained and with average nutrient

concentration of 0.1% nitrogen, 1.9% carbon and 0.01% phosphorus (101.8 mg kg⁻¹) for the top 0–30 cm (Quesada et al., 2010). The vegetation of the site is classified as *terra firme* (upland) evergreen forest, characterized by high diversity of plant species, with an average canopy height of 30 m and canopy tree crowns close to each other (Pereira et al., 2019). On average, the irradiance reaching the understory of this site is less than 5% of that reaching the upper canopy, and over 75% of the understory daytime irradiance is below 25 μmol m⁻² s⁻¹ (Dos Santos et al., 2019). The climate of the region is classified as rainy tropical, according to Köppen-Geiger (Peel et al., 2007). The local mean annual temperature is 26.7°C with low seasonal variation (24.5–27.5°C—min and max, respectively), and mean annual precipitation of 2400 mm, with a drier period between July and September when monthly precipitation can reach less than 100 mm (Ferreira et al., 2005; Tanaka et al., 2014).

2.2 | Experimental design

Eight steel-polypropylene OTCs, with 2.5 m diameter and 3 m height each, surrounded by soil trenching of 30 cm wide and 50 cm deep—to isolate them from the surrounding soil and plant roots—(Supporting information: Figure S1), were installed at the experimental site. The chambers were designed to increase the [CO₂] inside them and were set up in pairs, with four control (ambient—aCO₂) and four treatment (+250 ppmv—eCO₂) chambers (Supporting information: Table S1). The distance between the OTC chambers varies between 14.7 and 219.4 m. The locations of the chambers were determined semi-randomly by using the Leaf Area Index (LAI) derived from hemispherical photos, aiming to homogenize the amount of light that reaches each area. Each pair of OTCs has a CO₂/H₂O nondispersive infrared (NDIR) gas analyser (LI-840A, Li-Cor® Biosciences) installed in a nearby central system that measures and records (Campbell Scientific CR1000 dataloggers) the CO₂ and water (H₂O) concentrations inside the chambers every 1 min. This system was programmed to control the CO₂ injection in the eCO₂ replicates whenever the difference between the pair of OTCs falls below 200 ppmv. The CO₂ is injected inside the eCO₂ chambers through a gas line connected to a central cylinder system and spread by fans installed close to the injection hose. The CO₂ injection into the eCO₂ chambers started on 1 November 2019, and since then, CO₂ injectors have been switched on during daytime hours, that is, from 6 AM to 6 PM, each day. The mean (±SD) daytime [CO₂] during 2020 was 466 ± 21 and 732 ± 24 ppmv among the control and treatment chambers, respectively.

In addition to [CO₂] and [H₂O] measurements inside the OTCs, we estimated the solar irradiance (mol m⁻² day⁻¹) from hemispherical photographs taken 1.5 m above the ground in the centre of each chamber using a Canon Rebel EOS T3 camera with Sigma fish-eye lens (8 mm) and further analysed using Gap Light Analyzer software (<https://www.caryinstitute.org/science/our-scientists/dr-charles-d-canham/gap-light-analyzer-gla>). For each OTC, the total solar radiation transmitted by the canopy (TSRT) was calculated as a function of the solar constant (1367 W m⁻²), geographical coordinates (latitude/longitude), cloudiness index ($kt = 0.5$) and canopy

openness (percentage of open sky seen from beneath the forest canopy), and then transformed into photosynthetically active radiation (PAR) (Supporting information: Table S1).

Considering all OTCs, we identified 56 different plant species, belonging to 26 distinct families. Among the OTCs, the number of individual plants varied from $n = 10$ to 18 (Supporting information: Table S1), with heights (considered here as the main stem length— H_t) between 0.28 and 3 m, diameter at the base (DB) ranging from 3.9 to 35 mm (Supporting information: Figure S2A and Table S2). Due to the high local biodiversity, there were no species that occurred in all OTCs (Supporting information: Table S2).

2.3 | Leaf level gas exchange measurements

Leaf level gas exchange parameters were determined through light-saturated CO_2 assimilation versus intercellular CO_2 concentration (A/C_i) curves in up to three leaves from each of three individuals of different species per OTC ($a\text{CO}_2 = 36$, $e\text{CO}_2 = 34$), and light response curves in one leaf from each of the individuals selected for A/C_i curves per OTC ($a\text{CO}_2 = 12$, $e\text{CO}_2 = 12$), during March 2020, 120 days after CO_2 enrichment started. These measurements were taken with a portable infrared gas analyser (IRGA-LI-6400XT; Li-Cor® Biosciences) on fully expanded leaves, between 8:00 AM and 3 PM, in individuals that either belonged to species that were more abundant or displayed a perceived larger contribution the LAI within a given OTC. Before the measurements, leaves were acclimated for at least 15 min by carefully monitoring assimilation rate and g_s values to ensure their stability. From each A/C_i response curve, the photosynthetic capacity parameters (V_{cmax} and J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) were calculated based on Farquhar et al. (1980) by a curve-fitting routine based on minimum least squares (Domingues et al., 2010) and adjusted to the standard temperature of 25°C (Bernacchi et al., 2001). From the light response curves, the A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$), g_s at A_{sat} ($\text{mol m}^{-2} \text{s}^{-1}$), E at A_{sat} ($\text{mmol m}^{-2} \text{s}^{-1}$) and $i\text{WUE}$ ($\mu\text{mol mol}^{-1}$) were quantified, and the LCP ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and the Φ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) were calculated.

For all measurements, the standardized set conditions inside the IRGA chamber were airflow of 400 $\mu\text{mol s}^{-1}$, relative humidity between 60% and 70%, and leaf temperature of 30°C. The A/C_i curves were performed at a saturating photosynthetic photon flux density (PPFD) of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and the reference $[\text{CO}_2]$ were controlled as follows: 400, 300, 200, 75, 50, 400, 600, 800, 1000, 1200 and 1500 $\mu\text{mol mol}^{-1}$. The light response curves were performed at $[\text{CO}_2]$ of 400 $\mu\text{mol mol}^{-1}$ for $a\text{CO}_2$ and 600 $\mu\text{mol mol}^{-1}$ for $e\text{CO}_2$, using a PPFD sequence of 250, 500, 750, 1000, 1500, 500, 250, 100, 75, 50, 25, 10, 5 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The A_{sat} , g_s , E and $i\text{WUE}$ parameters were taken from these curves, under PPFD of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and according to the CO_2 treatment, at 400 $\mu\text{mol mol}^{-1}$ for $a\text{CO}_2$ and 600 $\mu\text{mol mol}^{-1}$ for $e\text{CO}_2$. The LCP was calculated by the equation:

$$LCP = \frac{R_d}{\alpha}, \quad (1)$$

where R_d is the dark respiration rate (PPFD = 0) and α is the light response curve initial slope, between 0 and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD. The Φ was determined as the light response curve initial slope above LCP .

2.4 | Leaf production

We measured leaf production (Lf_p) of 55 individuals in $a\text{CO}_2$, and 47 individuals in $e\text{CO}_2$, from January to October 2020, with Lf_p defined as the number of newly produced leaves divided by the number of individual plants within a given OTC. Leaf production monitoring followed the methodology by Menezes et al. (2022) where, at the beginning of the experiment, all leaves from each individual were included as initial stock, and the leaves from both ends (proximal and terminal) of each branch were tagged to follow changes in leaf demography. Thereafter, the new flushed leaves were included in the demographic censuses when their leaf blade was nearly expanded, while scars left from an abscised petiole were considered to be dead leaves. Leaf demographic censuses were performed in November 2019, January, March, July and October 2020. The cumulative Lf_p was calculated as the sum of total flushed leaves during the sampled period, that is, 354 days after the start of the experiment.

2.5 | Leaf area

We quantified changes in individual leaf area (Lf_{area}) by measuring two fully expanded leaves from the same branch, one that flushed before (t_1) and another that flushed after (t_2) the onset of the experiment (November 2019), on all plants that flushed new leaves ($a\text{CO}_2 = 42$, $e\text{CO}_2 = 41$). Measurements of both leaves were taken in the same campaign (July 2020). Lf_{area} was determined from photographs taken from leaves overlaying graph paper and processed in the ImageJ software (<https://imagej.nih.gov/ij/>). The results are presented as the sum of the mean leaf area (m^2) per treatment, and the percentage change in leaf area ($Lf_{\text{area}}\%$) was calculated as the difference between the leaves that flushed before and after CO_2 enrichment starts, in the same branch.

2.6 | Height, diameter and relative growth rate

We measured the individual plants main stem length (which is often their height) (H_t ; $a\text{CO}_2 = 51$, $e\text{CO}_2 = 44$) and the stem base diameter (BD; $a\text{CO}_2 = 56$, $e\text{CO}_2 = 45$) in November 2019 and September 2020. The H_t was measured with a millimetre measuring tape, from the base of the stem to the apex of the main stem, even when the individual's growth pattern was not completely vertical (e.g., lianas). In each plant, two perpendicular measurements of the BD were taken using a digital calliper (Mitutoyo/Absolute). The BD measurement point was set in the first campaign, at 5 cm from the ground. Thereafter, subsequent measurements were taken at the same marked point. For subsequent analysis, we

divided the plants in three height classes: 20 ≤ 80, 80 < 140 and ≥ 140 cm, and three diameter classes: 3 ≤ 9, 9 < 15 and ≥ 15 mm.

Daily mean height (cm day⁻¹) and base diameter (mm day⁻¹) increment were calculated for each plant, as the difference between *Ht* and *BD*, respectively, measured in September 2020–*t*₂ and November 2019–*t*₁ (when CO₂ enrichment started). We also calculated the relative growth rate (RGR) for each plant, using *BD*² × *Ht* as a non-destructive surrogate for plant dry mass (Bloomberg et al., 2008). RGR was calculated as

$$RGR = [\ln(BD_{t_2}^2 \times Ht_{t_2}) - \ln(BD_{t_1}^2 \times Ht_{t_1})]/(t_2 - t_1), \quad (2)$$

where *BD*_{*t*₁} and *BD*_{*t*₂} are initial and final base diameter (mm), *Ht*_{*t*₁} and *Ht*_{*t*₂} are initial and final total height (cm), and *t*₁ and *t*₂ are initial and final time (days).

2.7 | Statistical analyses

We analysed the effects of eCO₂ on leaf-level gas exchange (*A*_{sat}, *gs*, *E*, *iWUE*, *V*_{cmax}, *J*_{max}, *J*_{max}·*V*_{cmax}, *LCP* and Φ), *Lf*_p, *Lf*_{area} and plant growth (*Ht*, *BD* and *RGR*). Due to the high diversity of species and considering the fact that there were no species that occurred in every OTC, it was not feasible to consider species identity in our analyses. Instead, we evaluated parameters averages (Supporting Information: Table S3) from each OTCs as sampling units (*n* = 8), establishing comparisons between the control (ambient–aCO₂, *n* = 4) and treatment (+250 ppmv–eCO₂, *n* = 4) OTCs. For the *Lf*_p data, we first averaged the rate of leaf production for each OTC (number of new leaves produced within the time interval divided by the number of individuals within a given OTC) and afterwards we calculated the mean and standard deviations among the four OTCs from each treatment. We also analysed the average absolute accumulation of new leaves as the sum of all leaves produced within each OTC (Section 2.4). In the case of *Ht* and *BD*, the analyses were conducted according to the previously mentioned size class division specified in Section 2.6.

Differences between the parameters measured under aCO₂ and eCO₂ were analysed by generalized linear mixed models, with the CO₂ treatment as categorical fixed effect with two levels. For all parameters, we found that models including species identity or ambient light levels as random effects were not significantly different from those that did not (*p* ≤ 0.05); therefore, these factors were not included in the final models. To account for the chambers' natural environmental variation, we included OTC pairs as a random effect in the mixed models, using the 'glmmTMB' package (Brooks et al., 2017). The leaf-level gas exchange parameters are presented as the mean ± SD and reported as the mean percentage change of the response ratio [(*r* - 1) × 100], where *r* = response under eCO₂/response under aCO₂. Significant differences were regarded at *p* ≤ 0.05. The statistical analyses were performed with R version 4.1.2 (R Core Team 2021).

3 | RESULTS

3.1 | CO₂ enrichment effects on leaf gas exchange

We evaluated the response of leaf gas exchange to eCO₂ 120 days after CO₂ enrichment started. Under eCO₂ the *A*_{sat} was 67% and the *iWUE* was 78% higher than aCO₂ (*p* ≤ 0.001 for both). *J*_{max} and the *J*_{max}·*V*_{cmax} ratio were 19% higher under eCO₂ (*p* ≤ 0.001 for both). For *V*_{cmax}, *gs* and *E*, no significant changes were observed under eCO₂ (*p* = 0.7, *p* = 0.5 and *p* = 0.3, respectively). The Φ was 56% higher (*p* ≤ 0.001), while *LCP* did not show significant difference between the treatments (*p* = 0.3) (Figure 1 and Table 1). Regarding the quality of the *A/C_i* curve fitting, the average root mean squared error (RMSE) was 0.11 (ranging from 0.04 to 0.28).

3.2 | CO₂ enrichment effects on leaf production, leaf area and plant growth

Plants under aCO₂ produced more leaves between January and March 2020 (Figure 2). However, between March and June, *Lf*_p was more than two times higher under eCO₂ than aCO₂ (59 vs. 18 leaves, Figure 2). Despite this, the cumulative *Lf*_p was higher under aCO₂ than eCO₂ for that period. Between June and October, *Lf*_p was 85% higher under eCO₂, although it was not statistically different from aCO₂ (*p* = 0.059). At the end of our study, we observed that the absolute accumulated *Lf*_p, between January and October, was 23% higher under eCO₂, although no significant difference was detected between treatments (*p* = 0.3) (Figure 2).

The *Lf*_{area} that flushed after the onset of CO₂ enrichment, compared to those already present before the experiment started (November 2019), increased by 51% under eCO₂ and 19% under aCO₂ (*p* ≤ 0.001) (Figure 3).

Although we observed a trend of higher total *Ht* of 23% under eCO₂, this increase was not statistically significant (Figure 4), except for the larger size class (≥140 cm) (*p* = 0.2, *p* = 0.9 and *p* ≤ 0.001, respectively). When all *BD* classes were considered together, increment was significant (*p* ≤ 0.001) and 65% higher at eCO₂. That was also true for the intermediary and largest size classes (9 < 15 and ≥15 mm) (*p* ≤ 0.001 for both). For the smallest size classes (3 < 9), no difference was detected (*p* = 0.02 (Figure 5).

Concerning the *RGR*, we detected a 29% increment under eCO₂ (*p* = 0.01) (Figure 6).

4 | DISCUSSION

4.1 | Carbon assimilation and growth responses to elevated CO₂

This Amazonian understory community showed higher potential carbon assimilation rates (*A*_{sat}) under eCO₂, as previously observed in

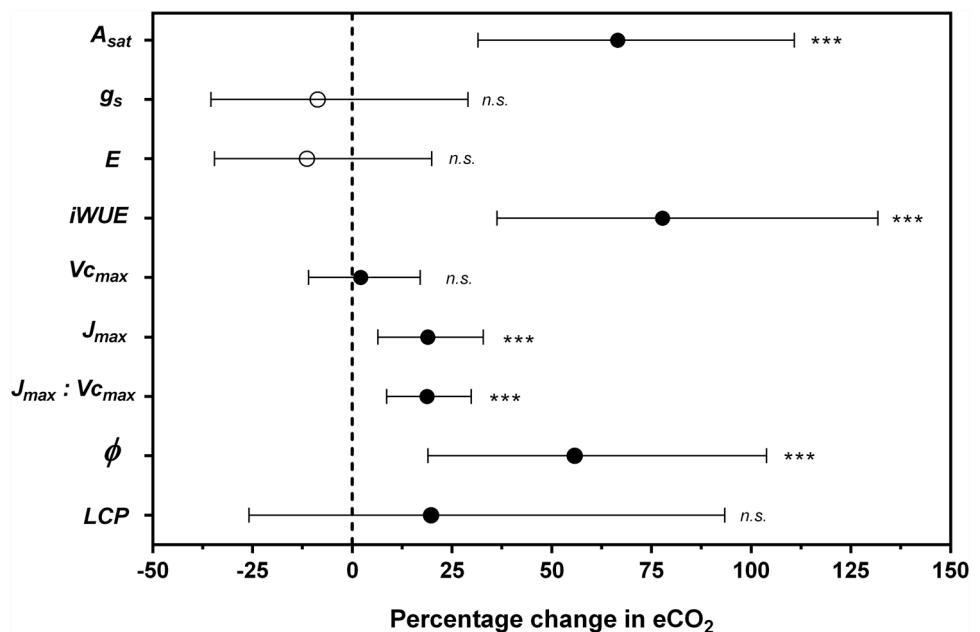


FIGURE 1 Mean response to eCO₂ (n = 8, ±95% CI) of net CO₂ assimilation at saturating light (A_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), transpiration (E , $\text{mmol m}^{-2} \text{s}^{-1}$), intrinsic water-use efficiency ($iWUE$, $\mu\text{mol mol}^{-1}$), apparent maximum carboxylation rate of Rubisco (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), apparent maximum electron transport rate for RuBP regeneration under saturating light (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), $J_{max} : V_{cmax}$ ratio, apparent quantum yield (ϕ , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and light compensation point (LCP , $\mu\text{mol m}^{-2} \text{s}^{-1}$). The dashed line represents no change, black circle (●) an increase and open circle (○) a decrease under eCO₂. The asterisks indicate significant treatment effect (***p ≤ 0.001) and n.s. = no significant, n = 8 OTCs (4—aCO₂ and 4—eCO₂).

TABLE 1 Gas exchange parameters of plants under ambient (aCO₂) and elevated (eCO₂) CO₂ concentration.

Variable	aCO ₂	eCO ₂	p
A_{sat}	3.7 ± 0.7	6.1 ± 0.8	≤0.001
g_s	0.077 ± 0.03	0.069 ± 0.008	0.5
E	1.11 ± 0.3	0.97 ± 0.12	0.3
$iWUE$	54.1 ± 9.9	96.2 ± 19.2	≤0.001
V_{cmax}	18.4 ± 1.3	18.8 ± 2.3	0.7
J_{max}	26.1 ± 0.7	31.1 ± 3.4	≤0.001
$J_{max} : V_{cmax}$	1.45 ± 0.10	1.72 ± 0.10	≤0.001
ϕ	0.02 ± 0.005	0.03 ± 0.003	≤0.001
LCP	8.7 ± 1.4	10.6 ± 4.9	0.3

Note: Mean ± SD and p value for each parameter analysed between treatments.

Net CO₂ assimilation at saturating light (A_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol m}^{-2} \text{s}^{-1}$), intrinsic water-use efficiency ($iWUE$, $\mu\text{mol mol}^{-1}$), maximum carboxylation rate of Rubisco (V_{cmax} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), maximum electron transport rate for RuBP regeneration under saturating light (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), $J_{max} : V_{cmax}$ ratio, apparent quantum yield (ϕ , $\mu\text{mol m}^{-2} \text{s}^{-1}$) and light compensation point (LCP , $\mu\text{mol m}^{-2} \text{s}^{-1}$).

shade plants (DeLucia & Thomas, 2000; Hättenschwiler, 2001; Kubiske & Pregitzer, 1996). The increase in A_{sat} was sustained by an enhancement in J_{max} , indicating a high energy demand for RUBP regeneration, which suggests an enhancement in the capacity of

these plants to utilize *sunflecks* (DeLucia & Thomas, 2000; Pearcy, 1990). Thus, eCO₂ may facilitate shaded plants in the understory to better exploit sunflecks which is the main, but erratic, light resource available. Despite previous eCO₂ studies reporting a reduction in V_{cmax} under eCO₂ (Ainsworth & Long, 2004; Leakey et al., 2002; Medlyn et al., 1999), we did not observe a downregulation of carboxylation capacity. This may be linked to the relatively short observation period of this study, implying insufficient time for the process of downregulation to occur in these plants (Ainsworth et al., 2004b; Moore et al., 1999; Sage, 1994). Alternatively, it might result from non-limiting nitrogen availability. Similar studies have previously demonstrated a reduction in LCP and an increase in ϕ under eCO₂ and limited light (Hättenschwiler, 2001; Kubiske & Pregitzer, 1996). We hypothesized that under eCO₂ and understory conditions, plants decrease their LCP and increase ϕ to optimize light use and carbon assimilation (Drake et al., 1997). Here, the eCO₂ did not lead to a decrease in the LCP , a result also recorded by Norby et al. (2003) for understory shaded leaves. Instead, there was a high variability among plants in both treatments, which can be related to the diversity of species present within the community (Drake et al., 1997; Kubiske & Pregitzer, 1996) but not accounted for by our experimental design. However, the higher values of ϕ reiterate the increase in assimilation and consequent carbon gain observed in response to eCO₂ (Kubiske & Pregitzer, 1996).

Such an increase in carbon assimilation (A_{sat} , J_{max} and ϕ) resulted in a higher Lf_{area} , BD and RGR under eCO₂, which implies that these plants prioritize investments towards increasing light capture and processing into fixed carbon, perhaps boosting their performance in

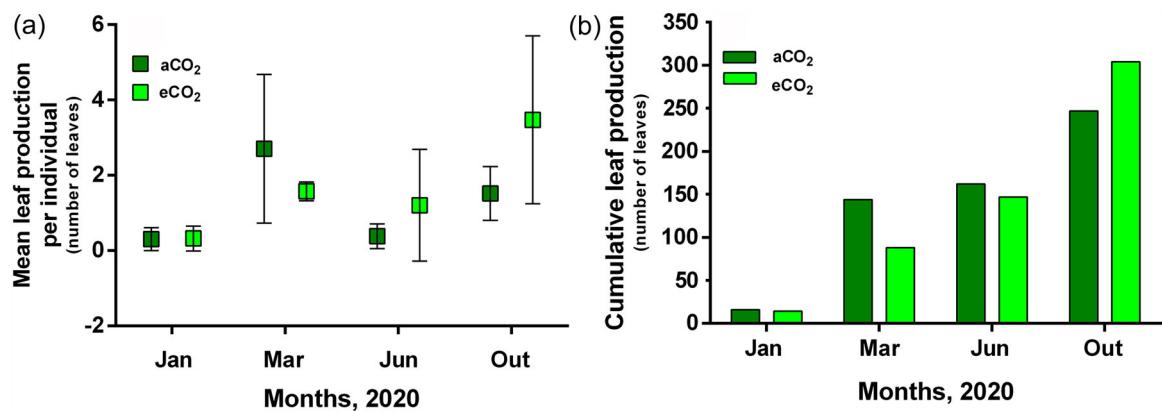


FIGURE 2 (a) Mean \pm SD and (b) cumulative leaf production leaf production measured over four field campaigns (January, March, June and October 2020) across treatments: dark green is aCO₂ and light green is eCO₂. Bars indicate the standard deviation of the means. [Color figure can be viewed at wileyonlinelibrary.com]

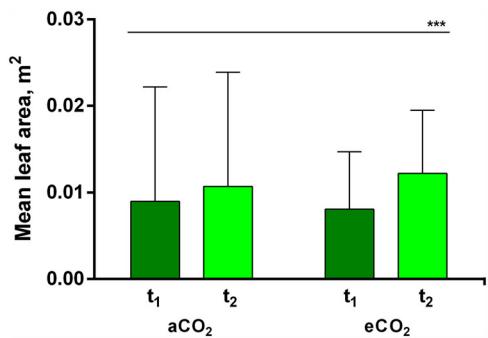


FIGURE 3 Mean leaf area (m²) of the open-top chambers (OTC, $n=8$) of leaves that flushed before (dark green bars—t₁) and after (light green bars—t₂) the onset of CO₂ enrichment (November 2019). Bars indicate the standard deviation of the means. The asterisks indicate significant treatment effect (** $p \leq 0.001$), $n=8$ OTCs (4—aCO₂ and 4—eCO₂). [Color figure can be viewed at wileyonlinelibrary.com]

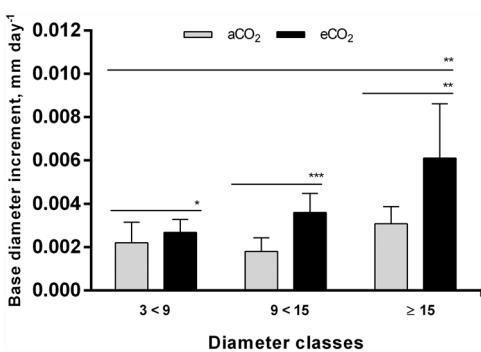


FIGURE 5 Daily mean base diameter increment (mm day⁻¹) of plants under aCO₂ (grey bars) and eCO₂ (black bars), divided into three diameter classes (3 < 9, 9 < 15 and ≥ 15 mm). Error bars indicate the standard deviation of the means. The asterisks indicate significant treatment effect (* $p \leq 0.05$), $n=8$ OTCs (4—aCO₂ and 4—eCO₂).

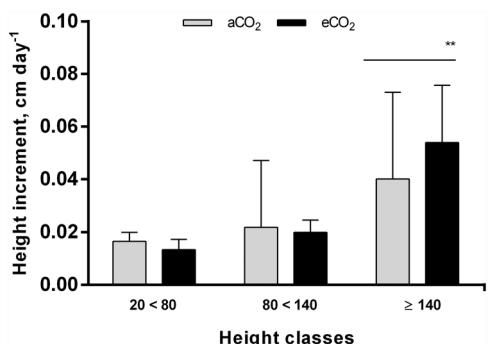


FIGURE 4 Daily mean height increment (cm day⁻¹) of plants under aCO₂ (grey bars) and eCO₂ (black bars), divided into three height classes (20 < 80, 80 < 140 and ≥ 140 cm). Error bars indicate the standard deviation of the means. The asterisks indicate significant treatment effect (** $p \leq 0.01$), $n=8$ OTCs (4—aCO₂ and 4—eCO₂).

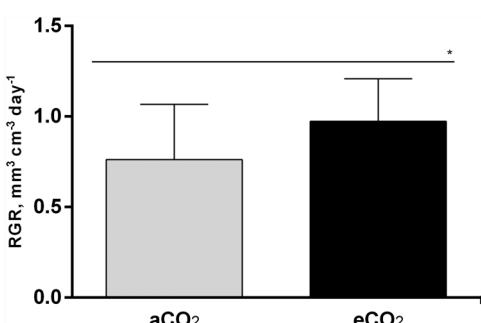


FIGURE 6 Mean daily relative growth rate (RGR, mm³ cm⁻³ day⁻¹) of plants under aCO₂ (grey bars) and eCO₂ (black bars). Error bars indicate the standard deviation of the means. The asterisks indicate significant treatment effect (* $p \leq 0.05$), $n=8$ OTCs (4—aCO₂ and 4—eCO₂).

the understory environment (Valladares & Niinemets, 2008). Higher BD and a weak *Ht* response of the individuals indicate that in this forest, the understory community may be more responsive to eCO₂ in terms of growth to acquire resources (Givnish, 1988). Plants adapted to shade conditions may invest resources to optimize light capture (Gommers et al., 2013) at the expense of vertical growth (Valladares & Niinemets, 2008), in contrast to canopy species that survive by waiting for better conditions, such as canopy openings, to further develop (Swaine & Whitmore, 1988). Indeed, plants growing under shaded conditions are expected to benefit disproportionately from eCO₂ as they live closer to their *LCP* and are known to be more responsive to eCO₂ (Curtis & Wang, 1998; Lloyd & Farquhar, 2008), with our observations supporting the view that eCO₂ can stimulate and change growth performance in the Amazonian understory. We observed wide variations occurring in *LCP*, as well as growth responses that could indicate very different overall eCO₂ responses by different species in our plant community and suggest that some species may develop competitive advantages over others under eCO₂ scenarios in the future. While our study did not focus on specific species, the results presented here reinforce the idea that, regardless of the species or habit, a tropical plant community can respond to environmental changes, such as eCO₂. Marvin et al. (2015), in an experiment with tropical liana and tree seedlings, showed that both life forms had significant responses to eCO₂, but no difference between them, which supports the effect of eCO₂ on tropical plant communities. This goes against the idea that increasing atmospheric [CO₂] is causing an expansion of the lianas in tropical forests (Schnitzer and Bongers, 2011). However, the extent to which such changes could alter the whole forest species composition in the future remains speculative, and an in-depth evaluation of species or functional group responses to eCO₂ is needed (Lapola et al., 2009; Marvin et al., 2015). If species respond differently to eCO₂, some species could show a disproportional change in performance compared to others. Perhaps species at the acquisitive end of the conservative-acquisitive spectrum will benefit more from the increasing atmospheric [CO₂]. Their increased performance might reflect on their population dynamics within the community (i.e., reproduction, recruitment and mortality rates), likely culminating in alterations in community structure (i.e., species composition and/or dominance and rarity patterns).

4.2 | Water-use efficiency under elevated CO₂

We found that the increase in *iWUE* was driven primarily by increased *A_{sat}*, with little variation in *g_s* and *E*. Although it is widely known that eCO₂ tends to reduce *g_s* (Ainsworth & Long, 2004; Ainsworth & Rogers, 2007; Medlyn et al., 2011), this is not true in all cases (Ellsworth, 1999). The smaller or nonsignificant changes in *g_s* under eCO₂ are more commonly observed in plants with low rates of metabolism (Saxe et al., 1998), and the CO₂-induced reductions in *g_s* decrease from the top to the bottom of the canopy (Domingues et al., 2007; Gunderson et al., 2002; Wullschleger et al., 2002), which was the case of this study. The no response of *g_s* to

eCO₂ in our study may also have been due to the limited variation of other environmental factors, such as humidity and temperature (and consequently the vapour pressure deficit—VPD), since the stomata are sensitive to environmental conditions (Grossiord et al., 2020; Gunderson et al., 2002) and these variables are, in general, reasonably constant in the understory of tropical forests (Mendes & Marenco, 2017). In these cases, when the increase in *iWUE* is due only to the increase in *A_{sat}*, there is no improvement in the water economy (Saxe et al., 1998). However, there could be changes in the understory water fluxes to the atmosphere since we observed a significant increase in *Lf_{area}* in our study. Even without significant changes in *g_s*, a higher transpirative foliar area may enhance the contribution of the understorey stratum to ecosystem evapotranspiration rates, which may in turn affect land–atmosphere fluxes.

Maximizing *E* rates is a possible mechanism for plants to ensure sufficient nutrient uptake when under competition, especially under low phosphorus availability (Cernusak et al., 2011b). There are experimental and modelling studies showing that the Central Amazon Forest is limited by phosphorus (Cunha et al., 2022; Fleischer et al., 2019). The lack of response in both *g_s* and *E* observed in our experiment might be the reflex of these variables being more strongly influenced by phosphorus competition at the forest understory. Such strategy might be facilitated by the fact that tropical forest understory plants tend to maintain high *g_s* to minimize the stomatal limitation of assimilation rates during brief sunflecks (Pearcy, 1990). It makes sense that at the humid tropics such acquisitive strategies are favoured, although drought events during El Niño years certainly favours the conservative species (Domingues et al., 2018). We still have very limited understanding of how variable are tropical plant species regarding their functional strategies, although species can be remarkably different (Thompson et al., 2019).

Ecosystem level manipulation experiments are scientifically challenging and financially demanding. Many ecosystem processes are dependent on relatively long timescales, especially for forests. Even more challenging is the particular way that each species contributes to ecosystem functioning. Considering the large diversity of species that are present in tropical forests, replication of experimental units is the limiting factor on extrapolations of single experiments to the whole tropical forest biome. That is certainly the case for our experimental design. A way forward is recognizing that groups of species converge are often redundant on their functional ecology, forming functional groups. In the past, species were simply grouped by anecdotal knowledge of their distribution along the successional changes that communities undergo after disturbances. Nowadays, the characterization of species based on their functional traits is an interesting possibility of forming truly functional groups that can simplify ecosystem studies and enable extrapolation of local studies to larger scales.

5 | CONCLUSION

The Amazon forest understory, despite growing in a light-limited environment, responded positively to CO₂ enrichment. We showed that these understory plants improve their C gain, through higher *A_{sat}* and Φ ,

and their growth, through higher BD increment and Lf_{area} under eCO₂. These results depict how this light and phosphorus-limited ecosystem can increase assimilation rates and modulate investments of resources to enhance the capture and efficient use of light and potentially have significant impacts on the structure and composition of the Amazon in the future. No decrease in g_s was observed and, together with the increase in Lf_{area} , this result suggests an enhancement in the contribution of understory to leaf-to-atmosphere moisture flux, predicted to decrease in upper canopy trees. These results, together with several studies that have already been carried out with eCO₂, demonstrate the flexibility of plant communities to adjust to the current scenario of increased atmospheric CO₂ and its impact on global climate change. Still, a better grasp of individual species abilities is a knowledge gap that needs addressing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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