



PG·ECO·INPA
PÓS-GRADUAÇÃO EM ECOLOGIA

INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Aumento da concentração de CO₂ e adição de fósforo nos fluxos de carbono e energia do processo fotossintético de plântulas de *Inga edulis* Mart. no sub-bosque da Amazônia Central

Vanessa Rodrigues Ferrer

Manaus – Amazonas

Maio/2021

Vanessa Rodrigues Ferrer

Aumento da concentração de CO₂ e adição de fósforo nos fluxos de carbono e energia do processo fotossintético de plântulas de *Inga edulis* Mart. no sub-bosque da Amazônia Central

Orientador: Dr. Carlos Alberto Nobre Quesada

Coorientadora: Dra. Sabrina Garcia

Coorientador: Dr. Vinicius Fernandes de Souza

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Ecologia do Instituto Nacional de Pesquisas da Amazônia – INPA como parte dos requisitos para obtenção do título de Mestre em Ecologia.

Manaus – Amazonas

Mai/2021



PG·ECO·INPA
PÓS-GRADUAÇÃO EM ECOLOGIA



MINISTÉRIO DA
CIÊNCIA, TECNOLOGIA
E INOVAÇÕES



PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 31 dias do mês de Maio do ano de 2021, às 14h00min, por videoconferência. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: **Dr. Bart Kruijt**, da Universidade de Wageningen - WUR, o **Dr. João Victor Rodrigues**, da Universidade Federal do Amazonas - UFAM, e o **Dr. Giordane Martins**, do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes o Dr. José Luís Campana Camargo, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o Dr. Marciel José Ferreira, da Universidade Federal do Amazonas - UFAM, sob a presidência do orientador, a fim de proceder à arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** da **VANESSA RODRIGUES FERRER**, intitulado: **“AUMENTO DA CONCENTRAÇÃO DE CO₂ E ADIÇÃO DE FÓSFORO NOS FLUXOS DE CARBONO E ENERGIA DO PROCESSO FOTOSSINTÉTICO DE PLÂNTULAS DE INGA EDULIS MART. NO SUB-BOSQUE DA AMAZÔNIA CENTRAL”**, orientada pelo Dr. Carlos Alberto Nobre Quesada, do Instituto Nacional de Pesquisas da Amazônia – INPA, co-orientada pela Dra. Sabrina Garcia, do Instituto Nacional de Pesquisas da Amazônia - INPA e pelo Dr. Vinícius Fernandes de Souza, da Universidade do Estado do Amazonas - UEA.

Após a exposição, o(a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

APROVADO (A)

REPROVADO (A)

POR UNANIMIDADE

POR MAIORIA

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

DR. BART KRUIJT

DR. JOÃO VICTOR RODRIGUES

DR. GIORDANE MARTINS

DR. JOSÉ LUÍS CAMPANA CAMARGO

DR. MARCIEL JOSÉ FERREIRA

(Coordenação PPG-ECO/INPA)

F385e Ferrer, Vanessa Rodrigues

Aumento da concentração de CO₂ e adição de fósforo nos fluxos de carbono e energia do processo fotossintético de plântulas de *Inga edulis* Mart. no sub-bosque da Amazônia Central / Vanessa Rodrigues Ferrer; orientador Carlos Alberto Nobre Quesada; coorientadora Sabrina Garcia; coorientador Vinicius Fernandes de Souza. -- Manaus:[s.l.], 2021. 54 f.

Dissertação (Mestrado - Programa de Pós-Graduação em Ecologia) -- Coordenação do Programa de Pós-Graduação, INPA, 2021.

1. Fotossíntese. 2. Mudanças Climáticas. 3. Florestas Tropicais. 4. CO₂ elevado. 5. Limitação por Fósforo. I. Quesada, Carlos Alberto Nobre, orient. II. Garcia, Sabrina, coorient. III. de Souza, Vinicius Fernandes, coorient. IV. Título.

CDD: 598

Sinopse:

Foi investigado a resposta fotossintética de plântulas da espécie *Inga edulis* Mart. crescendo no sub-bosque da Amazônia Central em concentrações elevadas de dióxido de carbono e fertilizadas com fósforo. Aspectos das respostas de trocas gasosas e fluorescência da clorofila a esses fatores foram avaliados.

Palavras-chave: mudanças climáticas, fotossíntese, eCO₂, limitação nutricional, câmaras de topo aberto, floresta tropical

Agradecimentos

Aos meus pais, Bernadette Rodrigues Ferrer e Edson Caruso Ferrer e ao meu irmão Vítor Caruso Rodrigues Ferrer que sempre me incentivaram a dar o melhor de mim e me apoiaram para atravessar o Brasil e vir realizar este mestrado na Amazônia.

Ao meu amor, Murilo Moreira, que esteve presente durante todo o processo de desenvolvimento desta dissertação, pelos momentos de alegria, palavras de incentivo e companheirismo durante o mestrado.

Ao meu orientador Dr. Carlos Alberto Quesada pelo acolhimento no grupo, pela oportunidade e confiança ao me aceitar como sua orientanda e contribuição no meu amadurecimento acadêmico.

À minha coorientadora Sabrina Garcia pelo auxílio em toda esta caminhada desde o início do experimento, seja em campo, online, offline, por compartilhar seus conhecimentos e pelo incentivo nos momentos finais do mestrado.

Ao meu coorientador Vinicius Fernandes de Souza pelos ensinamentos, por compartilhar suas experiências da vida acadêmica e companheirismo em campo.

À Gabriela Ushida, que esteve sempre disposta a ajudar, que dividiu o experimento, a experiência, os meses de campo e muita convivência comigo.

À família biogeoquímica pelo acolhimento, amizade, as trocas de ideias, a convivência diária pré-pandemia, os momentos de descontração e todo o apoio em campo.

Ao pessoal do campo, França, Eliseu, Rose, que sempre me acolheram muito bem.

Ao Instituto Nacional de Pesquisas da Amazônia - INPA e ao Programa de Pós-graduação em Ecologia (PPG-ECO) pela estrutura que permitiu a realização do trabalho.

À CAPES e ao programa AmazonFACE pela concessão da bolsa de estudo.

Ao Programa AmazonFACE e respectivo comitê científico pela oportunidade de fazer parte desse grupo de trabalho e pelo apoio financeiro.

Resumo

O aumento da concentração de dióxido de carbono (CO₂) na atmosfera é um dos principais fatores que impulsionam as mudanças climáticas, sendo o CO₂ também o substrato primário para a fotossíntese das plantas. Na concentração atual de CO₂, a fotossíntese da maioria das plantas não está saturada, sugerindo que elas podem se beneficiar desse aumento. No entanto, a resposta fotossintética pode ser limitada por outros fatores, como a disponibilidade de nutrientes. De modo geral, as florestas tropicais são regiões com baixas concentrações de fósforo (P) no solo, e o efeito do aumento de CO₂ atmosférico pode ser limitado por esse nutriente. Devido à importância da Floresta Amazônica como grande sumidouro de CO₂, este estudo teve como objetivo investigar como os parâmetros fotossintéticos de plântulas de *Inga edulis*, uma espécie nativa da Amazônia, respondem ao aumento de CO₂ (eCO₂) e adição de P em um ambiente de sub-bosque. As plântulas foram cultivadas em câmaras com CO₂ ambiente (aCO₂ - 500 ppm) e elevado (eCO₂ - 700 ppm), em vasos com e sem adição de P, durante dez meses no sub-bosque da Amazônia Central. Para avaliar os efeitos dos tratamentos sobre os parâmetros fotoquímicos e bioquímicos da fotossíntese, foram realizadas medidas de trocas gasosas e fluorescência da clorofila α . Os resultados mostraram que as plântulas cultivadas em eCO₂ aumentaram a eficiência do uso da luz na fotossíntese e tiveram um aumento nas taxas de assimilação. Plântulas que cresceram em solos não fertilizados podem estar adaptadas ao baixo P disponível, pois conseguem manter suas taxas fotoquímicas e bioquímicas, e a adição de P não teve interação significativa com o eCO₂. Esses resultados mostraram que o estímulo do processo fotossintético promovido pelo eCO₂ não foi limitado pelo P, e que a adição de P não teve efeito na resposta fotossintética das plantas estudadas.

Palavras-chave: CO₂ elevado, limitação por fósforo, fotossíntese, mudanças climáticas, florestas tropicais.

Abstract

The increase in the concentration of carbon dioxide (CO₂) in the atmosphere is one of the main factors that drive climate change, with CO₂ also being the primary substrate for plants photosynthesis. At the current CO₂ concentration, the photosynthesis of most plants is not saturated, suggesting that they can benefit from this increase. However, the photosynthetic response can be limited by other factors, such as the availability of nutrients. In general, tropical forests are regions with low concentrations of phosphorus (P) in the soil, and the effect of increasing atmospheric CO₂ can be limited by this nutrient. Due to the importance of the Amazon Forest as a large CO₂ sink, this study aimed to investigate how the photosynthetic parameters of seedlings of *Inga edulis*, an Amazon native species, respond to the elevation in CO₂ (eCO₂) and the addition of P in an understory environment. The seedlings were grown in chambers with ambient (aCO₂ - 500 ppm) and high (eCO₂ - 700 ppm) CO₂, in pots either fertilized or non-fertilized with P, for ten months in the understory of Central Amazonia. To evaluate the treatments effects on the photochemical and biochemical parameters of photosynthesis, measurements of gas exchange and chlorophyll α fluorescence were performed. The results showed that seedlings grown in eCO₂ increased the light use efficiency in photosynthesis and had an increase in assimilation rates. Seedlings that grew in non-fertilized soils may be adapted to the low P available, as they manage to maintain their photochemical and biochemical rates, and the addition of P had no significant interaction with eCO₂. These results showed that the stimulation of the photosynthetic process promoted by eCO₂ was not limited by P, and that the addition of P had no effect on the photosynthetic response of the studied plants.

Keywords: elevated CO₂, phosphorus limitation, photosynthesis, climate response, tropical forests.

Sumário

Lista de Tabelas	VIII
Lista de Figuras	IX
Lista de Abreviações e Siglas	XI
Introdução Geral	13
Objetivos	14
<i>Objetivo Geral</i>	14
<i>Objetivos Específicos</i>	14
Referências Bibliográficas	15
<i>Effects of CO₂ and phosphorus on carbon and energy flux in seedlings of Inga edulis Mart. growing in the understory of a Central Amazon Forest</i>	18
Abstract	19
Introduction	20
Material and Methods	22
<i>Study Site</i>	22
<i>Open-Top Chambers</i>	23
<i>Plant material and experiment set-up</i>	24
<i>Experimental Design</i>	25
<i>Environmental data</i>	25
<i>Leaf-level Gas Exchange</i>	25
<i>Chlorophyll Fluorescence</i>	27
<i>Statistical Analysis</i>	27
Results	28
Discussion	29
<i>Elevated CO₂ effects on photosynthetic parameters</i>	29
<i>Does soil P availability modify the seedlings' photosynthetic response under different CO₂ concentrations?</i>	31
Conclusions	32
References	33
Tabelas	42
Figuras	44
Appendix A	48
<i>Determination of light saturation point</i>	48
<i>Results and Discussion</i>	49
<i>References</i>	50
<i>Figures</i>	52

Lista de Tabelas

Table 1: Gas exchange and fluorescence α parameters of *Inga edulis* for each treatment. Means \pm SD of $n = 8$ per treatment. The measurements were made in the following environmental conditions: 21% oxygen, constant air flow rate of $700 \mu\text{mol s}^{-1}$, with pressure between 0-0.1 Pa, fan at 10.000 rpm, relative humidity between 65 to 70%, leaf temperature of $30 \text{ }^\circ\text{C}$, $250 \mu\text{mol m}^{-2}\text{s}^{-1}$ of PPFD. $[\text{CO}_2]$ according to treatment: 500 ppm if ambient, 700 ppm if elevated..... 39

Table 2: Results of p values of each treatment obtained by fitting linear or generalized linear mixed-effects model. The marginal R^2 (R^2_m) describes the goodness of model fit given fixed effects only, while the conditional R^2 (R^2_c) describes the goodness of model fit including fixed and random effects. VWC is soil volumetric water content. TRST is total solar radiation transmitted..... 40

Lista de Figuras

Figure 1. Total solar radiation transmitted estimated through hemispherical photos (a); and soil volumetric water content (b) obtained for each OTC. aCO₂ represents control OTCs (500 ppm of CO₂) and eCO₂ represents treatment OTCs (700 ppm of CO₂). Means are represented by the blue point. Black point represent outlier. n = 4 per OTC..... 41

Figure 2. Boxplots represent the light-saturated assimilation (A_{sat}); (a) and stomatal conductance (g_{sw}) (b) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. Gas-exchange measurements were made at the [CO₂] for each treatment at 30 °C and 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF_D. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. n = 8 per treatment..... 42

Figure 3. Maximum rate of Rubisco carboxylation (V_{cmax}) (a); maximum electron transport rate to regeneration of ribulose-1,5-biphosphate (J_{max}) (b); and maximum rate of electron transport to the maximum rate of Rubisco carboxylation ratio (J_{max}/V_{cmax}) (c) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. V_{cmax} and J_{max} were calculated from A/Ci curves. Gas-exchange measurements were made at the [CO₂] for each treatment at 30 °C and 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPF_D. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. n = 8 per treatment..... 43

Figure 4. Maximum efficiency of PSII photochemistry in light-adapted leaf (F_v'/F_m') (a); and non-photochemical quenching (NPQ) (b) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. Chlorophyll fluorescence measurements were made at the growth CO₂ for each treatment. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. n = 8 per treatment..... 44

Figure A1. Curves of the response of photosynthetic CO₂ assimilation rate (A) to increasing photosynthetic photon flux density (PPFD) obtained for each treatment at 30 °C and 500 ppm CO₂ (control and P) or 700 ppm CO₂ (eCO₂ and eCO₂ + P). Each point represents the mean of 3 measures (n = 3 per treatment)..... 49

Figure A2. Curves of the response of photochemical quenching (qP , filled points) and non-photochemical quenching (qN , open points) coefficients to increasing photosynthetic photon flux density ($PPFD$), at control (a), P fertilization (b), elevated CO_2 (c), and elevated $CO_2 + P$ fertilization (d) treatments obtained by fluorescence measurements at $30^\circ C$ and 500 ppm CO_2 (control and P) or 700 ppm CO_2 (e CO_2 and e $CO_2 + P$). Means \pm standard deviation, $n = 3$ per treatment.....50

Figure A3. Curves of the response of quantum yield of PSII photochemistry (Φ_{PSII} , filled points) and non-photochemical quenching (NPQ , open points) coefficients to increasing photosynthetic photon flux density ($PPFD$), at control (a), P fertilization (b), elevated CO_2 (c), and elevated $CO_2 + P$ fertilization (d) treatments obtained by fluorescence measurements at $30^\circ C$ and 500 ppm CO_2 (control and P) or 700 ppm CO_2 (e CO_2 and e $CO_2 + P$). Means \pm standard deviation, $n = 3$ per treatment.51

Lista de Abreviações e Siglas

[CO₂] – CO₂ concentration

A/C_i – light-saturated CO₂ assimilation curve

aCO₂ – Ambient carbon dioxide

A_{sat} – Net light-saturated CO₂ assimilation rate

ATP – Adenosine triphosphate

C_i – Internal CO₂ sub-stomatal concentration

CO₂ – Carbon dioxide

eCO₂ – Elevated carbon dioxide

FACE – Free Air CO₂ Enhancement

F_m' – Light-adapted maximum fluorescence

F_m – Maximum fluorescence

F_o' – Light-adapted minimal fluorescence

F_s – Steady-state fluorescence

F_v'/F_m' – Maximum quantum efficiency of PSII at light-adapted leaves

g_{sw} – Stomatal conductance

IPCC – Intergovernmental Panel on Climate Change

J_{max} – Maximum electron transport rate

N – Nitrogen

NADPH – Reduced form of nicotinamide adenine dinucleotide phosphate

NPQ – Non-photochemical quenching

OTC – Open Top Chambers

P – Phosphorus

PCR – Photosynthetic carbon reduction cycle

PPFD – Photosynthetic photon flux density

PSII – Photosystem II

Rubisco – Ribulose-1,5-bisphosphate carboxylase/oxygenase

RuBP – Ribulose-1,5-biphosphate

TSRT – Total solar radiation transmitted

V_{cmax} – Maximum rate of carboxylation of rubisco

VWC – Volumetric water content

Φ_{PSII} – Quantum yield of PSII photochemistry

Introdução Geral

Nas últimas décadas, as mudanças climáticas têm preocupado os cientistas e a comunidade internacional que documentam e testemunham o aumento do dióxido de carbono (CO₂) e de outros gases de efeito estufa na atmosfera (Cramer *et al.*, 2001; Cassia *et al.*, 2018; Dusenke *et al.*, 2019). Muitos esforços estão sendo feitos para entender os efeitos regionais e globais que surgem a partir dessas mudanças, e as projeções do Painel Intergovernamental para Mudanças Climáticas (IPCC) são de que a elevação de CO₂ pode chegar a até 900 ppm em 2100 (IPCC, 2014). O aumento do CO₂ na atmosfera pode afetar a temperatura do ar e os padrões de precipitação na superfície da Terra (Alexander *et al.*, 2006), impactar a distribuição, composição e os aspectos funcionais da vegetação (Richardson *et al.*, 2013), bem como a fisiologia e o crescimento das plantas (Long *et al.*, 2004; Norby & Zak, 2011).

Em 2016, Hofhansl *et al.* destacaram as atuais lacunas na compreensão da resposta da Floresta Amazônia às mudanças climáticas previstas, e Fleischer *et al.* (2019) sugeriram que os efeitos do CO₂ elevado (eCO₂) sob a Floresta Amazônica podem ser menor do que anteriormente suposto, uma vez que as projeções de modelos que não consideram a baixa disponibilidade de fósforo (P) no solo, encontrada em grande parte da bacia amazônica, observam um maior potencial na resposta das florestas tropicais ao eCO₂, em relação aos modelos que utilizam o ciclo do P em suas simulações (Cox *et al.*, 2013; Huntingford *et al.*, 2013). Além disso, a compreensão das respostas da Floresta Amazônica, e seus diferentes estratos e complexos ambientes de luz, ao aumento de CO₂, também se faz necessária a fim de aumentar a acurácia de modelos preditivos, e compreender o funcionamento do ecossistema da floresta como um todo.

A assimilação de carbono pelas plantas, via o processo de fotossíntese, não está saturada nos níveis atuais de CO₂ atmosférico (Long *et al.*, 2004). Com o aumento da concentração de CO₂ ([CO₂]) previsto, espera-se um estímulo na taxa fotossintética, e uma maior assimilação de carbono, gerando o efeito chamado de “fertilização de CO₂” (Norby *et al.*, 2005). O processo fotossintético, como um todo, também depende de outros fatores e recursos, como a disponibilidade de água, luz e nutrientes, e uma possível resposta desse processo, ao eCO₂, pode ser influenciada quando há limitação desses recursos.

Entre os nutrientes minerais, o P é um dos mais limitantes em ecossistemas tropicais, como a região da Amazônia Central (Quesada *et al.*, 2010). O P está presente em quase todos os aspectos do metabolismo vegetal, e está diretamente relacionado com a fotossíntese através

de seu papel na transferência de energia, e no metabolismo de carboidratos no cloroplasto (Singh & Reddy, 2015), sendo o principal elemento dos recursos energéticos (adenosina trifosfato - ATP e nicotinamida adenina dinucleotídeo fosfato - NADPH), na formação de intermediários fosforilados e atividade enzimática no ciclo de redução do carbono fotossintético, além de integrar diversos compostos e reações metabólicas (Lambers & Plaxton, 2015).

Diante desse cenário, estudos que investiguem como esses dois recursos (CO₂ e P) influenciam a assimilação de carbono e a resposta fotossintética em espécies de florestas tropicais contribuirão para o conhecimento funcional dessas espécies frente às mudanças ambientais previstas, bem como para auxiliar no aperfeiçoamento dos parâmetros utilizados em modelos de previsão sobre a Amazônia e os ciclos globais. O objetivo desse trabalho foi investigar como os parâmetros fotossintéticos de plântulas de *Inga edulis* Mart. responderão ao aumento da concentração CO₂ e adição de P em um ambiente de sub-bosque.

Objetivo Geral:

Avaliar os efeitos do eCO₂ e da concentração de P nos fluxos de carbono e energia do processo fotossintético de plântulas de *Inga edulis*.

Objetivos Específicos:

- Investigar o efeito do eCO₂ e da concentração de P no solo nos mecanismos de captura de energia, e na eficiência fotoquímica das folhas.
- Compreender os mecanismos fotossintéticos associados à assimilação de carbono sob eCO₂ e diferentes concentrações de P no solo.
- Avaliar se a adição de P no solo altera a resposta dos parâmetros fotossintéticos ao eCO₂.

Referências Bibliográficas

- Alexander, L. V., Zhang, X., Peterson, T. C., Caesar, J., Gleason, B., Klein Tank, A. M. G., ... & Vazquez-Aguirre, J. L. (2006). Global observed changes in daily climate extremes of temperature and precipitation. *Journal of Geophysical Research: Atmospheres*, 111(D5).
- Cassia, R., Nocioni, M., Correa-Aragunde, N., & Lamattina, L. (2018). Climate change and the impact of greenhouse gasses: CO₂ and NO, friends and foes of plant oxidative stress. *Frontiers in plant science*, 9, 273.
- Cramer, W., Bondeau, A., Woodward, F. I., Prentice, I. C., Betts, R. A., Brovkin, V., ... & Young-Molling, C. (2001). Global response of terrestrial ecosystem structure and function to CO₂ and climate change: results from six dynamic global vegetation models. *Global change biology*, 7(4), 357-373.
- Cox, P. M., Pearson, D., Booth, B. B., Friedlingstein, P., Huntingford, C., Jones, C. D., & Luke, C. M. (2013). Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. *Nature*, 494(7437), 341-344.
- Dusenge, M. E., Duarte, A. G., & Way, D. A. (2019). Plant carbon metabolism and climate change: elevated CO₂ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist*, 221(1), 32-49.
- Fleischer, K., Rammig, A., De Kauwe, M. G., Walker, A. P., Domingues, T. F., Fuchslueger, L., ... & Lapola, D. M. (2019). Amazon forest response to CO₂ fertilization dependent on plant phosphorus acquisition. *Nature Geoscience*, 12(9), 736-741.
- Hofhansl, F., Andersen, K. M., Fleischer, K., Fuchslueger, L., Rammig, A., Schaap, K. J., ... & Lapola, D. M. (2016). Amazon forest ecosystem responses to elevated atmospheric CO₂ and alterations in nutrient availability: filling the gaps with model-experiment integration. *Frontiers in Earth Science*, 4, 19.
- Huntingford, C., Zelazowski, P., Galbraith, D., Mercado, L. M., Sitch, S., Fisher, R., ... & Cox, P. M. (2013). Simulated resilience of tropical rainforests to CO₂-induced climate change. *Nature Geoscience*, 6(4), 268-273.
- IPCC (2014) Summary for Policymakers. *Climate Change 2014 Synthesis Report Contribution of Work Groups I, II III to the Fifth Assessment Report of the Intergovernmental Panel on*

Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Lambers, H., & Plaxton, W. C. (2015). Phosphorus: back to the roots. *Annual plant reviews*, 48, 3-22.

Long, S. P., Ainsworth, E. A., Rogers, A., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: plants FACE the future. *Annu. Rev. Plant Biol.*, 55, 591-628.

Norby, R. J., & Zak, D. R. (2011). Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Annual review of ecology, evolution, and systematics*, 42.

Norby, R. J., DeLucia, E. H., Gielen, B., Calfapietra, C., Giardina, C. P., King, J. S., ... & Oren, R. (2005). Forest response to elevated CO₂ is conserved across a broad range of productivity. *Proceedings of the National Academy of Sciences*, 102(50), 18052-18056.

Quesada, C. A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T. R., Czimczik, C., ... & Paiva, R. (2010). Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences*, 7(5), 1515-1541.

Richardson, A. D., Keenan, T. F., Migliavacca, M., Ryu, Y., Sonnentag, O., & Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. *Agricultural and Forest Meteorology*, 169, 156-173.

Singh, S. K., & Reddy, V. R. (2015). Response of carbon assimilation and chlorophyll fluorescence to soybean leaf phosphorus across CO₂: Alternative electron sink, nutrient efficiency and critical concentration. *Journal of Photochemistry and Photobiology B: Biology*, 151, 276-284.

Capítulo 1

Ferrer, V.R.; Neves, G.U.; Garcia, S.; de Souza, V.F.; Quesada, C.A.N. Effects of CO₂ and phosphorus on carbon and energy flux in seedlings of *Inga edulis* Mart. growing in the understory of a Central Amazon Forest.

**Effects of CO₂ and phosphorus on carbon and energy flux in seedlings of *Inga edulis*
Mart. growing in the understory of a Central Amazon Forest.**

Vanessa R. Ferrer¹, Gabriela U. Neves¹, Sabrina Garcia², Vinícius F. de Souza³, Carlos Alberto N. Quesada²

1. Graduate Program in Ecology, National Institute of Amazonian Research, Manaus, Amazonas, Brazil

2. National Institute of Amazonian Research (INPA), Manaus, Brazil.

3. State University of Amazonas, Manaus, Brazil

Correspondence: Vanessa Rodrigues Ferrer. e-mail: vanessa-ferrer@live.com

Abstract

Carbon dioxide (CO₂) is one of the main drivers of climate change and the primary substrate for photosynthesis. Under the current atmospheric CO₂ concentration, most plants are not photosynthetically saturated, suggesting that they can benefit from the CO₂ increases. However, the photosynthetic response may be constrained by nutrients availability, and tropical forests are thought to be limited by soil phosphorus (P). Due to the importance of the Amazon Forest as a global CO₂ sink, this study investigated photosynthetic parameters response to elevated CO₂ (eCO₂) and different P concentrations, in an understory environment. Seedlings of *Inga edulis* Mart. were grown inside Open Top Chambers (OTCs) under ambient (500 ppm) and elevated (700 ppm) CO₂ concentrations [CO₂], in either fertilized or non-fertilized P treatments, in the understory of a Central Amazon Forest. To evaluate the effects of the treatments on photochemical and biochemical parameters of photosynthesis, gas exchange and chlorophyll α fluorescence measurements were carried out. The results showed that seedlings growing under eCO₂ increased the light use efficiency of photosynthesis and their assimilation rates. Despite P addition did not significantly change photosynthetic response, an increase in electron transport rates and slight decrease in carboxylation rates, resulted in an increase in the ratio between these parameters, suggesting a contribution of electron transport to RuBP regeneration, adjusting photosynthetic machinery to optimize the use of sporadic sunflecks in a low irradiance environment. These results showed the increases of the photosynthetic process owing to eCO₂ was not limited by P, and that P addition had no effect on the photosynthetic rate response of the studied plants.

Keywords: elevated CO₂, phosphorus limitation, photosynthesis, climate response, tropical forests.

Introduction

Current atmospheric carbon dioxide concentration ($[\text{CO}_2]$) already surpassed 400 ppm (Tans & Keeling, 2018) and is projected to reach 600–700 ppm in 2050 (IPCC, 2014), causing significant global atmospheric and climate changes. Several researches efforts are being taken to comprehend how the $e\text{CO}_2$ will affect ecosystems, mainly the plants' response to these changes, which is a great challenge due to the highly heterogeneity of species and environmental conditions (Ainsworth & Long, 2005). The $e\text{CO}_2$ has direct impacts on photosynthesis, the main process of carbon uptake in the biosphere, and can positively affect the productivity of terrestrial vegetation (Cox *et al.*, 2013; Huntingford *et al.*, 2013), helping to reduce the global changes impacts.

The impacts of $e\text{CO}_2$, on plant metabolism, have been studied in the past decades and include increased efficiency in light using, reduction in stomatal conductance and transpiration, and enhanced water-use efficiency and photosynthetic rates (Drake *et al.*, 1997). While these results guide our current knowledge, most of the focus was on temperate ecosystems (Ainsworth & Long, 2005), and still are knowledge gaps for tropical regions (Cernusak *et al.*, 2013, Ainsworth & Long, 2021). Tropical forests have a diverse system, with dense vegetation and different strata and environments. The lower stratum of tropical forests has a proper dynamic that is often underestimated, although comprehends many species in different development stages, before they reach the upper strata of the forest, and contributes significantly to leaf biomass and carbon balance of these forests (Nilsson & Wardle, 2005). For these reasons, is important to understand how species that inhabit this complex light environment will respond to $e\text{CO}_2$.

Photosynthesis is a complex physiological process that occurs in the chloroplasts and involves photosynthetic pigment synthesis, light energy electron transport, and the photosynthetic carbon reduction (PCR) cycle, to convert light energy into chemical energy, using CO_2 as the primary substrate to produce sugar and other organic compounds (Blankenship, 2014). In the photochemistry phase, photosynthetic pigments connected to protein complexes (referred to as photosystems) absorb the light and transfer the excitation energy to a chain of electron carriers, which works in series, to generate energetic compounds such as adenosine triphosphate (ATP) and reduced form of nicotinamide adenine dinucleotide phosphate (NAPDH) that will be used for CO_2 fixation, on the biochemistry phase.

The increase in atmospheric $[\text{CO}_2]$, predicted in the coming years, may enhance photosynthesis rates, via so-called CO_2 fertilization (Schimel *et al.*, 2015). This enhancement is primarily related to the kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the main enzyme in the photosynthetic process, which reacts with both CO_2 , starting the PCR cycle, and with oxygen (O_2), starting the photorespiratory carbon oxidation (PCO) cycle. Under $e\text{CO}_2$, the CO_2/O_2 ratio increases, optimizing the carbon fixation and reducing the photorespiration (Drake *et al.*, 1997, Makino & Mae, 1999). To support the increase in photosynthetic rate, the amount of energy that plants capture and transport must be sufficient to maintain the extra CO_2 fixation.

The increase in CO_2 assimilation (A), through the photosynthetic process, increases plants' demand for mineral nutrients, once nutrients are essential for the functioning of plant metabolism (Singh & Reddy, 2014). The importance of nutrient availability has been shown in experiments performed in temperate ecosystems, where nitrogen (N) limits ecosystem response to $e\text{CO}_2$ (Norby & Zak, 2011). Strong evidence indicates that in tropical rainforests, where soils are old and frequently highly weathered, P can be the primary nutritional limitation instead of N (Vitousek & Sanford 1986; Lloyd *et al.*, 2001), due to its low concentration in total and available for the plant (Quesada *et al.* 2010; 2011).

P is an essential plant nutrient, present in membranes, nucleic acids, and energy compounds (Armstrong *et al.*, 1999). P-containing metabolites such as ADP, ATP, sugar phosphates, and inorganic phosphates have key roles in photochemistry and biochemistry, and insufficient metabolic P could limit maximum photosynthetic rates (Ågren *et al.*, 2012), therefore affecting photosynthetic electron transport, photosynthetic phosphorylation, and the PCR cycle (Singh *et al.*, 2018). The plant demand for P can increase under $e\text{CO}_2$ and an optimal leaf P concentration ($[\text{P}]$) is critical to obtain maximal photochemistry and photosynthesis efficiency across $[\text{CO}_2]$ levels (Singh & Reddy, 2015).

While plants can benefit from the rising CO_2 and increase their photosynthetic efficiency, the interactions with other abiotic factors such as nutrients availability may limit the positive effects of $e\text{CO}_2$ (Campbell and Sage, 2006). Low $[\text{P}]$ in the soil and increased $[\text{CO}_2]$ may coexist under natural environments in the future, given that it is predicted that several ecosystems will become limited by P with increasing atmospheric CO_2 (Vance *et al.*, 2003), including the Amazon Forest. The role that the Amazon Forest has in mitigate CO_2 emissions effects, acting as a carbon sink (Brienen *et al.*, 2015; Philips & Brienen, 2017) enhances the necessity to understand its photosynthetic response under the interaction between these two

factors, supported by recent models' predictions that indicate that the increase in CO₂ fixation will be limited by P in the Amazon Forest (Fleischer *et al.*, 2019).

To investigate the isolated and interactive effects of eCO₂ and soil P addition in the efficiency of carbon and energy fluxes of photosynthesis, we design a pot experiment inside open top chambers (OTCs) installed on Central Amazon Forest's understory. We measured gas exchange and chlorophyll α fluorescence parameters, which are important indicators of photosynthetic performance, in seedlings of *Inga edulis* Mart., to test the following hypotheses:

- (1) eCO₂ will alter the photosynthetic capacity and the photochemical efficiency driving a reinvestment of resources from carboxylation to electron transport;
- (2) P addition will affect photosynthesis rates mostly by supporting the photosynthetic reducing power and energy necessary to attend the demand to carbon assimilation; and
- (3) interaction of eCO₂ and P addition will maximize the photosynthetic efficiency.

Materials and Methods

Study Site

This study was carried out at the AmazonFACE (Free-Air CO₂ Enrichment - <https://amazonface.inpa.gov.br>) experimental site, located at the Experimental Station of Tropical Forestry, at 60 km northwest of Manaus, Brazil (2°36'32,67"S; 60°12'33,48"W), and managed by the National Institute of Amazonian Research (INPA) (Lapola & Norby, 2014). The AmazonFACE is a multi-institutional effort experiment, that aims to assess the effects of elevated atmospheric CO₂ on the ecology and resilience of the Amazon forest (Lapola & Norby, 2014). The study site is located in an old-growth closed-canopy *terra firme* (non-flooded) forest, with local variations in soil type, topography and drainage status that created distinct patterns in forest vegetation composition.

This region is characterized by a warm and humid equatorial climate, with mean temperature of 26 °C and mean annual precipitation around 2400 mm (Malhi & Wright, 2004), with a short dry season between July and September, when the precipitation may be less than 100 mm per month (Dias & Marenco, 2016; Camargo & Marenco, 2017; Ferreira *et al.*, 2005).

The relative humidity varies from 70% at noon to 100% at night (Magalhães *et al.*, 2014). The monthly average of solar radiation ranges from 165 W m⁻² in the rainy season, to 230 W m⁻² in the dry season (da Rocha *et al.*, 2009).

The soil type is a Ferralsol, rich in clay, with an acidic pH, low cation exchange capacity, and low total P (~100 mg kg⁻¹), calcium and potassium concentrations (Chauvel *et al.*, 1987; Vieira *et al.*, 2004). This soil type corresponds to ~32% of the Amazon basin soil (~60% of Brazilian Amazonia) and represents the low end of the plant available P spectrum in the Amazon (Quesada *et al.*, 2010, 2011).

Open-Top Chambers

This work was performed inside of open-top chambers (OTC), installed in the understory of the AmazonFACE experimental site, enclosing species that inhabit the understory of the forest.

OTCs are cylindrical greenhouse-like, made of aluminum and polycarbonate, with 3 m diameter and 2.5 m height, and the top completely open in frustum-shaped to allow air circulation. The edges of each chamber were trenched to a depth of 1 m and surrounded by a 2-m wide buffer. Pressurized CO₂ cylinders connected to a system of fans distribute the CO₂ in the chambers according to [CO₂] measuring sensors installed inside each chamber. There are eight paired OTCs, four control (ambient [CO₂] - aCO₂) and four treatment (+200 ppm [CO₂] - eCO₂), where the control chamber of each pair is used as a reference for ambient concentration and the other is injected with 200 ppm of CO₂ above the control. There are, at least, two to three changes of air per minute inside the OTCs, through forced ventilation, to avoid overheating damage to individuals.

The OTC system is composed of four gas analyzers sensors (LI-840A, LICOR Inc., Lincoln, Nebraska, USA), one for each pair of OTCs, a data logger (CR1000, Campbell Scientific Ins., Logan, Utah, USA), and an electropneumatic switching system with 8 relays, also from Campbell Scientific, together with four Parker solenoid valves, two regulators flow and energy sources cables, tubes, and cylinders of pressurized CO₂, that use natural gas as raw material, to inject CO₂ in the treatment chambers. The CO₂ is injected by an automatic system between 6 am and 6 pm, and algorithms computer-controlled feedback and feedforward

maintain the [CO₂] 200 ppm above the control chambers. CO₂ readings are taken every minute by the sensors. The CO₂ fumigation started in November 2019.

Plant material and experiment set-up

In this work, the study organism used was *Inga edulis* Mart., known as Ingá-cipó, a species of Inga, native to the Amazon. *I. edulis* is a leguminous tree with wide distribution in the region (Lojka *et al.*, 2010), belonging to the Fabaceae family, subfamily Mimosoideae. It is also a hyper-dominant genus in the Amazon (ter Steege *et al.*, 2013), a representative of nitrogen fixers and an important functional group.

Seeds of *I. edulis* were collected on November 5, 2019, in the Experimental Silviculture Station of INPA, from 3 different parent trees (named as “a”, “b”, and “c”), to avoid biased responses. The seeds were equally distributed, and a pair from each parent tree was germinated per OTC. The seeds germinated inside the OTCs, in pots of 60 cm height, filled with experimental site soil. The bottom end of the pots was closed with covers and a 2 m hose, installed at the cover, was used to drain the soil water. Seedlings’ growth was monitored every 15 or 30 days with a millimeter ruler, and the leaves’ birth was followed to standardize leaf age to the measurements.

The soil used in the experiment was collected in an area close to the OTCs and sieved in a 2 mm mesh sieve before being placed in the pots. Half of the pots were fertilized with 53.28 g of solid triple superphosphate Ca(H₂PO₄)₂, to reach the [P] of 600 mg/kg, which is representative of the fertile soils found in Western Amazonia (Quesada *et al.*, 2010).

To determine the amount of Ca(H₂PO₄)₂ added to the soil, we used the following equation:

$$\text{Ca(H}_2\text{PO}_4)_2 \text{ g} = \frac{([\text{P}] * \text{Volume (pot)} * \text{Soil density}) / \text{Conversion factor}}{1000}$$

Where [P] is the P concentration (600 mg/kg), volume corresponds to pot volume of 15.5 L, soil density is 1 g cm⁻³, and conversion factor of Ca(H₂PO₄)₂ is 0.192 (Malavolta *et al.*, 2002).

As P is rapidly immobilized in soil, we added 10% more of Ca(H₂PO₄)₂ to ensure enough amount of P for the plants (Cordell and White 2015).

Experimental Design

The experiment was composed of 48 pots, equally distributed among the chambers (six per OTC), and installed using a 2 x 2 factorial design, with 2 levels of CO₂ (ambient – aCO₂ and elevated – eCO₂), and 2 levels of phosphorus (non-P fertilized and P fertilized). The pots were separated into four treatments: control (aCO₂ x low P), P addition (aCO₂ x high P), elevated CO₂ (eCO₂ x low P) and elevated CO₂ with P addition (eCO₂ x high P), with 12 replicates per treatment. In this work, we measured 32 (eight replicates) from the 48 seedlings.

Environmental data

Superficial soil moisture (5 cm depth) was measured as soil volumetric water content (VWC, % volume), using a portable soil moisture sensor kit (SM 150T, Delta-T Devices, Cambridge UK). Three measurements were made in each pot in September 2020, during four distinct days.

To estimate the amount of radiation that reaches each pot, hemispheric photographs were taken using a Canon EOS Rebel T3i, coupled with a fisheye lens (Sigma's 8mm F3.5 EX DG Circular Fisheye), under the highest resolution (5184 × 3456 pixels), between 6 and 7 am, or after 5:30 pm. The photographs were analyzed using the Gap Light Analyzer (GLA) software (<https://www.caryinstitute.org/science/our-scientists/dr-charles-d-canham/gap-light-analyzer-gla>; Frazer *et al.*, 1999) to calculate the total solar radiation transmitted (TSRT, sum of direct and diffuse radiation) in mol m⁻² d⁻¹. Configuration settings were adjusted with image orientation and projection distortion, using the magnetic north, the Lambert's Equal Area, and the coordinates and elevation of each OTC. Growing season length, sky-region brightness, and atmospheric conditions were maintained according to the default for the region.

We calculated one value of radiation (TSRT) and soil moisture (VWC) for each pot to use as a covariate in our model analyzes (Figure 1).

Leaf-level Gas Exchange

Leaf-level gas exchange of light-saturated CO₂ assimilation (A/C_i response curves), and chlorophyll α fluorescence measurements were taken simultaneously from fully expanded

attached leaves, with approximately 10 months old. For that, we measured one leaf per seedling using a portable infrared gas exchange analyzer system (LI-6800 F, LI-CoR, Inc., Lincoln, NE, USA) equipped with a fluorometer chamber (6800-01 Fluorometer). Measurements were performed between 8 am and 4 pm (local time), in September/October 2020.

The measurements were taken under standard chamber environmental conditions of 21% oxygen concentration, constant air flow rate through the cuvette of $700 \mu\text{mol s}^{-1}$, pressure between 0-0.1 Pa, fan at 10.000 rpm, relative humidity between 65 to 70%, leaf temperature of $30 \pm 2 \text{ }^\circ\text{C}$, reference CO_2 concentration in accordance with the OTC treatment (500 or $700 \mu\text{mol mol}^{-1}$), and photosynthetically photon flux density (*PPFD*) of $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (10% blue radiation to maximize stomatal opening). To determine the value of *PPFD*, we carried out light response curves, prior to A/C_i curves, in three seedling per treatment (Appendix A). Before each measurement, the leaves were acclimated for at least 30 minutes inside the equipment chamber until they reach steady-state, when assimilation rate, stomatal conductance and fluorescence signal were stable. CO_2 concentration used during leaf acclimation was in accordance with the OTC treatment, 500 or $700 \mu\text{mol mol}^{-1}$. The A/C_i curves were performed following the protocol described by Sharkey (2019), with reference $[\text{CO}_2]$ starting at ambient concentration (500 or $700 \mu\text{mol mol}^{-1}$) proceeding the following order: 50, 75, 100, 200, 300, 400, 500, 600, 700, 1000, 1200, 1500, 1800, 2000 $\mu\text{mol mol}^{-1}$. The curve was performed with equipment's autoprogram where the maximum time in each step was 3 minutes.

Net light-saturated CO_2 assimilation rates (A_{sat}), stomatal conductance (g_{sw}), and internal CO_2 sub-stomatal concentration (C_i) were obtained from the A/C_i curves, at $[\text{CO}_2]$ of 500 or $700 \mu\text{mol mol}^{-1}$, according to the OTC treatment.

The A_{sat} and C_i were used to fit the model developed by Farquhar *et al.* (1980) and subsequent modifications (von Caemmerer, 2000). The biochemical parameters maximum carboxylation of rubisco (V_{cmax}) and the maximum electron transport rate (J_{max}) were calculated with a curve fitting routine based on minimum least-squares (Domingues *et al.*, 2010), through the following equations:

$$A = V_{cmax} \left[\frac{C_c - \Gamma^*}{C_c + K_C (1 + O/K_O)} \right] - Rd$$

$$A = J_{max} \frac{C_c - \Gamma^*}{4C_c + 8\Gamma^*} - Rd$$

where C_c is the CO₂ partial pressure at Rubisco, Γ^* is the CO₂ compensation point in the absence of day respiration, Rd is the mitochondrial respiration in the light, K_C and K_O are the Michaelis constant of Rubisco for CO₂ and O₂, respectively, and O is the partial pressure of oxygen at Rubisco. The enzymatic kinetic constants used were proposed by von Caemmerer (2000), assuming an infinite internal conductance where $C_c = C_i$.

Chlorophyll α Fluorescence

Chlorophyll α fluorescence parameters were measured by applying a saturation rectangular pulse of 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.5 s, to determine the light-adapted maximum fluorescence (F_m') and the steady-state fluorescence (F_s), followed by a far-red light pulse of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 5 s, to obtain the light-adapted minimal fluorescence (F_o'). The far-red radiation drives photosystem I (PSI) momentarily to help drain electrons from PSII.

After the sunset, between 6:30 and 8:00 pm, leaves already adapted to the dark were measured to obtain the maximum (F_m) fluorescence, under a measuring beam at 50 Hz of frequency, and a saturation rectangular pulse of 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The quantum yield of PSII photochemistry (Φ_{PSII}) was calculated according to Genty *et al.* (1989): $\Phi_{PSII} = (F_m' - F_s)/F_m'$.

Maximum quantum efficiency of PSII at light-adapted leaves (F_v'/F_m') was calculated as: $F_v'/F_m' = (F_m' - F_o')/F_m'$. Non-photochemical quenching (NPQ) was calculated as: $NPQ = (F_m - F_m')/F_m'$ (Maxwell & Johnson, 2000; Roháček, 2002).

Statistical Analysis

Linear and generalized linear mixed models were used to test whether elevated CO₂, P fertilization, and the CO₂ x P interaction affected the different response variables. The CO₂ (categorical with two levels: “ambient CO₂” and “elevated CO₂”), and P (categorical with two levels: “no P addition – low P” and “P addition - high P”) treatments, as well as the CO₂ x P interaction, were set as fixed effects, and total solar radiation transmitted (continuous) and soil volumetric water content (continuous) as covariates. To incorporate the dependence among observations, OTCs (categorical with eight levels) and parent trees (categorical with three levels: “a”, “b”, and “c”) were set as random effects. If the CO₂ x P interaction or the covariates

were not statistically significant ($p \leq 0.05$), it was removed, and the model fitted again keeping the simplest one.

For the A_{sat} , V_{cmax} , J_{max} , J_{max}/V_{cmax} and NPQ a Gamma distribution with a log-link function was used. The log link function ensures positive fitted values. For Φ_{PSII} , F_v'/F_m' and g_{sw} , normal distribution with an identity-link function was used. Each model calculated two R^2 values, to quantify the relative influence of the fixed compared to the random effects: the marginal R^2 , which is the R^2 associated with the fixed effects; and the conditional R^2 , which includes the fixed and the random effect (Nakagawa & Schielzeth, 2013).

All statistical analyses were performed using R version 4.0.2 (R Core Team, 2020). The package “glmmTMB” (Brooks *et al.*, 2017) was used to fit the models. The model assumptions and residual diagnostics were verified with “DHARMA” package (Hartig, 2000), and R^2 was calculated using “MuMin” (Barton, 2016). For graphical display, we used “ggplot2” (Wickham, 2016). The figures and data were presented as means \pm standard deviation of 32 measurements (eight replicates for treatment).

Results

By performing gas exchange in simultaneous with chlorophyll α fluorescence measurements, we evaluated how the raise in atmospheric CO_2 and the addition of P, in a naturally low-P soil, would affect the photosynthetic parameters of *Inga edulis*' leaves in the understory (Table 1).

We observe that the seedlings grown under e CO_2 treatment had a significant increase of 15% in A_{sat} rates ($p = 0.005$; Fig. 2a, Table 2) but did not respond under P treatment ($p = 0.926$), and soil volumetric water content (VWC) influenced significantly and positively A_{sat} ($p = 0.014$). The CO_2 also affected g_{sw} (Fig. 2b) that decreased by 10% under e CO_2 ($p = 0.046$). Although the seedlings subjected to both treatments ($CO_2 \times P$) decreased g_{sw} in 25%, this response was due to CO_2 treatment since P treatment was not statistically significant ($p = 0.291$) neither the interaction between them. VWC had a significant effect on g_{sw} ($p = 0.003$).

The V_{cmax} response to e CO_2 , despite of showed a slight decrease of 5.5%, were not statistically significant ($p = 0.217$) (Table 2, Figure 3a). Although J_{max} values increased 7.5% with P addition and 5.5% under e CO_2 (Fig. 3b), this enhance was not statistically significant (p

= 0.158 and $p = 0.636$, respectively), and P addition do not alter the J_{max} under eCO₂. Total solar radiation transmitted, however, positively affected J_{max} ($p = 0.024$). These results influenced the J_{max}/V_{cmax} ratio that were 12.5% higher under eCO₂ ($p = 0.006$) and 9% under P treatment ($p = 0.049$). Seedlings subjected to both eCO₂ and P addition had an increase of 14.5%, but the interaction was not statistically significant (Fig. 3c).

For fluorescence variables, maximum efficiency of PSII photochemistry in light-adapted leaf (F_v'/F_m') (Fig. 4a) was significantly raised by eCO₂ ($p = 0.017$) but not by P treatment ($p = 0.728$). Non-photochemical quenching (NPQ) (Fig. 4b) was significantly affected only by eCO₂ ($p = 0.033$) and not by P ($p = 0.793$). Quantum yield of PSII photochemistry (Φ_{PSII}) was not affected by eCO₂ ($p = 0.697$) nor P ($p = 0.815$, Table 2).

Discussion

We investigated the effects of eCO₂ and P addition on the efficiency of carbon and energy fluxes of photosynthesis through gas exchange and chlorophyll α fluorescence measurements.

Elevated CO₂ effects on photosynthetic parameters

We observed a 15% increase in A_{sat} in response to eCO₂, an enhance that has been reported for understory plants in different magnitudes (Hattenschwiler & Körner, 2000; DeLucia & Thomas 2000; Springer & Thomas, 2007; Tomimatsu *et al.*, 2014), and can change accordingly with plant developmental stages and the environmental heterogeneity of ecosystems (Leakey *et al.*, 2009). Since low irradiance is predominant in forest understories, even a small change in rates of photosynthesis significantly impacts daily carbon gain when integrated over time (Leakey *et al.*, 2002).

The increase in A_{sat} rates and decrease g_{sw} , observed under eCO₂, are in accordance with other studies, since, in general, photosynthesis increases with eCO₂ and reduces the g_{sw} (Ainsworth & Rogers, 2007; Ainsworth & Long, 2021). , maximizing its water use efficiency and its assimilation. Previous studies concluded that the reduce in stomatal conductance is likely due to changes in stomatal openness rather than reduction in stomatal density (Ainsworth & Rogers, 2007; Cernusak *et al.*, 2011).

We expected to see an eCO₂ effect on photosynthetic capacity driving a reinvestment of resources from carboxylation to electron transport, since it is more likely that a rise in the atmospheric CO₂ can create an excess in carboxylation capacity and intensify RuBP limitation unless Rubisco is downregulated and/or RuBP regeneration is upregulated (Bowes, 1993; DeLucia & Thomas, 2000; Sage & Kubien, 2007). The changes on V_{cmax} and J_{max} , however, were small and not significant. Still J_{max}/V_{cmax} ratio was significantly higher in the eCO₂ chambers.

This increase in J_{max}/V_{cmax} ratio suggests that the leaves are optimizing photosynthetic capacity by investing resources, besides the CO₂ capture, to other limited processes as the capacity for RuBP regeneration (Leakey *et al.*, 2009). The downregulation of V_{cmax} , which is determined by the amount and activity of the Rubisco enzyme, may occur because the leaves can decrease their Rubisco content without losing efficiency in their carboxylation rate since CO₂ availability is no longer a constraint (Ainsworth & Long, 2005; Wang *et al.*, 2020). During the sunflecks events, photosynthesis can yield most of the daily carbon gain and determine rates of growth in understory plants (Leakey *et al.*, 2002; Valladares & Niinemets, 2008), so the greater investment in J_{max} under eCO₂ can be associated with a better light use efficiency and utilization of sunflecks to support the increased demand for RuBP regeneration (DeLucias & Thomas, 2000).

Photosynthesis is a complex process that is strongly influenced by the environment (Blankenship, 2014) and, under optimal conditions, maintain the equilibrium between the biochemical and biophysical processes. Photochemistry reactions must be quite adaptive to a dynamic environment, such as forest's understory, to coordinate the energetic demands for ATP and NADPH while simultaneously regulating light capture (Avenson *et al.*, 2005), whilst subjected to low light intensity, oscillating below 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and inconstant sunflecks of varying intensities (Mendes *et al.*, 2017). Indeed, we observed an increase in Fv'/Fm' , and a decrease in NPQ under e[CO₂], but Φ_{PSII} was not affected by the treatment.

Φ_{PSII} estimates the efficiency at which light absorbed by PSII is used to initiate the electron transport and can be used as a proxy to quantify the actual flux of electrons through PSII (Genty *et al.*, 1989; Earl & Ennahli, 2004). We did not see an eCO₂ effect on Φ_{PSII} that could indicate an increase in the energy flux with eCO₂, to support the increase in A_{sat} . Even though we have not seen a rise in the electron flux, we observed that the light use efficiency is higher under eCO₂ due to the increase in Fv'/Fm' , suggesting a better efficiency of PSII photochemistry when the leaf is exposed to light (Baker, 2008), and the decrease in NPQ , which

represents the heat loss from PSII, and can correspond to a photoprotective mechanism which prevents highly reactive oxygen species formation due to the excess of light energy absorbed (Muller *et al.*, 2001). These results indicate a better efficiency of light-harvesting and energy transduction by open PSII centers since there was no excess energy. Thus, increased photoprotection through heat dissipation was not necessary (Riikonen *et al.*, 2005, Wujeska-Klaue *et al.*, 2019).

Does soil P availability modify the seedlings' photosynthetic response under different CO₂ concentrations?

We investigate the effects of P addition on carbon and energy flux of photosynthesis in an Amazonian species seedlings and, except for J_{max}/V_{cmax} ratio, no analyzed parameter responded to P addition. Since photosynthesis involves a series of chemical and physical processes that are extremely dependent of P supply, some degree of adaptation to P availability can be expected for plants growing on low-P soils (Mo *et al.*, 2019).

J_{max}/V_{cmax} ratio was significantly higher in the seedlings fertilized with P, due to an increase in J_{max} and a slight decrease in V_{cmax} , which can indicate that P addition is positively contributing to the rate of electron transport to RuBP regeneration, adjusting photosynthetic machinery to optimize the use of sporadic sunflecks in a low irradiance environment (Walters, 2005; Dietzel *et al.*, 2008). In response to a sunfleck, a rapid accumulation of high-energy metabolites, as RuBP and its immediate precursors, can occur in the PCR cycle, creating a pool to support a few seconds of continued CO₂ assimilation after the sunfleck (Way & Pearcy, 2012). It is consistent with the expectation that P be more often limiting for J_{max} than for V_{cmax} (Domingues *et al.*, 2010) because RuBP regeneration is affected by various factors such as the cyclic phosphorylation rates and by the activity of PCR cycle enzymes (Long *et al.*, 2004), all of which could benefit of the P fertilization.

We worked with the hypothesis that photosynthetic rates of *I. edulis* seedlings would respond more strongly to elevated CO₂ when fertilized with P, which was not the case since we did not observe any significant interaction effect. Recent studies corroborate our results that soil P addition was not a key factor modifying A_{sat} response to eCO₂ (Duan *et al.*, 2019; Thompson *et al.*, 2019) because of the various ways plants have to obtain P to use in photosynthesis. Although the increases in A_{sat} rates, under eCO₂, demand more nutrients, species adapted to low soil P availability have developed different strategies to acquire P, such

as increases in carbon exudation, phosphatase secretion, carbon trade with mycorrhizal partners, and root surface area (Steidinger *et al.*, 2015; Zemunik *et al.*, 2015; Nasto *et al.*, 2017). At the leaf-level, foliar P allocation patterns can be shifted to meet the P demand to maintain photosynthetic capacity (Mo *et al.*, 2019) while the extra P can be used in strategies to reduce light limitation, which can limit carbon absorption (Mendes & Marengo, 2010; 2014), such as increase leaf area to enhance the light acquisition capacity (Bloom *et al.*, 1985; Giardina *et al.*, 2003).

Conclusions

Our study brings to light the complexity of the photosynthetic process under eCO₂ and soil P addition, in seedlings of an Amazonian species. Our results showed that eCO₂ increases the efficiency of absorbed light energy to sustain the assimilation increased rates. We did not observe a P limitation in leaf photosynthetic rates and these results may be more common as previously assumed, due to plant adaptation mechanisms in low P tropical forests, although the response to P availability can be specie specific and not all plants may have a mechanism to overcome the low P soil concentration. We did not find a significant interaction between the P and CO₂ treatments, and P addition does not stimulate the response of plant photosynthesis to CO₂ fertilization in this species. We suggest that future experiments be carried out with different species to fully understand the photosynthetic response of tropical forests to eCO₂ and P availability.

References

- Ågren, G. I., Wetterstedt, J. M., & Billberger, M. F. (2012). Nutrient limitation on terrestrial plant growth—modeling the interaction between nitrogen and phosphorus. *New Phytologist*, *194*(4), 953-960.
- Ainsworth, E. A., & Long, S. P. (2005). What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New phytologist*, *165*(2), 351-372.
- Ainsworth, E. A., & Long, S. P. (2021). 30 years of free-air carbon dioxide enrichment (FACE): What have we learned about future crop productivity and its potential for adaptation?. *Global change biology*, *27*(1), 27-49.
- Ainsworth, E. A., & Rogers, A. (2007). The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant, cell & environment*, *30*(3), 258-270.
- Armstrong, D. L., Griffin, K. P., Danner, M., Mees, C., & Nguyen, D. (1999). Functions of phosphorus in plants. *Better Crops with Plant Food*, *83*(1), 6-7.
- Avenson, T. J., Kanazawa, A., Cruz, J. A., Takizawa, K., Ettinger, W. E., & Kramer, D. M. (2005). Integrating the proton circuit into photosynthesis: progress and challenges. *Plant, Cell & Environment*, *28*(1), 97-109.
- Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.*, *59*, 89-113.
- Barton, K. (2020). MuMIn: Multi-Model Inference. R package version 1.43.17. <https://CRAN.R-project.org/package=MuMIn>
- Blankenship, R. E. (2014). *Molecular mechanisms of photosynthesis*. Pp. 312 John Wiley & Sons.
- Bloom, A. J., Chapin III, F. S., & Mooney, H. A. (1985). Resource limitation in plants-an economic analogy. *Annual review of Ecology and Systematics*, *16*(1), 363-392.
- Bowes, G. (1993). Facing the inevitable: plants and increasing atmospheric CO₂. *Annual review of plant biology*, *44*(1), 309-332.

- Brienen, R. J., Phillips, O. L., Feldpausch, T. R., Gloor, E., Baker, T. R., Lloyd, J., ... & Zagt, R. J. (2015). Long-term decline of the Amazon carbon sink. *Nature*, *519*(7543), 344-348.
- Brooks, M. E., Kristensen, K., Van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., ... & Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *The R journal*, *9*(2), 378-400.
- Camargo, M. A. B., & Marenco, R. A. (2017). Tree growth over three years in response to monthly rainfall in central Amazonia. *Dendrobiology*, *78*(1), 10-17.
- Campbell, C. D., & Sage, R. F. (2006). Interactions between the effects of atmospheric CO₂ content and P nutrition on photosynthesis in white lupin (*Lupinus albus* L.). *Plant, Cell & Environment*, *29*(5), 844-853.
- Cernusak, L. A., Winter, K., Dalling, J. W., Holtum, J. A., Jaramillo, C., Körner, C., ... & Wright, S. J. (2013). Tropical forest responses to increasing atmospheric CO₂: current knowledge and opportunities for future research. *Functional Plant Biology*, *40*(6), 531-551.
- Cernusak, L. A., Winter, K., Martínez, C., Correa, E., Aranda, J., Garcia, M., ... & Turner, B. L. (2011). Responses of legume versus nonlegume tropical tree seedlings to elevated CO₂ concentration. *Plant physiology*, *157*(1), 372-385.
- Chauvel, A., Lucas, Y., & Boulet, R. (1987). On the genesis of the soil mantle of the region of Manaus, Central Amazonia, Brazil. *Experientia*, *43*(3), 234-241.
- Cordell, D., & White, S. (2015). Tracking phosphorus security: indicators of phosphorus vulnerability in the global food system. *Food Security*, *7*(2), 337-350.
- Cox, P. M., Pearson, D., Booth, B. B., Friedlingstein, P., Huntingford, C., Jones, C. D., & Luke, C. M. (2013). Sensitivity of tropical carbon to climate change constrained by carbon dioxide variability. *Nature*, *494*(7437), 341-344.
- Da Rocha, H. R., Manzi, A. O., Cabral, O. M., Miller, S. D., Goulden, M. L., Saleska, S. R., ... & Maia, J. F. (2009). Patterns of water and heat flux across a biome gradient from tropical forest to savanna in Brazil. *Journal of Geophysical Research: Biogeosciences*, *114*(G1).
- DeLucia, E. H., & Thomas, R. B. (2000). Photosynthetic responses to CO₂ enrichment of four hardwood species in a forest understory. *Oecologia*, *122*(1), 11-19.

- Dias, D. P., & Marenco, R. A. (2016). Tree growth, wood and bark water content of 28 Amazonian tree species in response to variations in rainfall and wood density. *Volume 9, Número JUNE2016, Pags. 445-451*.
- Dietzel, L., Bräutigam, K., & Pfannschmidt, T. (2008). Photosynthetic acclimation: State transitions and adjustment of photosystem stoichiometry–functional relationships between short-term and long-term light quality acclimation in plants. *The FEBS journal*, 275(6), 1080-1088.
- Domingues, T. F., Meir, P., Feldpausch, T. R., Saiz, G., Veenendaal, E. M., Schrodte, F., ... & Lloyd, J. O. N. (2010). Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant, Cell & Environment*, 33(6), 959-980.
- Drake, B. G., González-Meler, M. A., & Long, S. P. (1997). More efficient plants: a consequence of rising atmospheric CO₂?. *Annual review of plant biology*, 48(1), 609-639.
- Duan, H., Onteddu, J., Milham, P., Lewis, J. D., & Tissue, D. T. (2019). Effects of elevated carbon dioxide and elevated temperature on morphological, physiological and anatomical responses of *Eucalyptus tereticornis* along a soil phosphorus gradient. *Tree physiology*, 39(11), 1821-1837.
- Earl, H. J., & Ennahli, S. (2004). Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. *Photosynthesis Research*, 82(2), 177-186.
- Farquhar, G. D., von Caemmerer, S. V., & Berry, J. A. (1980). A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta*, 149(1), 78-90.
- Ferreira, S. J. F., Luizão, F. J., & Dallarosa, R. L. G. (2005). Precipitação interna e interceptação da chuva em floresta de terra firme submetida à extração seletiva de madeira na Amazônia Central. *Acta Amazonica*, 35(1), 55-62.
- Fleischer, K., Rammig, A., De Kauwe, M. G., Walker, A. P., Domingues, T. F., Fuchslueger, L., ... & Lapola, D. M. (2019). Amazon forest response to CO₂ fertilization dependent on plant phosphorus acquisition. *Nature Geoscience*, 12(9), 736-741.
- Frazer, G. W., Canham, C. D., & Lertzman, K. P. (1999). Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-colour fisheye photographs, users manual and program documentation. *Simon Fraser*

University, Burnaby, British Columbia, and the Institute of Ecosystem Studies, Millbrook, New York, 36.

Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 990(1), 87-92.

Giardina, C. P., Ryan, M. G., Binkley, D., & Fownes, J. H. (2003). Primary production and carbon allocation in relation to nutrient supply in a tropical experimental forest. *Global Change Biology*, 9(10), 1438-1450.

Hartig, F. (2020). DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.3.3.0. <http://florianhartig.github.io/DHARMA/>

Hättenschwiler, S. E., & Körner, C. R. (2000). Tree seedling responses to in situ CO₂-enrichment differ among species and depend on understorey light availability. *Global Change Biology*, 6(2), 213-226.

Huntingford, C., Zelazowski, P., Galbraith, D., Mercado, L. M., Sitch, S., Fisher, R., ... & Cox, P. M. (2013). Simulated resilience of tropical rainforests to CO₂-induced climate change. *Nature Geoscience*, 6(4), 268-273.

IPCC (2014) Summary for Policymakers. *Climate Change 2014 Synthesis Report Contribution of Work Groups I, II III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Lapola, D. M., & Norby, R. J. (2014). Amazon-FACE: Assessing the Effects of Increased Atmospheric CO₂ on the Ecology and Resilience of the Amazon Forest-Science Plan and Implementation Strategy. *Brasília: Ministério de Ciência, Tecnologia e Inovação*, 56.

Leakey, A. D., Ainsworth, E. A., Bernacchi, C. J., Rogers, A., Long, S. P., & Ort, D. R. (2009). Elevated CO₂ effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. *Journal of experimental botany*, 60(10), 2859-2876.

Leakey, A. D. B., Press, M. C., Scholes, J. D., & Watling, J. R. (2002). Relative enhancement of photosynthesis and growth at elevated CO₂ is greater under sunflecks than uniform irradiance in a tropical rain forest tree seedling. *Plant, Cell & Environment*, 25(12), 1701-1714.

- Lloyd, J., Bird, M. I., Veenendaal, E. M., & Kruijt, B. (2001). Should phosphorus availability be constraining moist tropical forest responses to increasing CO₂ concentrations?. In *Global biogeochemical cycles in the climate system* (pp. 95-114). Academic Press.
- Lojka, B., Dumas, L., Preininger, D., Polesny, Z., & Banout, J. (2010). The use and integration of *Inga edulis* in agroforestry systems in the amazon—Review article. *Agricultura Tropica et Subtropica*, 43(4), 352-359.
- Long, S. P., Ainsworth, E. A., Rogers, A., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: plants FACE the future. *Annu. Rev. Plant Biol.*, 55, 591-628.
- Magalhães, N. D. S., Marengo, R. A., & Camargo, M. A. B. (2014). Do soil fertilization and forest canopy foliage affect the growth and photosynthesis of Amazonian saplings?. *Scientia Agricola*, 71(1), 58-65.
- Makino, A., & Mae, T. (1999). Photosynthesis and plant growth at elevated levels of CO₂. *Plant and Cell Physiology*, 40(10), 999-1006.
- Malavolta, E., Pimentel-Gomes, F., & Alcarde, J. (2002). Adubos e adubações. NBL Editora.
- Malhi, Y., & Wright, J. (2004). Spatial patterns and recent trends in the climate of tropical rainforest regions. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 359(1443), 311-329.
- Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, 51(345), 659-668.
- Mendes, K. R., & Marengo, R. A. (2014). Is stomatal conductance of Central Amazonian saplings influenced by circadian rhythms under natural conditions?. *Theoretical and Experimental Plant Physiology*, 26(2), 115-125.
- Mendes, K. R., & Marengo, R. A. (2010). Leaf traits and gas exchange in saplings of native tree species in the Central Amazon. *Scientia Agricola*, 67(6), 624-632.
- Mendes, K. R., Marengo, R. A., & Nascimento, H. C. S. (2017). Velocidade de carboxilação da rubisco e transporte de elétrons em espécies arbóreas em resposta a fatores do ambiente na Amazônia Central. *Ciência Florestal*, 27(3), 947-959.

- Mo, Q., Li, Z. A., Sayer, E. J., Lambers, H., Li, Y., Zou, B. I., ... & Wang, F. (2019). Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. *Functional Ecology*, *33*(3), 503-513.
- Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant physiology*, *125*(4), 1558-1566.
- Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in ecology and evolution*, *4*(2), 133-142.
- Nasto, M. K., Osborne, B. B., Lekberg, Y., Asner, G. P., Balzotti, C. S., Porder, S., ... & Cleveland, C. C. (2017). Nutrient acquisition, soil phosphorus partitioning and competition among trees in a lowland tropical rain forest. *New Phytologist*, *214*(4), 1506-1517.
- Nilsson, M. C., & Wardle, D. A. (2005). Understorey vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Frontiers in Ecology and the Environment*, *3*(8), 421-428.
- Norby, R. J., & Zak, D. R. (2011). Ecological lessons from free-air CO₂ enrichment (FACE) experiments. *Annual review of ecology, evolution, and systematics*, *42*.
- Perez-Martin, A., Michelazzo, C., Torres-Ruiz, J. M., Flexas, J., Fernández, J. E., Sebastiani, L., & Diaz-Espejo, A. (2014). Regulation of photosynthesis and stomatal and mesophyll conductance under water stress and recovery in olive trees: correlation with gene expression of carbonic anhydrase and aquaporins. *Journal of experimental botany*, *65*(12), 3143-3156.
- Phillips, O. L., & Brienen, R. J. (2017). Carbon uptake by mature Amazon forests has mitigated Amazon nations' carbon emissions. *Carbon balance and management*, *12*(1), 1-9.
- Quesada, C. A., Lloyd, J., Schwarz, M., Patiño, S., Baker, T. R., Czimczik, C., ... & Paiva, R. (2010). Variations in chemical and physical properties of Amazon forest soils in relation to their genesis. *Biogeosciences*, *7*(5), 1515-1541.
- Quesada, C. A., Lloyd, J., Anderson, L. O., Fyllas, N. M., Schwarz, M., & Czimczik, C. I. (2011). Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences*, *8*(6), 1415-1440.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

- Riikonen, J., Holopainen, T., Oksanen, E., & Vapaavuori, E. (2005). Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO₂ and O₃ in the field. *Tree Physiology*, *25*(5), 621-632.
- Roháček, K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, *40*(1), 13-29.
- Sage, R. F., & Kubien, D. S. (2007). The temperature response of C₃ and C₄ photosynthesis. *Plant, cell & environment*, *30*(9), 1086-1106.
- Schimel, D., Stephens, B. B., & Fisher, J. B. (2015). Effect of increasing CO₂ on the terrestrial carbon cycle. *Proceedings of the National Academy of Sciences*, *112*(2), 436-441.
- Seneweera, S., Makino, A., Mae, T., & Basra, A. S. (2005). Response of rice to p (CO₂) enrichment: the relationship between photosynthesis and nitrogen metabolism. *Journal of Crop Improvement*, *13*(1-2), 31-53.
- Sharkey, T. D. (2019). Is triose phosphate utilization important for understanding photosynthesis?. *Journal of experimental botany*, *70*(20), 5521-5525.
- Singh, S. K., & Reddy, V. R. (2014). Combined effects of phosphorus nutrition and elevated carbon dioxide concentration on chlorophyll fluorescence, photosynthesis, and nutrient efficiency of cotton. *Journal of Plant Nutrition and Soil Science*, *177*(6), 892-902.
- Singh, S. K., & Reddy, V. R. (2015). Response of carbon assimilation and chlorophyll fluorescence to soybean leaf phosphorus across CO₂: Alternative electron sink, nutrient efficiency and critical concentration. *Journal of Photochemistry and Photobiology B: Biology*, *151*, 276-284.
- Singh, S. K., Reddy, V. R., Fleisher, D. H., & Timlin, D. J. (2018). Phosphorus nutrition affects temperature response of soybean growth and canopy photosynthesis. *Frontiers in plant science*, *9*, 1116.
- Springer, C. J., & Thomas, R. B. (2007). Photosynthetic responses of forest understory tree species to long-term exposure to elevated carbon dioxide concentration at the Duke Forest FACE experiment. *Tree Physiology*, *27*(1), 25-32.
- Steidinger, B. S., Turner, B. L., Corrales, A., & Dalling, J. W. (2015). Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. *Functional Ecology*, *29*(1), 121-130.

- Tans P. & Keeling R. (2018) Trends in atmospheric carbon dioxide. NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/)
- Ter Steege, H., Pitman, N. C., Phillips, O. L., Chave, J., Sabatier, D., Duque, A., ... & Vásquez, R. (2006). Continental-scale patterns of canopy tree composition and function across Amazonia. *Nature*, *443*(7110), 444-447.
- Thompson, J. B., Slot, M., Dalling, J. W., Winter, K., Turner, B. L., & Zalamea, P. C. (2019). Species-specific effects of phosphorus addition on tropical tree seedling response to elevated CO₂. *Functional Ecology*, *33*(10), 1871-1881.
- Tomimatsu, H., Iio, A., Adachi, M., Saw, L. G., Fletcher, C., & Tang, Y. (2014). High CO₂ concentration increases relative leaf carbon gain under dynamic light in *Dipterocarpus sublamellatus* seedlings in a tropical rain forest, Malaysia. *Tree physiology*, *34*(9), 944-954.
- Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 237-257.
- Vance, C. P., Uhde-Stone, C., & Allan, D. L. (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New phytologist*, *157*(3), 423-447.
- Vieira, S., de Camargo, P. B., Selhorst, D., Da Silva, R., Huttyra, L., Chambers, J. Q., ... & Martinelli, L. A. (2004). Forest structure and carbon dynamics in Amazonian tropical rain forests. *Oecologia*, *140*(3), 468-479.
- Vitousek, P. M., & Sanford Jr, R. L. (1986). Nutrient cycling in moist tropical forest. *Annual review of Ecology and Systematics*, *17*(1), 137-167.
- Von Caemmerer, S. (2000). *Biochemical models of leaf photosynthesis*. Csiro publishing.
- Walters, R. G. (2005). Towards an understanding of photosynthetic acclimation. *Journal of experimental botany*, *56*(411), 435-447.
- Wang, W., Cai, C., He, J., Gu, J., Zhu, G., Zhang, W., ... & Liu, G. (2020). Yield, dry matter distribution and photosynthetic characteristics of rice under elevated CO₂ and increased temperature conditions. *Field Crops Research*, *248*, 107605.
- Way, D. A., & Pearcy, R. W. (2012). Sunflecks in trees and forests: from photosynthetic physiology to global change biology. *Tree Physiology*, *32*(9), 1066-1081.
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wujeska-Klaue, A., Crous, K. Y., Ghannoum, O., & Ellsworth, D. S. (2019). Leaf age and eCO₂ both influence photosynthesis by increasing light harvesting in mature *Eucalyptus tereticornis* at EucFACE. *Environmental and Experimental Botany*, *167*, 103857.

Zemunik, G., Turner, B. L., Lambers, H., & Laliberté, E. (2015). Diversity of plant nutrient-acquisition strategies increases during long-term ecosystem development. *Nature plants*, *1*(5), 1-4.

Tables

Table 1: Gas exchange and fluorescence α parameters of *Inga edulis* for each treatment. Means \pm SD of $n = 8$ per treatment. The measurements were made in the following environmental conditions: 21% oxygen, constant air flow rate of $700 \mu\text{mol s}^{-1}$, with pressure between 0-0.1 Pa, fan at 10.000 rpm, relative humidity between 65 to 70%, leaf temperature of $30 \text{ }^\circ\text{C}$, $250 \mu\text{mol m}^{-2}\text{s}^{-1}$ of PPFD. $[\text{CO}_2]$ according to treatment: 500 ppm if ambient, 700 ppm if elevated.

<i>Parameters</i>	<i>Ambient CO₂</i>		<i>Elevated CO₂</i>	
	<i>Low P</i>	<i>High P</i>	<i>Low P</i>	<i>High P</i>
A_{sat} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	4.488 ± 0.235	4.544 ± 0.208	5.159 ± 0.358	5.000 ± 0.207
g_{sw} ($\text{mol m}^{-2} \text{s}^{-1}$)	0.150 ± 0.016	0.144 ± 0.011	0.132 ± 0.011	0.113 ± 0.015
V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	20.135 ± 0.921	19.917 ± 0.86	19.027 ± 1.26	18.623 ± 0.841
J_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	27.262 ± 1.23	29.341 ± 1.4	28.752 ± 1.75	28.661 ± 0.997
J_{max}/V_{cmax}	1.356 ± 0.026	1.473 ± 0.029	1.524 ± 0.068	1.550 ± 0.052
Φ_{PSII}	0.263 ± 0.012	0.262 ± 0.015	0.260 ± 0.022	0.254 ± 0.012
F_v/F_m'	0.557 ± 0.006	0.559 ± 0.006	0.584 ± 0.008	0.579 ± 0.014
NPQ	1.27 ± 0.08	1.35 ± 0.058	1.06 ± 0.103	1.15 ± 0.11

Table 2: Results of p values of each treatment obtained by fitting linear or generalized linear mixed-effects model. The marginal R^2 (R^2_m) describes the goodness of model fit given fixed effects only, while the conditional R^2 (R^2_c) describes the goodness of model fit including fixed and random effects. VWC is soil volumetric water content. TRST is total solar radiation transmitted.

<i>Variable</i>	<i>CO₂</i> <i>treatment</i>	<i>P</i> <i>treatment</i>	<i>VWC</i>	<i>TRST</i>	<i>CO₂</i> <i>x P</i>	<i>R²_m</i>	<i>R²_c</i>
<i>A_{sat}</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.005	0.926	0.014	-	-	0.301	0.308
<i>g_{sw}</i> ($\text{mol m}^{-2} \text{s}^{-1}$)	0.046	0.291	0.003	-	-	0.304	0.37
<i>V_{cmax}</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.189	0.729	-	-	-	0.05	0.059
<i>J_{max}</i> ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.636	0.158	-	0.024	-	0.156	0.156
<i>J_{max}/V_{cmax}</i>	0.006	0.049	-	-	-	0.282	0.3
<i>Φ_{PSII}</i>	0.697	0.815	-	-	-	0.006	0.021
<i>F_v'/F_m'</i>	0.017	0.728	-	-	-	0.217	0.52
<i>NPQ</i>	0.033	0.793	-	-	-	0.201	0.666

Statistical significance of each treatment is noted in bold. $p \leq 0.05$.

Figures

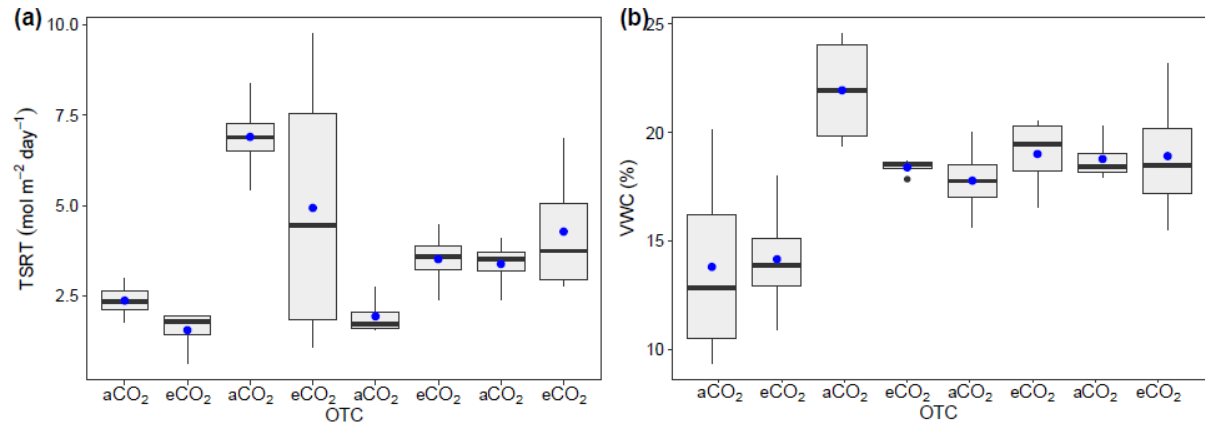


Figure 1. Total solar radiation transmitted estimated through hemispherical photos (a); and soil volumetric water content (b) obtained for each OTC. aCO₂ represents control OTCs (500 ppm of CO₂) and eCO₂ represents treatment OTCs (700 ppm of CO₂). Means are represented by the blue point. Black point represent outlier. n = 4 per OTC.

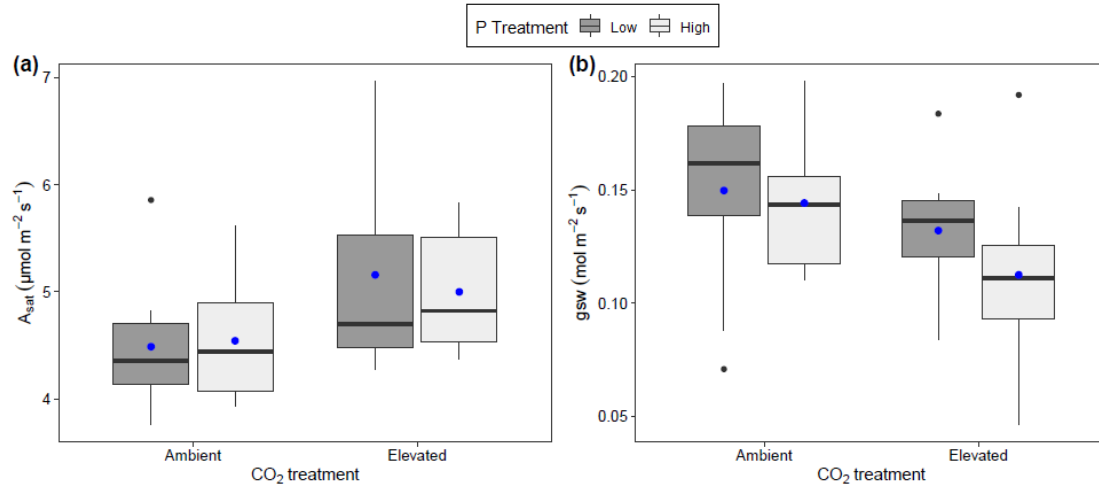


Figure 2. Boxplots represent the light-saturated assimilation (A_{sat}); (a) and stomatal conductance (g_{sw}) (b) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. Gas-exchange measurements were made at the [CO₂] for each treatment at 30 °C and 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of *PPFD*. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. $n = 8$ per treatment.

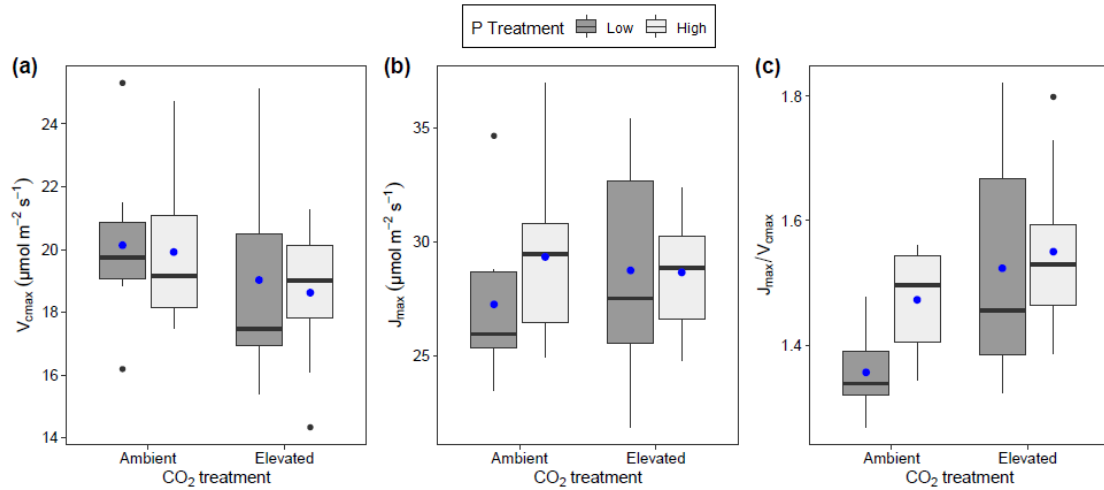


Figure 3. Maximum rate of Rubisco carboxylation (V_{cmax}) (a); maximum electron transport rate to regeneration of ribulose-1,5-biphosphate (J_{max}) (b); and maximum rate of electron transport to the maximum rate of Rubisco carboxylation ratio (J_{max}/V_{cmax}) (c) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. V_{cmax} and J_{max} were calculated from A/Ci curves. Gas-exchange measurements were made at the [CO₂] for each treatment at 30 °C and 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. n = 8 per treatment.

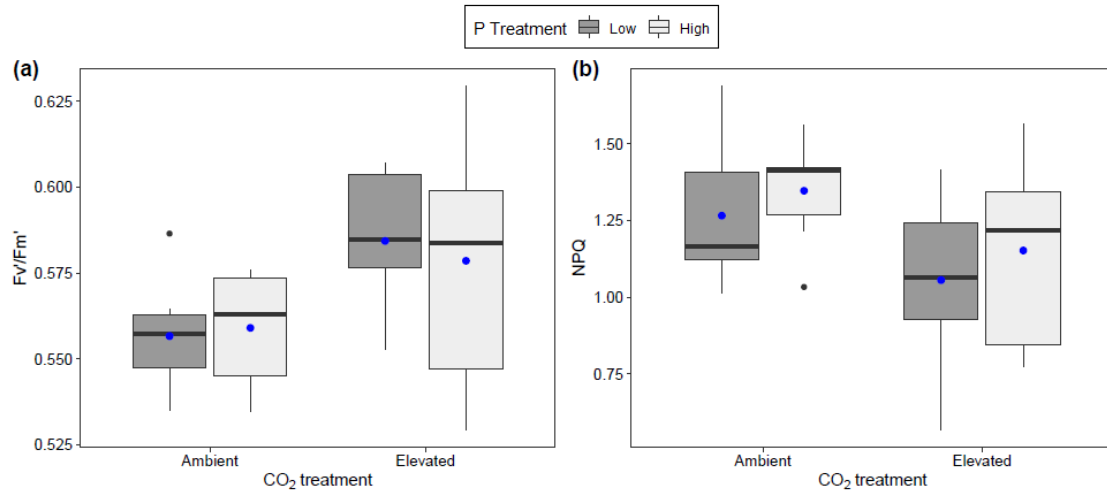


Figure 4. Maximum efficiency of PSII photochemistry in light-adapted leaf (F_v'/F_m') (a); and non-photochemical quenching (NPQ) (b) of seedlings grown at ambient CO₂ (500 ppm) or elevated CO₂ (700 ppm) in unfertilized (low P) or fertilized (high P) soil. Chlorophyll fluorescence measurements were made at the growth CO₂ for each treatment. Means are represented by the blue point, the bold horizontal line indicate the median, the top and bottom of each box indicate the first and third quartiles, the whiskers extending to the highest and lowest values are within 1.5 the interquartile range (IQR) of the upper and lower quartiles, respectively. Black points represent outliers. n = 8 per treatment..

Appendix A

Determination of light saturation point

Along the tropical rainforests profile, light is spatially and temporally heterogeneous and not much photosynthetically active radiation reaches the understory where the seedlings were grown (Chazdon *et al.*, 1988). Because light is a key resource for photosynthesis, both low and high sunlight can limit and influence photosynthetic capacity of plants (Valladares & Niinemets, 2008), so tests were made to discover the point where photosynthesis was light-saturated to use in the CO₂ response curves. Shade leaves may saturate at low photosynthetic photon flux density (*PPFD*) (Bellasio *et al.*, 2016), so we made curves of the response of photosynthetic CO₂ assimilation rate to photosynthetic photon flux density (*A/PPFD* curves) in three leaves for each treatment.

The measurements were taken under standard chamber environmental conditions of 21% oxygen concentration, constant air flow rate through the cuvette of 700 $\mu\text{mol s}^{-1}$, pressure between 0-0.1 Pa, fan at 10.000 rpm, relative humidity between 65 to 70%, leaf temperature of 30 ± 2 °C, reference CO₂ concentration in accordance with the OTC treatment (500 or 700 $\mu\text{mol mol}^{-1}$). Before each measurement, the leaf was allowed to reach full photosynthetic induction for 40-50 minutes inside the equipment chamber until they reach steady-state, when assimilation rate, stomatal conductance and fluorescence signal were stable. A light response curve auto-program was initiated with the *PPFD* steps: 700, 600, 500, 400, 350, 300, 250, 200, 150, 100, 75, 50, 25, 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with minimum 3 min and maximum 5 minutes between each step and matching the IRGAs at each step.

Chlorophyll α fluorescence parameters were measured at the end of each step of *A/PPFD* curves, by applying a saturation rectangular pulse of 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 0.5 s, to determine the light-adapted maximum fluorescence (F_m') and the steady-state fluorescence (F_s), followed by a far-red light pulse of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 5 s, to obtain the light-adapted minimal fluorescence (F_o').

After the sunset, between 6:30 and 8 pm, leaves already adapted to the dark were measured to obtain the maximum (F_m) fluorescence, under a measuring beam at 50 Hz of frequency, and a saturation rectangular pulse of 8000 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The quantum yield of PSII photochemistry (Φ_{PSII}) was calculated according to Genty *et al.* (1989): $\Phi_{PSII} = (F_m' - F_s)/F_m'$. Photochemical quenching (qP) was calculated as: $qP = (F_m'$

$- F_s)/(F_m' - F_o')$; Non-photochemical quenching (NPQ and qN) was calculated as: $NPQ = (F_m - F_m')/F_m'$ and qN as: $qN = (F_m - F_m')/(F_m - F_o')$. (Maxwell & Johnson, 2000; Roháček, 2002).

Results and Discussion

According to Long & Bernacchi (2003), photosynthesis parameters became more informative when gas exchange and chlorophyll fluorescence measurements are made in simultaneous. To determine the light saturating point of photosynthesis, the point beyond which no significant change in assimilation occurs (Lobo *et al.*, 2013), we analyzed both the $A \times PPFD$ curves, and the fluorescence parameters carried out simultaneously to identify the saturating point with an adequate balance between the photochemical (qP and Φ_{PSII}) and non-photochemical (qN and NPQ) processes of energy dissipation (Pimentel *et al.*, 2011).

It is possible to identify that after the curvature, an increase in $PPFD$ does not stimulated a proportional increase in assimilation (Fig. A1). By looking at fluorescence parameters we saw that above $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ of $PPFD$, qN is higher than qP for all treatments (Fig. A2). These parameters of quenching analysis measured in the same scale represents the photochemical capacity of photosystem II (PSII) in a light-adapted state by estimating the fraction of PSII reaction centers that can receive an electron (qP), a number that decreases as a function of light saturation, and the activation of non-photochemical light energy conversion leading to non-radiative thermal dissipation of excitation energy (qN) (Maxwell and Johnson 2000).

Another way to analyze where the balance between photochemical and non-photochemical processes happens is through $\Phi_{PSII} \times NPQ$ graphics which show a reduction in 50% of the quantum yield of PSII photochemistry (Φ_{PSII}) and proportional increase in non-photochemical quenching (NPQ) above $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ of $PPFD$ (Fig. A3). Φ_{PSII} estimates the efficiency at which light absorbed by PSII is used to initiate the linear electron transport (Genty *et al.*, 1989; Earl & Ennahli, 2004) and are generally high under low light conditions because a large proportion of the absorbed light is used in photochemistry. NPQ represents the heat loss from PSII, it is a parameter more sensible than qN and can represent a photoprotective mechanism which prevents highly reactive oxygen species formation due the excess of light energy absorbed (Müller *et al.*, 2001).

The decline in fluorescence parameters Φ_{PSII} , and qP with increasing $PPFD$ indicates that excess energy absorbed by photosynthetic pigments was not used for CO_2 fixation and

needs to be dissipated through non-photochemical processes (qN and NPQ) that increases with $PPFD$. The decreases in PSII and qP efficiency leads to a protective non-photochemical mechanism activation, because a decrease in qP is strongly associated with increased risk of photoinhibition (Ögren and Rosenqvist 1992).

$qP \times qN$ and $\Phi_{PSII} \times NPQ$ graphics showed that the intersection point was around $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, but looking at $A \times PPFD$, we see that there is still an increase in assimilation rates, until $250 \mu\text{mol m}^{-2} \text{s}^{-1}$ of $PPFD$. So, a balance between gas exchange and fluorescence measurements was made to assure that most of the absorbed energy is being directed to primary photochemical process. Throughout this, we can have the $PPFD$ value, where A is light-saturated and not stressed by overexcitation, to use in CO_2 response curves.

References

- Bellasio, C., Beerling, D. J., & Griffiths, H. (2016). An Excel tool for deriving key photosynthetic parameters from combined gas exchange and chlorophyll fluorescence: theory and practice. *Plant, cell & environment*, 39(6), 1180-1197.
- Chazdon, R. L., Williams, K., & Field, C. B. (1988). Interactions between crown structure and light environment in five rain forest Piper species. *American Journal of Botany*, 75(10), 1459-1471.
- Earl, H. J., & Ennahli, S. (2004). Estimating photosynthetic electron transport via chlorophyll fluorometry without Photosystem II light saturation. *Photosynthesis Research*, 82(2), 177-186.
- Genty, B., Briantais, J. M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 990(1), 87-92.
- Lobo, F. D. A., De Barros, M. P., Dalmagro, H. J., Dalmolin, Â. C., Pereira, W. E., De Souza, E. C., Vourlitis, G. L. & Ortíz, C. R. (2013). Fitting net photosynthetic light-response curves with Microsoft Excel—a critical look at the models. *Photosynthetica*, 51(3), 445-456.

- Long, S. P., & Bernacchi, C. J. (2003). Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *Journal of experimental botany*, *54*(392), 2393-2401.
- Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of experimental botany*, *51*(345), 659-668.
- Müller, P., Li, X. P., & Niyogi, K. K. (2001). Non-photochemical quenching. A response to excess light energy. *Plant physiology*, *125*(4), 1558-1566.
- Ögren, E., & Rosenqvist, E. (1992). On the significance of photoinhibition of photosynthesis in the field and its generality among species. *Photosynthesis Research*, *33*(1), 63-71.
- Pimentel, C., Oliveira, R. F. D., Ribeiro, R. V., Santos, M. G. D., & Machado, E. C. (2011). Características fotossintéticas de *Phaseolus vulgaris* L. *Hoehnea*, *38*(2), 273-280.
- Roháček, K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, *40*(1), 13-29.
- Valladares, F., & Niinemets, Ü. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annual Review of Ecology, Evolution, and Systematics*, *39*, 237-257.

Figures

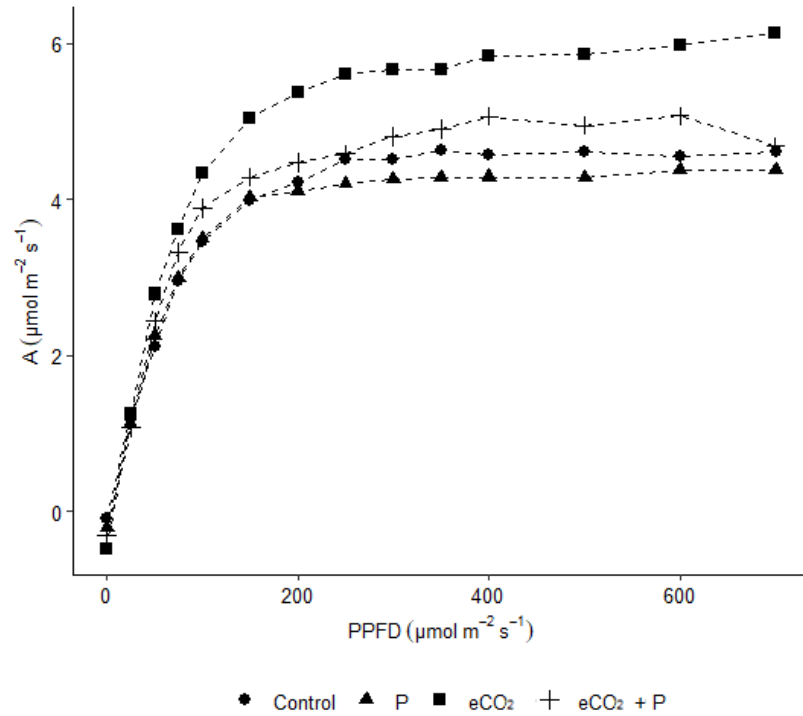


Figure A1. Curves of the response of photosynthetic CO₂ assimilation rate (*A*) to increasing photosynthetic photon flux density (*PPFD*) obtained for each treatment at 30 °C and 500 μmol mol⁻¹ CO₂ (control and P) or 700 μmol mol⁻¹ CO₂ (eCO₂ and eCO₂ + P). Each point represents the mean of 3 measures (*n* = 3 per treatment).

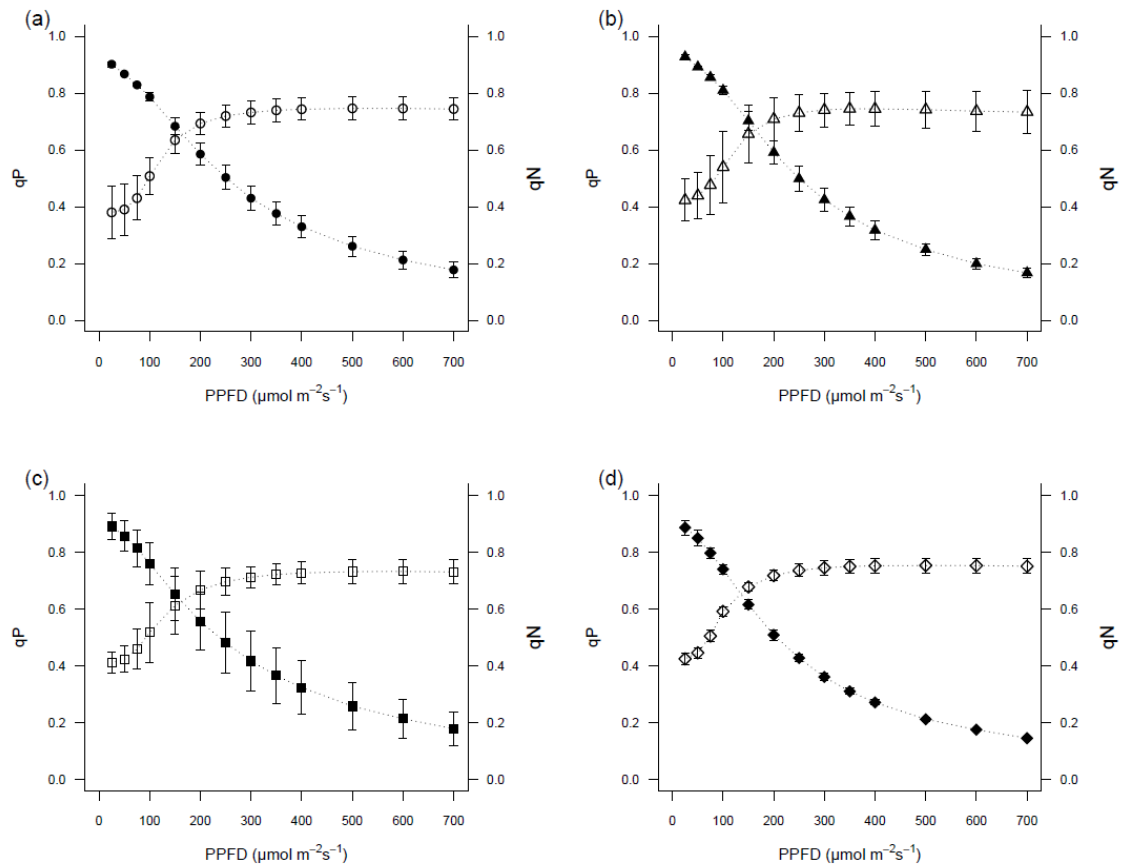


Figure A2. Curves of the response of photochemical quenching (qP , filled points) and non-photochemical quenching (qN , open points) coefficients to increasing photosynthetic photon flux density ($PPFD$), at control (a), P fertilization (b), elevated CO_2 (c), and elevated CO_2 + P fertilization (d) treatments obtained by fluorescence measurements at $30^\circ C$ and 500 ppm CO_2 (control and P) or 700 ppm CO_2 (e CO_2 and e CO_2 + P). Means \pm standard deviation, $n = 3$ per treatment.

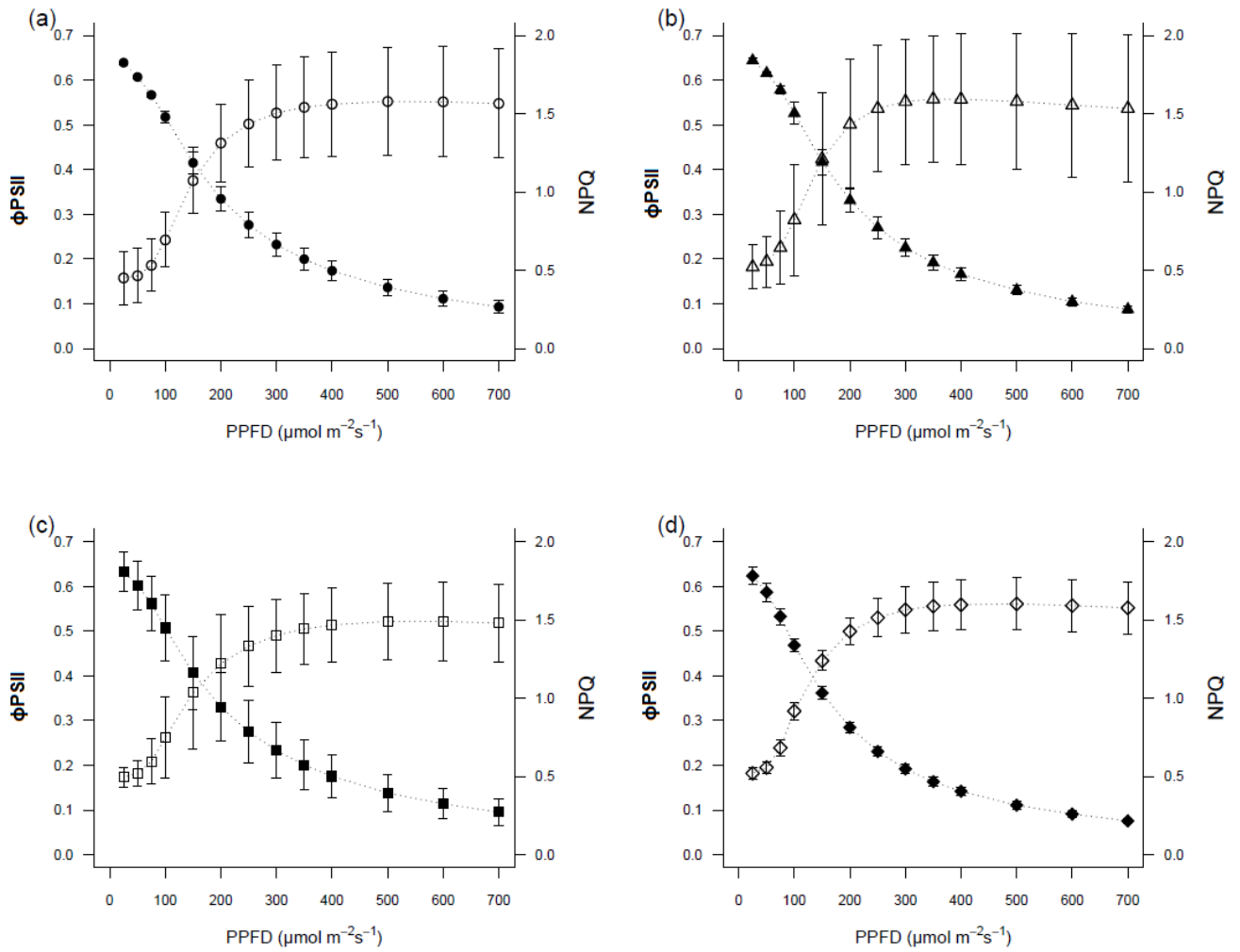


Figure A3. Curves of the response of quantum yield of PSII photochemistry (Φ_{PSII} , filled points) and non-photochemical quenching (NPQ, open points) coefficients to increasing photosynthetic photon flux density (PPFD), at control (a), P fertilization (b), elevated CO_2 (c), and elevated CO_2 + P fertilization (d) treatments obtained by fluorescence measurements at 30°C and 500 ppm CO_2 (control and P) or 700 ppm CO_2 (e CO_2 and e CO_2 + P). Means \pm standard deviation, $n = 3$ per treatment.