INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

FÓSFORO RESTRINGE RESPOSTAS ALOMÉTRICAS, MAS NÃO FISIOLÓGICAS, DE PLÂNTULAS DE *Inga edulis* Mart. SOB CRESCIMENTO EM CO_2 ELEVADO

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MANAUS, AMAZONAS

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Aos 25 dias do mês de Maio do ano de 2021, às 13h30min, por videoconferência. Reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: **Dr. Bart Kruijt**, da Universidade de Wageningen - WUR, o **Dr. Samuel Cordeiro Vitor Martins**, da Universidade Federal de Viçosa - UFV, e o **Dr. João Victor Rodrigues**, da Universidade Federal do Amazonas - UFAM, tendo como suplentes a Dra. Flávia Delgado Santana, do Instituto Nacional de Pesquisas da Amazônia - INPA, e o Dr. David Montenegro Lapola, da Universidade Estadual de Campinas - UNICAMP, sob a presidência do orientador, a fim de proceder à arguição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** da **GABRIELA USHIDA NEVES**, intitulado: **"FÓSFORO RESTRINGE RESPOSTAS ALOMÉTRICAS, MAS NÃO FISIOLÓGICAS, DE PLÂNTULAS DE *INGA EDULIS* MART. SOB CRESCIMENTO EM CO_2 ELEVADO"**, 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.

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Sinopse:

Estudou-se os efeitos do aumento da concentração de dióxido de carbono (CO_2) e adição de fósforo (P) em respostas fisiológicas e alométricas em plântulas de uma espécie de leguminosa (*Inga edulis* Mart.) crescendo experimentalmente em câmaras de topo aberto instaladas no sub-bosque de uma floresta primária de terra-firme na Amazônia central.

Palavras-chave: Mudanças climáticas, Amazônia central, câmaras de topo aberto, metabolismo primário de carbono, A_{sat}, R_{dark}, crescimento acima do solo.

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Dedico esta dissertação à minha tia, Elza, que partiu muito cedo, deixando ainda uma vida inteira de saudades.

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"You once told me that the human eye is god's loneliest creation. How so much of the world passes through the pupil and still it holds nothing. The eye, alone in its socket, doesn't even know there's another one, just like it, an inch away, just as hungry, as empty.
Opening the front door to the first snowfall of my life, you whispered,
Look."

-*On Earth, we're briefly gorgeous*,
Ocean Voung

RESUMO

A floresta Amazônica é a maior floresta contínua tropical do mundo, exercendo importante papel no ciclo do carbono (C), ao assimilar CO_2 pela fotossíntese e fixá-lo em biomassa vegetal, representando um sumidouro de C. Nesse sentido, o aumento da concentração atmosférica de CO_2 nos últimos séculos pode trazer consequências, ainda pouco compreendidas, para o metabolismo e crescimento de plantas nessa região. Estudos realizados em ambientes temperados, onde há baixa disponibilidade de nitrogênio (N) no solo, mostraram estímulo à fotossíntese e crescimento em plantas submetidas a CO_2 elevado (eCO2), o que ficou conhecido como efeito de fertilização por CO_2. Na bacia Amazônica, devido à variação na idade dos solos e aos processos de intemperismo, existe um gradiente oeste-leste de disponibilidade de fósforo (P). Nas regiões com menor concentração de P no solo (leste), como a Amazônia Central, a limitação por P tem o potencial de restringir a fertilização por CO_2, afetando as respostas fisiológicas e de crescimento em ambientes de eCO_2. Nesse contexto, nós investigamos as respostas de variáveis fisiológicas (assimilação líquida (A_{sat}), fotorrespiração (P_R), respiração foliar no claro (R_{light}) e no escuro (R_{dark})) e alométricas acima do solo (altura e diâmetro de copa e da planta inteira, área foliar total, espessura foliar e taxa relativa de crescimento) de plântulas de *Inga edulis* Mart. submetidas a eCO_2 e adição de P, utilizando câmaras de topo aberto instaladas no sub-bosque de uma floresta primária de terra-firme na Amazônia Central. Nossos resultados mostraram ausência de interação entre eCO_2 e P, mas com um padrão claro de efeito significativo de eCO_2 no metabolismo (variáveis fisiológicas) e de P no crescimento e desenvolvimento (variáveis alométricas) das plântulas, o que indica que um papel diferencial de P nas respostas de plantas crescendo sob eCO_2, de acordo com a natureza do processo. Assim, se outras espécies na mesma região apresentarem respostas similares a eCO_2, isso poderia trazer implicações importantes para o ciclo de C.

ABSTRACT

The Amazon rainforest is the largest continuous tropical forest in the world. It plays an important role in the carbon (C) cycle, by assimilating CO_2 through photosynthesis, fixing it in plant biomass and working then as a terrestrial C sink. As [CO_2] rises, it brings potential consequences to plant metabolism and growth. Studies in temperate regions, where soil nitrogen (N) availability is low, showed stimulation of photosynthesis and growth in plants exposed to elevated [CO_2] (eCO_2), which is known as CO_2 fertilization. In the Amazon basin, due to variation in soil age and weathering processes, phosphorus availability (P) is distributed in an west-east gradient. In regions with lower P concentrations (east), such as Central Amazon, its limitation has the potential to restrict CO_2 fertilization, affecting physiological and growth responses to eCO_2. In that context, we investigated the responses of physiological variables (Net CO_2 assimilation, Photorespiration, Light and dark leaf respiration), linked to the primary carbon metabolism, and allometric variables (whole-plant and crown height and diameter, total leaf area, leaf thickness and relative growth rate) of seedlings of *Inga edulis* Mart. Our results showed that eCO_2 had an effect on carbon metabolism whereas high-P supply affected mostly aboveground growth and development (allometric variables), which indicates that P may have a differential impact on the responses of plants growing under eCO_2, depending on the nature of the processes. In that case, if other species in the region present similar responses to eCO_2, it could indicate major implications to C sink activity in the future.

SUMÁRIO

LISTA DE ABREVIAÇÕES E SIGLAS

Variable (abbreviation)	Definition	Unit
A_{net}	Net CO_2 assimilation	µmol CO_2 $m^{-2}s^{-1}$
A_{sat}	Net CO_2 assimilation at saturating light	µmol CO_2 $m^{-2}s^{-1}$
C_a	Ambient air CO_2 partial concentration	µmol CO_2 mol^{-1}
C_i	Intercellular CO_2 partial concentration	µmol CO_2 mol^{-1}
CD	Crown diameter	cm
CH	Crown height	cm
D	Seedling's diameter	cm
F	Steady-state fluorescence	-
Fs	Steady-state fluorescence at the time of measurement	-
Fm'	Maximum fluorescence achieved by artificially quenching PSII with a saturating pulse	-
Fm	Dark-adapted maximum fluorescence during a saturating pulse	-
Fo'	Minimum fluorescence during a dark pulse	-
g_{sw}	Stomatal conductance to water vapor	µmol H_2O mol^{-1}
H	Seedling's height	cm
J	Electron (e^-) transport rate through PSII	µmol e^- $m^{-2}s^{-1}$
Lth	Leaf thickness	mm
MLA	Mean leaf area	cm^2
NPQ	Non-photochemical quenching, as an estimate of the apparent rate constant heat loss from PSII	-
PPFD	Photosynthetic photon flux density	µmol photon $m^{-2}s^{-1}$
P_R	Photorespiration	µmol CO_2 $m^{-2}s^{-1}$
qP	Photochemical quenching, as an estimate of the proportion of photosystem II (PSII) open centers	-
R^2	Coefficient of determination of a linear model	-
R^2_{fixed}	Coefficient of determination of fixed effects calculated from a generalized linear mixed model	-
R^2_{random}	Coefficient of determination of random effects calculated from a generalized linear mixed model	-
R	Leaf respiration (CO_2 release other than from P_R)	µmol CO_2 $m^{-2}s^{-1}$
R_{dark}	Leaf respiration in the darkness	µmol CO_2 $m^{-2}s^{-1}$
R_{light}	Leaf respiration in the light	µmol CO_2 $m^{-2}s^{-1}$
RuBP	Rubisco's substrate, stands for ribulose-1,5-biphosphate	-
s	Lumped parameter as described in Yin *et al.* (2009)	-

TLA	Total leaf area	cm^2
V_o	Rubisco oxygenation rate	$\mu mol\ CO_2\ m^{-2}s^{-1}$
Φ_{PSII}	Quantum efficiency of PSII e- flow assessed from chlorophyll fluorescence measurements	$mol\ e^-\ (mol\ photon)^{-1}$
ρ	Spearman's correlation coefficient	-

Rubisco: Ribulose-1,5-biphosphate carboxylase/oxygenase

INTRODUÇÃO GERAL

A concentração atmosférica de CO_2 [CO_2] tem aumentado nas últimas décadas, sendo comumente associada a ações antrópicas, como queima de combustíveis fósseis, mudança de uso da terra e perda de cobertura vegetal (Hönisch et al. 2009, Pagani et al. 2009). De acordo com o Painel Intergovernamental de Mudanças Climáticas (IPCC 2014), é previsto que a [CO_2] aumente ainda mais, de 397 para 700 ppm até o fim do século XXI, contribuindo para o aquecimento global e mudanças no clima. Entretanto, durante a maior parte de sua história evolutiva recente, a vegetação terrestre foi exposta a [CO_2] inferiores às atuais e previstas (Lüthi et al. 2008, Leakey and Lau 2012), o que gera incertezas sobre como as plantas responderão a essa mudança. Considerando que o CO_2 é um dos principais substratos para a fotossíntese, estudos sugerem que os aumentos observados e projetados em sua concentração podem ter impacto positivo e direto no metabolismo, crescimento e produtividade de plantas ao redor do mundo (Cernusak et al. 2013). Entretanto, outros fatores como a quantidade de fósforo (P) no solo, podem limitar a capacidade de as plantas assimilarem CO_2, trazendo incertezas sobre as repostas a [CO_2] (eCO_2). Diante dessa perspectiva, tem-se buscado compreender como plantas responderão ao aumento de [CO_2] principalmente no que diz respeito aos processos de aquisição e alocação de C (Cernusak et al. 2011).

Os efeitos de eCO_2 no metabolismo e crescimento de plantas têm sido bastante estudados nas últimas quatro décadas (Drake et al. 1997, Ainsworth and Long 2005, Ainsworth and Rogers 2007, Ainsworth and Long 2021). No geral, as plantas respondem positivamente a eCO_2, com maior capacidade fotossintética (fertilização por CO_2), maior taxa metabólica e maior crescimento, apesar de essas respostas serem dependentes das características das espécies e condições ambientais (Drake et al. 1997, Ainsworth and Long 2005, Norby et al. 2005, Ainsworth and Rogers 2007, Norby and Zak 2011). O aumento da [CO_2] pode estimular a assimilação líquida de CO_2 (A_{net}) ao aumentar a disponibilidade de C para Rubisco e assim, aumentar a carboxilação de ribulose-1,5-bifosfato (RuBP), enquanto simultaneamente suprime a fotorrespiração (P_R) (Farquhar et al. 1980, Long 1991, Drake et al. 1997). Os aumentos observados em A_{net} são comumente acompanhados de diminuição de condutância estomática (g_{sw}) em plantas de metabolismo C_3 (Drake et al. 1997, Leakey et al. 2012). Entretanto, o grau em que A_{net} aumenta em eCO_2 e é sustentada, aumentando também o crescimento da planta, depende de processos complexos envolvidos com o metabolismo primário de carbono ao nível foliar. Tais processos, dentre os quais os dominantes são P_R e respiração mitocondrial (R),

resultam comumente na liberação de CO_2 fixado pela fotossíntese e podem ditar quanto C estará disponível para a planta usar em outros processos, influenciando o balanço de C na planta inteira (Productivity 1989, Kromer 1995, Hurry et al. 1996, O'Leary et al. 2019).

A P_R é um processo que envolve uma série de reações, começando com a oxigenação de RuBP no cloroplasto, ao invés de sua carboxilação pela Rubisco, com reações subsequentes no peroxissomo e na mitocôndria, onde CO_2 é então liberado como resultado de descarboxilação da glicina (Hurry et al. 2005). Essa reação de oxigenação representa de 15 a 35% das taxas líquidas de fotossíntese (Sharkey 1988, Atkin et al. 2006) e é responsável por mais descarboxilação na luz do que a respiração (Pärnik and Keerberg 1995), sendo o fluxo majoritário de descarboxilação em plantas C3 (Hurry et al. 2005). A supressão de P_R em eCO_2 costuma ser apontada como a principal razão para estímulo de A_{net} em eCO_2, já que, com mais CO_2 próximo aos sítios ativos da Rubisco, a afinidade da enzima por CO_2 aumenta, levando a maior carboxilação em detrimento de oxigenação (Farquhar et al. 1980, Long 1991). Além disso, P_R interage diretamente com a respiração foliar, compartilhando intermediários com o ciclo do ácido tricarboxílico (ciclo TCA) na mitocôndria (Hurry et al. 2005). Por causa de suas interações com esses dois importantes processos, a P_R pode ter grande impacto no balanço de carbono foliar (Hurry et al. 2005).

A respiração mitocondrial é responsável por produzir moléculas energéticas (ex. ATP), poder redutor (ex. NADPH) e esqueletos de carbono que serão usados na manutenção celular e biossíntese (Amthor 1991). De acordo com Loveys et al. (2002), entre 30-80% de todo CO_2 fixado pela fotossíntese a nível individual é respirado de volta à atmosfera diariamente. Mesmo que a respiração não ocorra somente em órgãos fotossintetizantes, aproximadamente metade de toda a respiração que ocorre na planta acontece nas folhas, considerando os dois tipos de R (Ayub et al. 2014). A respiração mitocondrial foliar ocorre tanto na luz (R_{light}), simultaneamente à fotossíntese e à fotorrespiração, quanto no escuro (R_{dark}). Entretanto, suas contribuições relativas à respiração total não são as mesmas, uma vez que R é inibida pela luz, resultando em menores taxas respiratórias na luz do que no escuro (Brooks and Farquhar 1985, Kromer 1995, Tcherkez et al. 2008). O grau no qual R é inibida pela luz varia bastante, de 17 a 66% (Ayub et al. 2014), com a inibição pela luz estritamente ligada às taxas subjacentes de R_{dark} e aos outros processos que ocorrem na folha durante o dia, como A_{net} e P_R (Wang et al. 2001, Atkin et al. 2006, Tcherkez et al. 2008, Ayub et al. 2011). As respostas de R a eCO_2 variam muito, de acordo com idade foliar, condições ambientais no momento da medição, métodos utilizados,

etc. (Way et al. 2015, Xu et al. 2015, Dusenge et al. 2019). Uma das razões para essa variação é a existência de duas vias principais reguladoras do metabolismo respiratório: a oferta de substrato (ex. fotossintatos) e a demanda por produtos respiratórios. Maiores concentrações de fotossintatos fornecidas por maior A_{net} têm sido correlacionadas com maiores taxas respiratórias, como resultado de maior disponibilidade de substrato (Azcón-Bieto and Osmond 1983). Por outro lado, a demanda por produtos respiratórios é relacionada tanto ao aumento de R, para acompanhar maiores taxas metabólicas e de crescimento, como também a reduções em R, amplamente ligadas a menor consumo de ATP, NADPH e esqueletos de carbono (Amthor 1991, Wullschleger et al. 1994).

Na luz, as maiores liberações de CO_2 mitocondriais provêm da P_R e da R_{light}. As necessidades celulares por energia e intermediários de carbono podem ser satisfeitos por esses dois processos, uma vez que equilibram as necessidades da célula e respondem plasticamente às demandas metabólicas (Hurry et al. 2005). Mesmo que as vias de descarboxilação sejam bem compartimentalizadas em relação aos intermediários e carbono, a interação das vias na mitocôndria sugere uma possível ligação, especialmente em relação à inibição de R pela luz (Wang et al. 2001, Hurry et al. 2005). De fato, essa ligação foi mostrada em alguns estudos (ex. (Tcherkez et al. 2008, Ayub et al. 2011, Crous et al. 2012), apesar de ainda não ser inteiramente compreendida. Tais estudos diferem na direção da relação entre esses dois processos, com os mecanismos subjacentes ainda incertos. Maiores taxas de R_{light} (menor inibição pela luz) têm sido relacionadas a maiores taxas de P_R (Crous et al. 2012, Griffin and Turnbull 2013, Ayub et al. 2014) possivelmente como resultado de maior demanda por transferidores de NH_2 durante a recuperação de intermediários do ciclo fotorrespiratório (Tcherkez et al. 2008). Por outro lado, P_R também tem sido associada a menores taxas de R_{light} (Wang et al. 2001, Zaragoza-Castells et al. 2007), sendo explicada pela dependência de reduções fotorrespiratórias de atividade de enzimas respiratórias e transição para um ciclo TCA parcial na luz (Tcherkez et al. 2005). Como é esperado que P_R seja suprimida por eCO_2, tal mudança impactaria a inibição de respiração foliar pela luz, independentemente da direção. Nesse caso, eCO_2 pode mudar razões R_{light}:R_{dark} além das variações esperadas em R_{dark}, o que pode levar a maiores variações no balanço de carbono da folha e da planta inteira.

As interações entre os processos entrada (fotossíntese) e saída (fotorrespiração e respiração) de CO_2 na folha são complexas e dependentes da direção de cada resposta a eCO_2 sob diferentes condições ambientais. Se as taxas de processos fisiológicos variarem

diferentemente em relação a sua sensitividade a eCO_2 (ex. A_{net} sendo mais sensível do que R_{dark}), um novo balanço de carbono pode surgir. Por exemplo, o balanço entre fotossíntese e respiração, que são os dois maiores fluxos de carbono entre atmosfera e biosfera, é crucial para compreender o quanto de carbono fixado está realmente disponível para crescimento (Crous et al. 2012). De fato, a maioria dos estudos apontam para maior crescimento em eCO_2, medido como maior produção de biomassa, incremento em altura e diâmetro e maiores copas (maior área foliar), graças ao carbono extra proveniente de maiores taxas de A_{net} (Norby et al. 1995, Ainsworth and Long 2005, Norby and Zak 2011).

Condições ambientais, como disponibilidade de nutrientes no solo, podem exercer papel fundamental na modificação de respostas fisiológicas e de crescimento a eCO_2 (Cernusak et al. 2013). Assim como fotossíntese e crescimento aumentam em eCO_2, a demanda pelos nutrientes necessários para sustentar esses processos também aumenta, o que eventualmente pode levar à depleção de nutrientes a longo prazo (Leakey et al. 2012). Como o crescimento é normalmente controlado pelo recurso limitante (e não pela quantidade total de recursos disponíveis), as respostas a eCO_2 podem ser modificadas se a disponibilidade natural de nutrientes não acompanhar as maiores demandas causadas por eCO_2 (Norby et al. 2010, Ellsworth et al. 2017). Em ambientes terrestres, nitrogênio (N) e fósforo (P) são os elementos que mais limitam a produtividade primária e crescimento vegetal (Lambers et al. 2008, Vitousek et al. 2010). Nos ambientes temperados, onde N é normalmente escasso, graças à menor fixação biológica, a menor quantidade de N restringiu respostas positivas de eCO_2 ao longo do tempo (Oren et al. 2001, Reich et al. 2006, Norby et al. 2010). Já florestas tropicais, onde os solos são geologicamente antigos e já bastante intemperizados, costumam apresentar menores quantidades de P (Lambers et al. 2008). Mesmo com menos evidências experimentais, já existe um consenso de que menor disponibilidade de P também pode restringir respostas a eCO_2 (Ellsworth et al. 2017, Jiang et al. 2020).

Fósforo é um macronutriente essencial para o metabolismo e crescimento vegetal, sendo componente de ácidos nucleicos (ex. DNA), moléculas energéticas (ex. ATP), fosfatos de açúcar (ex. RuBP) e fosfolipídeos (Crous et al. 2017). Assim, o fornecimento de P pode afetar tanto processos metabólicos (fotossíntese, (foto)respiração e interações entre eles), como alocação de carbono na planta inteira (Rao and Terry 1995, Plaxton and Podestá 2006, Pandey et al. 2015). A baixa disponibilidade de P no solo pode levar a menores taxas de regeneração de RuBP e consequentemente menores taxas fotossintéticas e fotorrespiratórias (Rao and Terry

1995, Ellsworth et al. 2015). A limitação por P pode levar ainda a menores taxas respiratórias através de baixos níveis de ATP intracelulares, dado que a razão ATP:ADP regula a fosforilação de enzimas do ciclo TCA assim como a atividade enzimática e passagem de elétrons pela cadeia de transporte de elétrons na mitocôndria (Bykova et al. 2003, Plaxton and Podestá 2006). Além disso, a baixa disponibilidade de P pode influenciar o crescimento tanto pela redução direta da fotossíntese (limitação pela fonte) quanto pelo acúmulo de fotossintatos nas folhas que não podem ser utilizados para crescimento, o que levaria a *down-regulation* da fotossíntese (limitação pelo dreno, Paul and Foyer 2001).

As florestas tropicais possuem grande importância na regulação do clima, na produtividade primária líquida (PPL) e no estoque de carbono global, estocando cerca de metade da biomassa vegetal, apesar de cobrir apenas 7% da superfície terrestre (Bonan 2008, Pan et al. 2011). A maior floresta tropical contínua do mundo encontra-se na bacia amazônica e é responsável por 14% do CO_2 fixado pela fotossíntese terrestre, evidenciando sua relevância global (Zhao and Running 2010). Assim como em outras florestas tropicais (Santiago et al. 2012), estima-se que a produtividade da Amazônia seja limitada pela disponibilidade de P no solo, onde P é o fator que melhor explica a variação de produtividade de madeira na bacia (Quesada et al. 2012). Estudos recentes ressaltaram incertezas relacionadas à falta de conhecimento sobre como espécies da região responderiam a eCO_2 graças à disponibilidade de P no solo (Leakey et al. 2009, Cernusak et al. 2013, Hofhansl et al. 2016) e projeções de modelos estimam reduções severas na PPL e acúmulo de biomassa na vegetação quando a limitação por nutrientes é levada em consideração (Fleischer et al. 2019). Tais modelos assumem que espécies de florestas tropicais podem responder mais fortemente a eCO_2 do que espécies de florestas temperadas, devido a sensitividade de processos eco-fisiológicos à maior temperatura dos trópicos (Hofhansl et al. 2016). Entretanto, a disponibilidade de P pode influenciar de diversas formas as respostas a eCO_2, em função das habilidades das espécies em lidar com as limitações nutricionais a que estão submetidas (Thompson et al. 2019). Ellsworth et al. (2017) não observaram diferença de produtividade em florestas de *Eucalyptus* submetidas a eCO_2, atribuindo a falta de resposta à baixa disponibilidade de P na região. Ainda, Zalamea et al. (2016) observaram que espécies que ocorrem em solos com baixa disponibilidade natural de P não responderam à sua adição, indicando que as afinidades espécie-específicas por condições ambientais podem ser mais importantes do que previamente presumido e impactar também o comportamento de respostas a eCO_2.

Neste trabalho, investigamos os efeitos de eCO_2 e disponibilidade de P no metabolismo primário de carbono e desenvolvimento acima do solo de plântulas *Inga edulis* Mart., uma espécie da família leguminosa, sob crescimento experimental em câmaras de topo aberto instaladas no sub-bosque de uma floresta primária na Amazônia central. Nós abordamos as seguintes perguntas: (1) Os processos que constituem o metabolismo primário de carbono na planta - como assimilação líquida de CO_2 sob luz saturante (A_{sat}), respiração foliar (R_{light} e R_{dark}) e fotorrespiração (P_R), respondem a eCO_2? (2) A disponibilidade de P modifica essas respostas a eCO_2? (3) O grau de inibição de respiração pela luz é afetado por eCO_2 e disponibilidade de P? Como parte dessa análise, investigamos o papel da P_R na determinação e predição de taxas de R_{light} e da razão R_{light}:R_{dark} e (4) A resposta de desenvolvimento acima do solo é afetada pela disponibilidade de P?

OBJETIVOS

Objetivo geral

Determinar a influência do aumento da concentração de CO_2 atmosférico e disponibilidade de P no metabolismo primário de carbono e na alometria de plântulas de *Inga edulis* Mart. em condições de campo.

Objetivos específicos

1. Avaliar a resposta de parâmetros fisiológicos ligados ao metabolismo primário de carbono (Assimilação líquida de CO_2, Fotorrespiração, Respiração foliar no claro e Respiração foliar no escuro) de plântulas de *I. edulis* Mart. ao aumento de CO_2 atmosférico e sua sensitividade à disponibilidade de P.
2. Avaliar a resposta de inibição de respiração pela luz de plântulas de *I. edulis* Mart. ao aumento de CO_2 atmosférico e sua sensitividade à disponibilidade de P.
3. Investigar o papel da fotorrespiração na determinação e predição de taxas de Respiração foliar no claro e da razão Respiração foliar no claro:Respiração foliar no escuro em plântulas de *I. edulis* Mart.
4. Avaliar resposta de desenvolvimento acima do solo de plântulas de *I. edulis* Mart. ao aumento de CO_2 atmosférico e sua sensitividade à disponibilidade de P.

CAPÍTULO ÚNICO

Phosphorus constrains allometric but not physiological responses in seedlings of *Inga edulis* Mart. growing under elevated CO_2

Introduction

The atmospheric CO_2 concentration [CO_2] has risen since the beginning of the 18th century, from 280 to current 410 parts per million (ppm) (Hönisch et al. 2009, Pagani et al. 2009), due to increased anthropogenic emissions of greenhouse gases through the burning of fossil fuels and change in land usage (IPCC 2014). Likewise, [CO_2] is still expected to increase even more by the end of the 21st century, even in the most conservative projected scenario (IPCC, 2014). However, in most of its recent evolutionary history, Earth's vegetation has been exposed to lower [CO_2] (Lüthi et al. 2008), which brings uncertainties about how plants will respond to this change in the future (Leakey and Lau 2012). Considering that CO_2 is the primary substrate for photosynthesis, which is the main entry of carbon in the biosphere, elevated [CO_2] (eCO_2) may have a direct impact on metabolism, carbon economy and growth in plants around the world.

Forests are important components of the carbon (C) cycle and can help lessen negative effects of eCO_2, such as global warming and climate change, working as terrestrial sinks of C (Bonan 2008, Pan et al. 2011). For instance, plant biomass is estimated to store 450-650 gigatons (Gt) of C worldwide (Friedlingstein et al. 2020). At first, as photosynthesis is CO_2-limited, it usually enhances with eCO_2 ("CO_2 fertilization"), also resulting enhanced growth, reported as greater biomass production, increment in height and diameter and larger canopies (i.e. increased leaf area) (Norby et al. 1995, Drake et al. 1997, Ainsworth and Long 2005, Norby and Zak 2011). However, environmental conditions, such as climate or nutrient availability, may play a fundamental role in modifying physiological and growth eCO_2-induced responses (Norby et al. 2005). As photosynthesis and growth increases, so does the demand for nutrients, which can eventually lead to nutrient depletion in the long-term (Leakey et al. 2012). Considering that growth is controlled by the limiting resource ("Law of the Minimum"), responses to eCO_2 may become restrained, or even nonexistent, if nutrients' natural availabilities do not meet eCO_2-enhanced demands (Norby et al. 2010, Ellsworth et al. 2017).

In terrestrial environments, nitrogen (N) and phosphorous (P) are the nutrients that most limit productivity and growth in forests (Walker and Syers 1976, Lambers et al. 2008, Vitousek et al. 2010). In temperate regions, where biological activities are adversely affected by the low seasonal temperatures, decreasing N fixation (Vitousek and Howarth 1991), low-N supply can constrain positive eCO_2 effects on growth over time (i.e. Oren et al. 2001, Reich et al. 2006, Norby et al. 2010). Alternatively, tropical regions usually have high availability of N, but are P-limited, due to the strongly weathered soils, rock-derived nature of P pools and soil P occlusion, which turns it unavailable for plant use (Lambers et al. 2008). Although with less experimental evidence, there is a growing consensus that low-P availability can constrain plant responses to eCO_2 as well (Ellsworth et al. 2017, Jiang et al. 2019).

Tropical forests account for 33% of terrestrial net primary productivity (NPP) and store one fourth of the C in terrestrial biosphere, despite covering only 7% of land's surface (Bonan 2008, Phillips et al. 2009), which indicates their importance to the C cycle. The largest continuous tropical forest is comprised within the Amazon basin, accounting for 14% of the CO_2 fixed by terrestrial photosynthesis (Zhao and Running 2010). As in other tropical forests, the Amazon productivity is thought to be constrained by P availability (Santiago et al. 2012, Santiago 2015). For instance, a work of Quesada et al. (2012) observed that soil P status was the factor that better explained variation in growth and wood productivity across basin where soil P availability varies in a basin-wide gradient, with higher amounts of P found in the east and lower in the west (Quesada et al. 2011). Recent studies have brought to light uncertainties related to the lack of knowledge on how species in the region would respond to eCO_2 due to soil P availability (Leakey et al. 2009, Cernusak et al. 2013, Hofhansl et al. 2016) and model projections also estimated severe reductions in NPP and biomass accumulation in a Central Amazon forest when nutrient limitation was taken into account (Fleischer et al. 2019).

The effects of eCO_2 in plant metabolism and growth have been largely studied for the last four decades (Drake et al. 1997, Ainsworth and Rogers 2007, Norby and Zak 2011, Ainsworth and Long 2021). Net CO_2 assimilation (A_{net}) is stimulated by eCO_2 by increasing carbon availability to Rubisco and thus enhancing carboxylation of ribulose-1,5-biphosphate (RuBP), whilst simultaneously suppressing photorespiration (P_R) (Farquhar et al. 1980, Long 1991, Drake et al. 1997). However, the degree to which enhanced A_{net} is sustained and translated into productivity and growth is dependent upon a complex range of oxidative processes in primary carbon metabolism at the leaf-level. Such processes, of which the most

dominant are P_R and leaf mitochondrial respiration (R), often result in the release of photosynthetically fixed CO_2 and the balance between them can determine how much carbon will actually be available for growth (Productivity 1989, Kromer 1995, Hurry et al. 1996, O'Leary et al. 2019).

Mitochondrial respiration is responsible for producing energy molecules, reducing power and carbon skeletons that will be used in cellular maintenance and biosynthesis (Amthor 1991). According to Loveys et al. (2002), between 30-80% of all CO_2 fixed by photosynthesis at the individual level is then respired back to the atmosphere on a daily basis. Even though respiration does not only occur in photosynthetic organs, approximately half of the whole-plant respiration occurs in leaves (Ayub et al. 2014). Leaf respiration takes place both in the light (R_{light}), concurrently with photosynthesis and photorespiration, and in the darkness (R_{dark}). Yet, their relative contributions to total R are not the same, since R is inhibited by light, resulting in respiratory rates being lower in the light than in the darkness (Brooks and Farquhar 1985, Kromer 1995, Tcherkez et al. 2008). The responses of both types of R to eCO_2 are highly variable (Way et al. 2015, Xu et al. 2015, Dusenge et al. 2019) and are often associated to the supply of substrate (i.e. photosynthates) and the plants' demand for respiratory products (Azcón-Bieto and Osmond 1983, Amthor 1991, Wullschleger et al. 1994).

Photorespiration (P_R) is a process that involves an intricate set of reactions. It begins with the oxygenation of RuBP in the chloroplast, instead of its carboxylation by Rubisco (Hurry et al. 2005). This oxygenation reaction accounts for 15-35% of net photosynthetic rates (Sharkey 1988) and is the prevailing decarboxylation flux in C3 species (Hurry et al. 2005). In addition, P_R interacts directly with R_{light}, sharing intermediates with the tricarboxylic acid cycle (TCA cycle) in the mitochondrion (Hurry et al. 2005), which indicates a link between P_R and light inhibition of R (Wang et al. 2001, Hurry et al. 2005). The relationship between P_R and R has been shown in various studies (Tcherkez et al. 2008, Ayub et al. 2011, Crous et al. 2012), although its direction is still uncertain (Crous et al. 2017). As P_R is likely to be suppressed by eCO_2, such change will also impact light inhibition of R, irrespectively of the direction. In that case, eCO_2 may change R_{light}:R_{dark} ratios beyond eCO_2-caused variations of R, which could lead to larger changes in leaf carbon balance as well as in whole-plant.

The interplays between assimilation (photosynthesis) and loss (photorespiration and respiration) of CO_2 at the leaf level are highly complex and depend on how each related process responds to eCO_2 according to different environmental conditions (Norby and Zak 2011). As

most of the studies have been focusing on temperate regions, knowledge of plant responses to eCO_2 is biased in regards of how they interact with low soil nutrient availability (i.e. soil N supply; Leakey et al. 2012). Given the differences of nutrient cycling, climate regimes and biological complexity between temperate and tropical regions, the direction and sensitivity of plant physiological and growth responses to eCO_2 when soil P availability is low, such as in the Amazon rainforest, may differ from current knowledge (Cernusak et al. 2013, Hofhansl et al. 2016, Fleischer et al. 2019). Hence, it is essential to understand if and how the processes involved in plant primary carbon metabolism and growth will respond to eCO_2 in such context.

Here we investigated the effects of eCO_2 and P availability on leaf primary carbon metabolism and aboveground development of *Inga edulis* Mart. seedlings, a leguminous tree from the Amazon region, growing in CO_2 enrichment chambers in the understory of a central Amazon forest. Therefore, we tested the following hypotheses: (1) Growth under eCO_2 will affect the processes constituting plant primary carbon metabolism in opposite directions, as light-saturated net CO_2 assimilation (A_{sat}) and leaf respirations (R_{light} and R_{dark}) will increase, with R_{light} lower than R_{dark}, and P_R will decrease; (2) High soil P availability will intensify the physiological responses to eCO_2, except from P_R which is expected to decrease irrespective of P supply; (3) eCO_2 and P-induced changes in the underlying rates of R_{dark} and P_R will affect the degree of light inhibition of respiration, with P_R rates varying in the same direction of R_{light} (4) High-P and eCO_2 will induce aboveground responses separately, with seedlings exposed to both resources addition treatments being the most developed.

Material and Methods

Study site

We conducted this study in the AmazonFACE (Free-Air CO_2 Enrichment - https://amazonface.inpa.gov.br) experimental site, located at Reserva Experimental de Silvicultura Tropical (EEST/ ZF- 2; 2°36’32.67S 60°12’33.48W). The site is managed by Instituto Nacional de Pesquisas da Amazônia (INPA) and is situated approximately 70km north of Manaus (Amazonas, Brazil) (Lapola and Norby 2014).

The experimental site is established in a plateau of primary “terra-firme” (non-flooded) forest in Central Amazon, with mean annual temperature of 26ºC and altitude of 130 m (Ferreira et al. n.d., Chambers et al. 2000, Araújo et al. 2002, Tanaka et al. 2014). Rainfall is seasonally

distributed with a marked dry season mid-year between July and October (average rainfall of 556.4 mm) and a wet season between November and May (average rainfall of 1851.2 mm), with an average annual rainfall of 2407.6 mm (Tanaka et al. 2014). Average canopy height at the site reaches 30 m, with emergent trees reaching as far as 45 m (Vieira et al. 2004). The main plant families are Lecythidaceae, Sapotaceae, Arecaceae, Euphorbiaceae, Burseraceae, and Chrysobalanaceae (Carneiro et al. 2005). The site has a well-drained acidic and clay soil, characterized as Ferralsol (Oxisol), with low fertility due to its reduced phosphorus (P) and other rock derived elements concentration. These soil characteristics are found in approximately 32% of the forests in the Amazon basin (60% of Brazilian Amazon) and represents the lower end of the gradient of soil fertility in the Amazon basin (Quesada *et al*. 2010, 2011).

Eight Open Top Chambers (OTCs), with 2.5 m diameter x 3 m height each, were installed in the study site as part of Phase 1 of the AmazonFACE experiment (Lapola 2017). The OTCs are designed to increase the [CO_2] inside them and are commonly used to investigate the effects of eCO_2 in small-stature vegetation (Leadley and Drake 1993). To assess the effect of the CO_2 elevation in the understory, the chambers were set up in pairs (four control and four treatment), with one being the reference for natural ambient [CO_2] (without any addition of CO_2; ambient [CO_2] - aCO_2), whereas in the other, [CO_2] was held, on average, 200ppm above its aCO_2 pair (elevated [CO_2] - eCO_2). Two shapes of OTC structures were used, with different top (frustum) openings. One pair of steel-polypropylene OTCs were octagonal, with narrower frustum openings and three pairs of aluminum-polycarbonate OTCs were dodecagonal, with wider frustum openings (Figure S1A). Each pair of OTC was connected to a CO_2 sensor (Li-Cor 840A, Li-Cor Inc., Lincoln, NE, USA), installed in a nearby central system, that measured the [CO_2] inside the chambers every minute and controlled the CO_2 injection into the eCO_2 ones. Data of [CO_2] was recorded in Campbell Scientific CR1000 dataloggers. Whenever the difference between the pair of OTCs fell below 200 ppm, CO_2 was injected inside the eCO_2 chambers through a gas line, connected to a central cylinder (filled with high-pressurized gaseous CO_2) system, and spread by fans installed close to the CO_2 injection hose (at least twice every minute). The CO_2 injectors were switched on at 6 am and off at 6 pm. In the aCO_2 chambers, [CO_2] was around 500 µmol m^{-2} s^{-1}, while in the eCO_2 ones, [CO_2] was held around 700 µmol m^{-2} s^{-1}.

Experimental design

For this experiment, we chose the leguminous tree species *Inga edulis* Mart. (subfamily Mimosoideae). It is native to the American continent and presents a wide natural distribution that covers different regions of the Amazon rainforest, including the study site. Seeds were collected in November 2019 in an experimental station close to the study site from three different parent trees and equally distributed among CO_2 and P treatments. The seeds germinated in cylindrical shaped pots filled with soil (B horizon) collected locally at the experimental site (Figure S1B). To remove any fine roots previously existent, the soil was sifted twice with meshes of different sizes (10 x 10 mm and 2 x 2 mm). Half of the seedlings grew in pots with natural (P) availability (hereafter low-P treatment) and the other half grew in pots fertilized with 53.28 g of solid triple superphosphate (hereafter high-P treatment). To calculate the amount of triple superphosphate added to the soil, we used the standard conversion equation:

$$\text{Triple superphosphate (g)} = \frac{([P] * Volume\ (pot) * Soil\ density)/_{Conversion\ factor}}{1000}$$

where [P] is P concentration in ppm, volume (pot) is equal to 15.5 L, soil density is 1 gm/cm³ and conversion factor = 0.192. In addition to the calculated amount, it was added 10% more triple superphosphate (48.44 g + 4.84 g), considering the natural P occlusion in the soil. In low-P pots, average value of resin extractable P (an indicator of P available to plants) was 13 mg kg^{-1} whereas in high-P pots it was 240 mg kg^{-1}.

The experiment was composed of 48 pots, equally distributed among OTCs (six pots per OTC; three with low-P and three with high-P supply), resulting in 12 replicates for each combination of CO_2 and P treatments (Ambient CO_2/Low P (control), Ambient CO_2/High P, Elevated CO_2/Low P and Elevated CO_2/High P). *I. edulis* seeds germinated in the pots inside the OTCs on early November 2019 and grew for 9 months. During the experiment, two seedlings died (one in Ambient CO_2/Low P and one in Ambient CO_2/High P treatments), resulting in 46 seedlings for analysis.

Environmental characterization of OTCs

Light environment was assessed from hemispherical photographs using a Canon Rebel EOS T3 camera with Sigma fish-eye lens (8 mm), and further analyzed using Gap Light Analyzer software (https://www.caryinstitute.org/science/our-scientists/dr-charles-d-canham/gap-light-

analyzer-gla). For each OTC, the total solar radiation transmitted (TSRT; mol m^{-2} day^{-1}) was calculated as a function of solar constant (1367 W m^{-2}), geographical coordinates (latitude/longitude) (Table 1), and canopy openness (percentage of open sky seen from beneath the forest canopy), using the effective leaf area index integrated over the zenith angles 0 to 60º (LAI 4 Ring) as described in the software manual.

Since the OTCs are completely open at the top, pots were irrigated by natural rainfall. 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), which yields a 3% accuracy on soil moisture measurements and minimal soil disturbance. Three measurements were made in each pot, always close to the roots during the dry season, in September 2020 (Table 1).

Gas exchange and chlorophyll fluorescence measurements

Leaf-level gas exchange (GE) measurements of light-saturated CO_2 net assimilation (A_{sat}), stomatal conductance to water vapor (g_{sw}), leaf respiration in the darkness (R_{dark}), leaf respiration in the light (R_{light}) and photorespiration (P_R) were carried out on 32 seedlings (two per treatment in each OTC). These measurements were taken using an open gas exchange system (Li-Cor 6800; Li-Cor Inc., Lincoln, NE, USA) with a 6 cm^2 integrated fluorescence chamber head (Li-Cor 6800 Leaf Chamber Fluorometer). Measurements of A_{sat}, g_{sw} and R_{dark} were taken as point measurements whereas R_{light} and P_R were estimated using data from light curves. These measurements were performed under leaf temperature (T_{leaf}) of 30ºC, leaf-to-air vapor pressure deficit (VPD) of 0–0.1 Pa, fan speed of 10000 rpm, relative humidity (RH) inside the leaf chamber between 65 and 70% and red:blue light ratio of 9:1. Air flow rate was set at 700 μmolm^{-2} s^{-1} for A_{sat} and 300 μmolm^{-2} s^{-1} for R_{dark}, R_{light} and P_R (for an explanation on how reduced air flow rate was chosen, see Notes S2). Leaf chamber oxygen concentration [O_2] was set at ambient O_2 (21%), unless stated otherwise. For the measurements taken in control OTCs (aCO_2), the [CO_2] inside the leaf chamber (C_a) was set at 500 μmol mol^{-1}, whilst for treatment OTCs (eCO_2), it was set as 700 μmol mol^{-1}. Two sets of light response curves were performed using the decreasing photosynthetically photon flux density (PPFD) sequence of 80, 70, 60, 50, 40, 35, 30, 25, 20, 15, 10 and 5 μmol m^{-2} s^{-1}. At each step, readings were recorded when the stability parameters (ΔCO_2, ΔH_2O, A, g_{sw} and F) reached steady-state values (3-5min). In addition, steady-state fluorescence (Fs) and light-adapted maximal fluorescence (Fm') were taken by applying an instantaneous saturating light pulse (8000 μmol photon $m^{-2}s^{-1}$ for 0.5

seconds). Values of photosystem II (PSII) electron transport efficiency (Φ_{PSII}) were then calculated as $\Delta F/Fm' = (Fm'-Fs)/Fm'$ (Genty et al. 1989) for both sets of light response curves. In the first set, [O_2] was set as ambient O_2 (21%) and in the second set, [O_2] was set as low O_2 (0.5%) to suppress P_R. In the latter set, a gas cylinder with a mixture of 99.5% N_2 and 0.5% O_2 was used to ensure non-photorespiratory conditions. The measurements were made on fully expanded leaves with, approximately, the same age (290-310 days old), thus excluding any bias in this regard. Before starting all GE and chlorophyll fluorescence (CF) measurements, leaves were acclimated for at least 30min to ensure the stability of CO_2 assimilation (A), stomatal conductance (g_{sw}) and fluorescence (F) values.

Measurements of A_{sat} and g_{sw} were made simultaneously at light saturation point (LSP; PPFD= 250 μmol m^{-2} s^{-1}) in daylight, between 8 a.m. and 4 p.m. (for a thorough explanation on how LSP was estimated, see Notes S1). R_{dark} values were measured at the end of the day between 6 and 8 p.m., when leaves were in natural darkness conditions (PPFD = 0 μmol $m^{-2}s^{-1}$) for at least one hour, thus avoiding light enhanced dark respiration fluxes (LEDR; i.e. respiration of recently fixed photoassimilates) (Atkin et al. 1998, 2006) and post-illumination bursts with the usage of green lanterns (Atkin et al. 1998). Readings of A_{sat} and R_{dark} were recorded at a 10 second-interval for 2-3 min (A_{sat}) and 5 min (R_{dark}) after the acclimation period.

R_{light} was estimated by the Yin method (Yin et al. 2009, 2011) using the light curves under ambient O_2. R_{light} was estimated as the negative intercept of the linear regression of A against (PPFD * Φ_{PSII}/4) (Yin et al. 2009, 2011). We chose this method instead of the Kok method, because it accounts for variation in Φ_{PSII} as PPFD changes. To estimate R_{light}, we used only the GE and CF measurements made between 5-40 μmol PPFD $m^{-2}s^{-1}$ due to a non-linear relationship above 40 μmol PPFD m^{-2} s^{-1} and a non-clear Kok effect in our data. The Kok effect refers to a break (an abrupt change) in the slope of the A_{net}-PPDF relationship near the light compensation point (LCP; Ayub et al. 2011, Tcherkez and Atkin 2021). Our calculations of LCPs, as the x-intercept in the light curves, yielded results that ranged from 0 to 4.5 μmol PPFD $m^{-2}s^{-1}$. This could indicate that break points were below LCPs, enabling us to use the lower end of the light curves in R_{light} estimates.

P_R rates were estimated using data from the light curves under low O_2 and determined as 0.5 * Vo (Busch 2013), where Vo (Rubisco oxygenation rate) was calculated as (Bellasio et al. 2016):

$$V_o = \frac{J}{6} - \frac{2\,(Asat+Rlight)}{3}$$

and the electron transport rate (J) was calibrated as $J = s * PPFD * \Phi_{PSII}$ (Yin *et al*. 2009), with the lumped parameter *s* being the slope of the linear regression of A against ($PPFD * \Phi_{PSII}/4$) of the low O_2 light curves (Yin et al. 2009).

Allometric measurements

Non-destructive measurements of allometric data were made in all 46 seedlings. Plant height (H; cm) was measured as the shortest distance between the root collar and the apical bud. The circumference (C) of the stem was measured using a string wire positioned around the seedlings' stem, close to the root collar. The length of the string wire was then measured using a caliper and plant diameter (D; cm) was calculated with the equation $D=C/\pi$. Crown height (CH; cm) was measured as the shortest distance between the oldest lateral bud and the apical bud. Crown diameter (CD; cm) was measured as the longest distance between two diametrically opposite leaves. All the leaves of each seedling were counted and photographed, using a known scale. Mean leaf area (MLA; cm^2) and total leaf area (TLA; cm^2) for each seedling were calculated using the software ImageJ (https://imagej.nih.gov/ij/). As recommended by (Pérez-Harguindeguy et al. 2016) for compound leaves, leaf area was measured as the sum of each leaflet blade and their rachises areas. Mean leaf thickness (L_{th}, mm) was measured using a digital micrometer (Mitutoyo QuantuMike IP65), in three different regions of each leaf, always in the intermediate portion of the leaf blade, from the margin into the midrib, avoiding large secondary veins whenever possible.

Some seedlings exhibited branching at the base of the stem (n=12). All allometric variables were collected only from the main branch, except for number of leaves and total leaf area. All data, including environmental, GE, CF and allometric measurements, were collected between September and October 2020 (dry season).

Data analyses

Gas exchange data

Point measurements of A_{sat} and R_{dark} were screened for outliers (points 1.5 times greater than the interquantile range) and then averaged. The ratio R:A was calculated using data of A_{sat} and R_{dark}.

Aboveground plant size and relative growth rate

To analyze interrelationships and recognize patterns between allometric data related to carbon allocation, we performed a principal component analysis (PCA) using the following variables: H, D, CH, CD, TLA and Lth. The variables were scaled before analyses and the coordinates (scores) of the first axis (PC1) used as an index for aboveground development and size, in which their ordination was positively related to aboveground development. PCA was performed using the packages 'FactoMineR' (Husson et al. 2020) and 'factoextra' (Kassambara and Mundt 2020).

Relative growth rate (RGR) based on the height of the seedlings was calculated using the height measured at the end of the field campaign (October 2020) divided by the number of days of the experiment until allometric data collection (336 days). We used height growth instead of diameter growth because shaded environments (understory) are more likely to drive plant carbon investment in height or leaf area in order to enhance light capture (Poorter et al. 2018). Besides environment-driven strategies, ontogenetically, seedlings are more likely to allocate carbon in primary (height) over secondary (diameter) growth.

Statistical analyses

To analyze the effects of treatments on the response variables, we used generalized mixed effects models (GLMMs) using the R package 'glmmTMB' (Magnusson et al. 2020).We considered CO_2 (aCO_2 and eCO_2) and P (Low P and High P) treatments and their interactions as main fixed effects, parent tree and OTC as random effects. To account for variability between and within OTCs, due to OTC surrounding environment (i.e. different canopy openness), structure (i.e. frustum opening) or pot location inside the OTC, we used average soil moisture (VWC) and average total solar radiation (mol m^{-2} day^{-1}) as covariates in the models. Whenever a covariate was not significant ($p > 0.05$), we would remove it and refit the model. The probability distributions and link functions used in each model are indicated in Table 3.

We used the 'anova' function in R to compare the variance of the full models, including all of the fixed and random effects, and their nested variations until achieving the best fitted model (the model that better explained most of the variation of the response variables using less predictors). After selecting the best models, we tested them against their null model to ensure ours explained more than the model without predictors.

Considering that mixed models have more than one source of variance (random effects and residuals), we calculated marginal and conditional R^2 (Nakagawa and Schielzeth 2013), using the R package "MuMIn" (Bartón 2020). We chose the trigamma-estimate whenever possible, and delta-estimate when it was the only estimate available, as suggested by Bartón (2020). Marginal R^2 (hereafter R^2_{fixed}) is the coefficient of determination calculated considering only the variation explained by the fixed effects and conditional R^2 (hereafter R^2_{model}) is the coefficient calculated considering both fixed and random effects. R^2 from random effects only (hereafter R^2_{random}) was calculated as $R^2_{model} - R^2_{fixed}$.

To evaluate pairwise relationships between physiological variables, we used linear regression analyses and Spearman's correlation, where ρ is a rank-based correlation coefficient for non-parametric data and varies from -1 to 1. All statistical analyses were performed in the R statistical environment (R Core 2020).

Results

Treatment effect on physiological variables

Light-saturated net photosynthetic CO_2 assimilation (A_{sat}) was significantly higher in seedlings exposed to eCO_2 regardless of P treatment (*p-value*$_{CO_2}$ = 0.05 and *p-value*$_P$ = 0.926; Figure 1A, Table 3), followed by a slight, but not significant, decrease in stomatal conductance (g_{sw}) at A_{sat} under eCO_2 (*p-value*$_{CO_2}$ = 0.074, Table 3). Leaf respiration, both in the darkness (R_{dark}) and in the light (R_{light}), was significantly affected by eCO_2 treatment (Figure1B and C, Table 3), but in opposite directions, where R_{dark} decreased (*p-value*$_{CO_2}$ = 0.024) and R_{light} increased under eCO_2 (*p-value*$_{CO_2}$ = 0.045). The seedlings exposed to eCO_2 and high-P treatment showed the highest reduction in R_{dark} (52.33%). Some of the seedlings exposed to eCO_2 showed slightly positive R_{dark} values (i.e. - 0.056 ± 0.063). In addition, we did not observe the expected suppression of P_R under eCO_2 (*p-value*$_{CO_2}$ = 0.492) nor any change due to P addition (*p-value*$_P$ = 0.933; Figure 1D, Table 3). The R_{dark}:A_{sat} ratio decreased among CO_2 and P treatments (*p-value*$_{CO_2}$ = 0.011 and *p-value*$_P$ = 0.019; Table 3), but without an interaction between treatments (*p-value*$_{interaction}$ = 0.1). Variations in this ratio were strongly correlated to variations in R_{dark} (ρ = 0.95 *p-value* < 0.002), rather than in A_{sat} (ρ = - 0.07, *p-value* = 0.7).

We did not observe a significant relationship between R_{light} and R_{dark} (R^2 = 0.01, *p-value* = 0.24) and, under eCO_2, R_{light} was greater than R_{dark} (Figure 2A), resulting in R_{light}:R_{dark} ratios greater than 1 in 37.5% of the seedlings (n = 12). When R_{light}:R_{dark} ratios fell within the expected

range, from 0 to 1 (62.5%, n = 20), we observed a light inhibition of 30%. Spearman's correlation showed that variations in R_{light}:R_{dark} ratio were more related to changes in R_{light} (ρ = 0.87, *p-value* < 0.001) than in R_{dark} (ρ = -0.20, *p-value* = 0.27). The relationship between R_{light} and P_R did not show a predictive link between P_R and light inhibition of respiration irrespectively of the direction (R^2 = -0.031, *p-value* = 0.78, Figure 2B). Similarly, P_R did not influence R_{light}:R_{dark} ratio (R^2= 0.006, *p-value* = 0.28, Figure 2C).

Treatment effects on allometric variables

To assess the effect of eCO_2 and P addition on allometric variables, we investigated both the responses of separate aboveground components (stem and leaves) and a proxy for total aboveground development, using PCA scores. When analyzing isolated components, such as height (H), crown height (CH), crown diameter (CD), total leaf area (TLA) and relative growth rate (RGR), the variables showed a significant and positive response to P addition, irrespective of exposure to eCO_2 (Figure 3A, C and D; Table 3). Particularly, high-P seedlings showed greater crown development, both in terms of CH and CD and TLA, with CH following the same pattern as H, with taller seedlings also displaying taller crowns. High-P supply also had a significant effect on TLA ($p\text{-}value_P$ < 0.001; Table 3). Indeed, TLA was the variable that showed the greatest variation among treatments, with mean values ranging from 308.174 ± 151.615 cm² (mean ± sd) in control seedlings to 697.605 ± 343.14 cm² in seedlings exposed both to eCO_2 and P addition (Table 2, Figure 3C), which represents an increase of 126% in total leaf area. However, the number of leaves did not vary between treatments ($p\text{-}value_{CO_2}$ = 0.073, $p\text{-}value_P$ = 0.016; Table 3). In agreement with the results for H, high-P supply also had a significant and positive effect on RGR ($p\text{-}value_P$ = 0.004; Table 3).

The collective responses of different allometric variables were evaluated altogether with a PCA. The first axis of the PCA (PC1), which holds the greatest variation between variables, explained 52.75% of the variation between coordinated growth of stem and leaves, showing a grouping between treatments. Seedlings exposed to aCO_2 and low-P treatment were grouped on the top and bottom left (Figure S4), while seedlings exposed either to eCO_2 or P addition were grouped on the right, apparently showing two different growth strategies: growing taller or investing more in leaves and stem diameter. The second axis of the PCA (PC2) explained 17.3% of the variation. Combined, the first two axis explained 70% of total variation between variables related to carbon allocation. The variables that most contributed in grouping the

individuals were H and CH, both strongly related to the search for light. As far as the individual's contributions to the variation explanation, the seedlings that showed the most development (highest PCA score) were all in the high-P treatment (Figure S4). In agreement with isolated aboveground organs responses to treatments, seedlings' size was also greater in high-P treatment ($p\text{-}value_P < 0.001$; Table 3). Phosphorus addition had a significant and positive effect on aboveground development whilst eCO_2 treatment did not have any effect whatsoever. We also did not observe interactive effects between P availability and the response to CO_2 (Table 3).

Discussion

We investigated the influence of eCO_2 on the primary carbon metabolism, aboveground growth, and development of *I. edulis* seedlings, with and without P addition. In our study we were able to distinguish a pattern, where eCO_2 affected mostly physiological variables linked to primary carbon metabolism, while P addition affected positively aboveground development and size. Such results are somewhat consistent with other findings in plant responses to eCO_2 and/or P addition in tropical forests that share similar soil nutrient limitations (Winter et al. 2001, Cernusak et al. 2011*b*, Ellsworth et al. 2017, Thompson et al. 2019). Even without an interactive effect of eCO_2 and high P supply, physiological and allometric responses were enhanced (i.e. greater percentage change when compared to control; Table 2), when seedlings were exposed to both treatments. Such tendency, although not statistically significant, suggests that further investigation is needed to better understand the role of P in limiting responses to eCO_2.

Leaf gas exchange responds to eCO_2 but not to P addition

We observed that eCO_2 itself had a significant effect on A_{sat}, contrary to our hypothesis that P supply would limit this impact on photosynthetic rates. Despite the low-light availability environment, seedlings responded positively to eCO_2, even after 9-month exposure to eCO_2, This indicates that $[CO_2]$ can also limit CO_2 assimilation to some extent even in situations where light availability has a great influence on photosynthetic responses (Springer and Thomas 2007). The absence of P effect on A_{sat} could be explained by mechanisms usually used to cope with low nutrient availability, as the inorganic phosphate (Pi) recycling during plant metabolic reactions, which allows for carbon fixation to continue even under low-P supply. Examples of such mechanisms include up-regulation of Pi-transporters, differential expression of genes that

encode enzymes responsible for reprioritizing internal Pi among others (Plaxton and Tran 2011).

The reduction in P_R is the mechanism often used to explain the greater elevation of A_{sat} under eCO_2 (Farquhar et al. 1980, Long 1991). However, contrary to our expectation, we did not find an effect of neither CO_2 or P treatments on P_R nor a significant relationship between P_R and $A_{sat.}$ The lack of treatment effect can be explained by P recycling in leaf metabolism as pointed before, and the non-linear relationship of Rubisco activity to increases in [CO_2] (Sharkey et al. 2007). Since we measured P_R under 500 ppm, the CO_2:O_2 ratio could have already been high and the increase of another 200 ppm in the leaf cuvette would have little or no effect on it. Thus, we have no evidence to conclude that, in the circumstances of this study, the suppression of P_R is involved in A_{sat} increases.

The direct stimulation of photosynthesis associated with decreasing g_{sw} under eCO_2 has been widely reported under similar semi controlled-environment conditions (i.e. Norby et al. 1995, Ainsworth and Long 2005) and implies greater photosynthates supply usually followed by greater leaf respiratory metabolism (Ainsworth and Long 2005, Leakey et al. 2009). Differences in leaf R rates under eCO_2 are often considered the results of indirect effects of carbon uptake, as respiration itself seems to be insensitive to CO_2 concentration (Amthor 2000, Gonzàlez-Meler et al. 2001). While the response of A_{sat} and g_{sw} partially confirmed our initial hypothesis, R_{dark} decreased mediated by eCO_2, contrary to our hypothesis that it would increase under eCO_2 and high-P supply. What could explain this opposite response for R_{dark}? At first, we assumed that respiratory metabolism would increase, in response to either a higher supply of substrate for respiration (i.e. more photosynthates from increased A_{sat}) or enhanced need for respiratory products, such as ATP, reductants (i.e. NADPH), and carbon skeletons (Wullschleger et al. 1994). In fact, the rate at which these compounds are used to meet leaf demands, such as maintenance, transport, and nutrient uptake, could better regulate respiratory metabolism than the greater substrate supply (Amthor 1994). Although our result showed lower R_{dark} rates, which are usually associated with reduced rates of photosynthesis and foliar nutrient content, reduced rates of respiration can also be explained by lower constructions and maintenance costs mediated by eCO_2-induced leaf composition changes (i.e. protein and nutrient dilution; Wullschleger et al. 1992, 1994, Atkin et al. 2006).

The significant reduction in the R_{dark}:A_{sat} ratio, under both CO_2 and P treatments, indicates more carbon use efficiency in leaf metabolism. Moreover, correlation analysis

suggests that R_{dark}:A_{sat} was impacted to a greater extent by changes in R_{dark} in comparison to A_{sat}, suggesting a higher sensitivity of R_{dark} to eCO_2 and P addition than A_{sat}. In this case, respiratory processes would play a more important role in determining leaf carbon balance than assimilatory ones, bringing to light the importance of measurements of CO_2 as a whole in plant metabolism. Indeed, in tropical environments where natural P availability is low, nutrient supply has been suggested to be more important in determining R_{dark} than A_{sat} (Rowland et al. 2016). In addition, accounting for the importance of leaf respiration to whole-plant carbon balance, the reduction in this ratio indicate that *I. edulis* seedlings have more carbon to allocate to vital processes other than those that take place in the leaves, such as whole-plant metabolism and biomass production.

Relationships between carbon loss processes at the leaf level

The mechanisms that underline the metabolism of R_{light} are still not fully understood (Tcherkez and Atkin 2021), but most studies have found a consistent relationship between the two types of leaf R, with R_{light} being lower than R_{dark} (i.e. Tcherkez et al. 2008, 2012, 2017). Although respiratory rates in the light tend to vary greatly, as a result of the conditions during measurements, previous studies using isotope labelling, which also accounts for internal re-fixation of CO_2, observed a higher frequency that R_{light} was lower than R_{dark} (i.e. McCashin et al. 1988, Pärnik and Keerberg 1995). In our study, however, we did not find a close relationship between the two types of R rates (Figure S2A and S2B), suggesting that light and dark respiratory metabolisms were loosely linked, which is highly unlikely. In addition, in 37.5% of the seedlings light seems to stimulate leaf respiration (i.e. $R_{light} > R_{dark}$). Few studies have found similar results, suggesting an association between R_{light} and the amount and type of photosynthates available in the leaf for immediate consumption (Pärnik et al. 2002, Griffin and Turnbull 2013), in which high R_{light} rates were associated to the consumption of primary (i.e. triose-phosphate) and stored photosynthates (Pärnik et al. 2002). In the light, both types of photosynthates are used as substrates for respiratory and photorespiratory metabolisms. However, starch consumption seems to be inhibited by light (Atkin et al. 2006), which results in lower R_{light}:R_{dark} ratios in plants that synthesize more starch than those who synthesize more sucrose. For these plants, the total rate of respiratory decarboxylations in the light (primary + stored photosynthates) can be equal or even higher than R_{dark} (Pärnik et al. 2002, Hurry et al. 2005). Even though we cannot test this association, as we did not measure neither primary nor stored photosynthates, this hypothesis merits further investigation. Moreover, Griffin and Turnbull 2013 observed that R_{light}:R_{dark} ratios in wheat leaves were very close to a value that

suggested that R was not suppressed by light but may have been slightly stimulated when measured at leaf temperature (T_{leaf}) of 30ºC (the same used in this study), which implies that at high T_{leaf} more respiratory products would be needed to support enhanced A_{net}. If this was the case for our study, then R_{light} would have to be strongly associated to A_{sat}, which we did not observe (R^2=0.01, p-value= 0.24).

Considering the low R_{dark} values (Table 2) and that light inhibition of leaf respiration can vary from 0% to 80% according to environmental conditions (Atkin et al. 2000, Hurry et al. 2005), actual R_{light} values could be lower than the detection sensibility of the IRGA used in this study and our measurements of R_{light} may not have reflected the real values of R_{light}. Another explanation could be that there is not, in fact, light inhibition of R in these seedlings, suggesting that the low light environment in which the *I. edulis* seedlings grew would play an important role in explaining our findings. Considering that in the understory, sunflecks (brief, intermittent periods of high photosynthetic photon flux density) can represent 10–80% of the total photosynthetic light available for photosynthesis (Pfitsch and Pearcy 1989, Chazdon and Pearcy 1991, Leakey et al. 2005), R rates of the seedlings would have to be very low to guarantee a positive carbon balance. Light compensation points (the light intensity where photosynthetic carbon uptake matches carbon release from cellular respiration; LCPs) are good indicators of metabolic costs of basal metabolism and the degree of plant adaptation to light availability (Bellasio et al. 2016), in which plants in shaded environments tend to present lower LCPs, as an indication of greater efficiency in carbon assimilation. Indeed, estimated LCPs for these seedlings were very low, which, beyond mirroring the low light availability in which these seedlings grew, could also be an evidence of the lack of light inhibition in leaf R, with R_{light} rates close to R_{dark}. In both situations, light inhibition of R would be most dependent on underlying rates of R_{dark}, as an indication of the mitochondrion ability to release CO_2 through the tricarboxylic acid cycle (TCA cycle) (Ayub et al. 2011).

Pairwise relationships between R_{light}, R_{dark} and P_R revealed a lack of consistency in association between these response variables, in which treatment-driven variations of P_R did not play a predictive role, whether positive or negative, in light inhibition of R nor in R_{light}:R_{dark} ratio. The absence of evidence to support the hypothesis that respiratory fluxes would be impacted not only by eCO_2, but also by possible links with P_R might come from the lack of a significant treatment effect on P_R or that were unable to accurately detect R_{light} fluxes as previously raised.

Phosphorus limitation on aboveground carbon allocation

Our last hypothesis focused on the responses of aboveground organs to eCO_2 and to what extent soil P supply could impact such responses. We observed that P addition induced greater aboveground carbon allocation, while eCO_2 did not affect these parameters, suggesting that P supply limited *I. edulis* allometric responses to eCO_2. For instance, unlike previous studies (Norby et al. 1995, Ainsworth and Long 2005), here we did not find a significant effect of eCO_2 on TLA (Figure 3C), revealing a strong P-dependence in leaf growth even under eCO_2. In fact, the lack of P constrains leaf development, reducing leaf initiation, expansion and growth, which can reduce light interception and plant growth (Fredeen et al. 1989, Chiera et al. 2002). In the same way, seedlings exposed to eCO_2 did not show a significant increment in height (Figure 3A) or diameter (Figure 3C). These results denote that without enough nutrient supply, plants may not respond as expected to eCO_2, confirming the P limitation hypothesis. In the understory, where light seems to be the most limiting resource (Pearcy, 1983), plants are more likely to invest in height and/or leaf area driven by light search, which was observed in seedlings under high-P supply only. In fact, overall seedlings' H showed a significant positive relationship with D (R^2 = 0.1, *p-value* = 0.017) and TLA (R^2=0.4, *p-value* < 0.001; Figure S5A and B). If the absence of an eCO_2 effect on aboveground development under low-P supply repeats itself for other species and ontogenetic stages, it may have important future implications for limiting the CO_2 fertilization effect and carbon sinks in forests with low P natural availabilities.

The seedlings that showed the greatest photosynthetic rates and lower respiratory rates (lower R_{dark}: A_{sat} ratios) were not necessarily the ones that showed greater aboveground development. The extra carbon available was not allocated to aboveground development in seedlings exposed to eCO_2 and low-P supply. This could be explained by different patterns in carbon allocation driven by different soil nutrient availability. In fertile soils, plants allocate more carbon to aboveground growth and invest proportionally more of their photosynthates in plant biomass production (Vicca et al. 2012). On low-P soils, plants tend to invest more carbon on strategies to enhance P acquisition, which can lead to lower aboveground development, as observed in our results. Initially, P limitation can enhance root over shoot growth, increasing root:shoot ratios (Pandey et al. 2015). Root growth, especially fine roots, can increase P acquisition efficiency by investing in greater length and specific areas, thus broadening soil exploration (Kong et al. 2016, Lugli et al. 2020). Other strategies related to enhancing P acquisition can be the exudation of organic acids (carbon compounds that promote the direct liberation of inorganic P - P_i - from soil particles; Hinsinger 2001), phosphatases (enzymes

responsible for hydrolyzing organic P and turning it into Pi; Treseder and Vitousek 2001) and more carbon allocation to mycorrhizal symbionts (Jones et al. 2004). Since we did not measure belowground investment as part of this study, we cannot rule out the effects of differential above/belowground allocation between P availability treatments. Our results suggest that the low aboveground development observed in this study can represent a key role of P availability in carbon allocation of plants growing under eCO_2.

Conclusion

Our results show a clear pattern between the responses of physiological and allometric variables of *I. edulis* seedlings to CO_2 and P treatments. Elevated CO_2 showed significant effects on physiological responses whereas high-P supply affected significantly mostly allometric responses, although some allometric variables also tended (i.e. non-significant statistical effect) to respond positively to eCO_2. In all cases, the lack of evidence of an interaction between both treatments, as well as the P effect only on allometry, suggest that this nutrient may have a differential impact on the responses of plants to eCO_2, depending on the nature of the processes. As P limited aboveground size and growth of the seedlings, even under eCO_2, if other species in the region present similar growth patterns, it could indicate major implications to C sink activity in the future. Therefore, more comprehensive studies, accounting for the diversity of species and environments in the Amazon rainforest, merit further investigation.

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Figures and tables

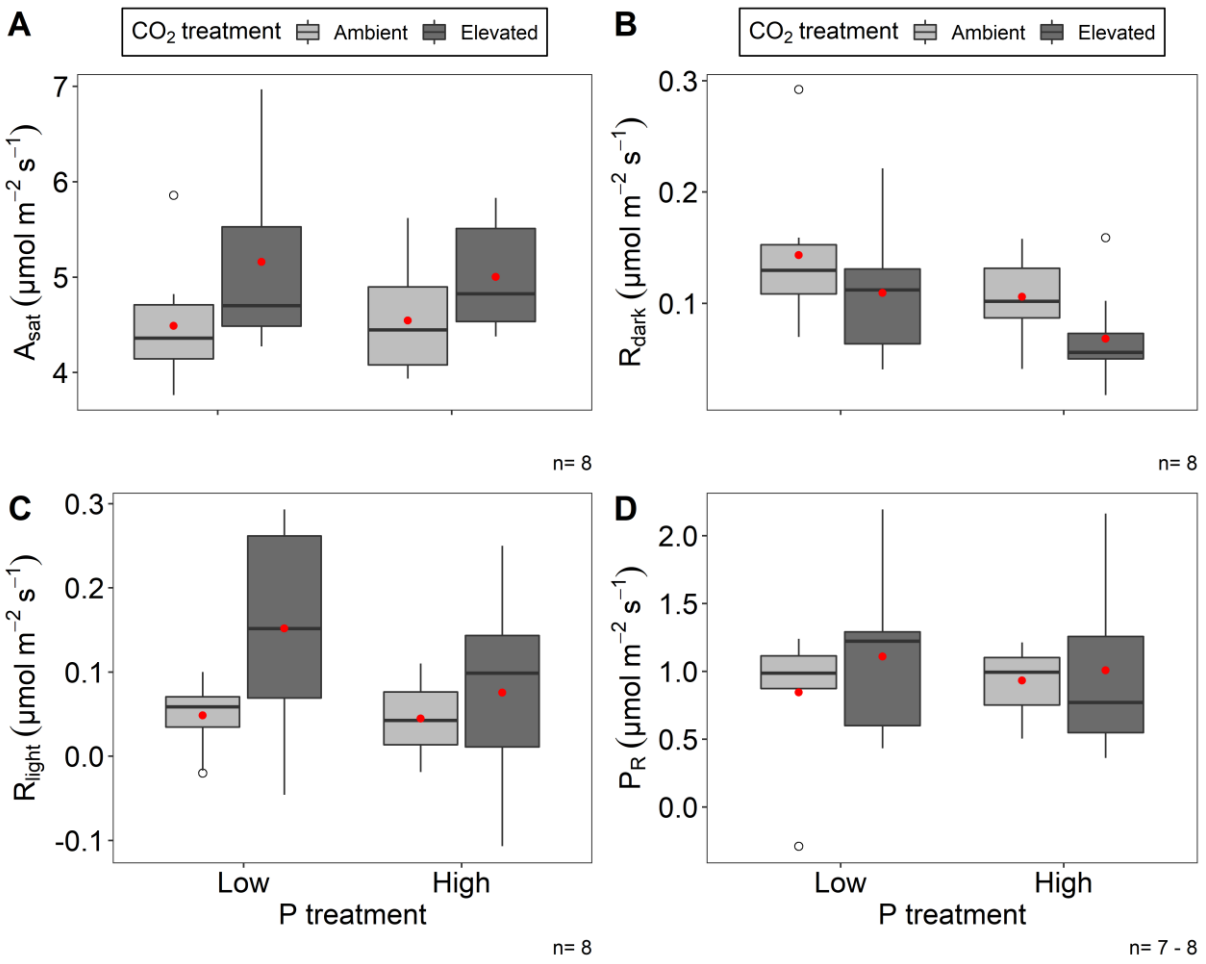

Figure 1 Boxplots showing the responses of leaf carbon metabolism for CO_2 and P treatments. **A)** Light-saturated net photosynthetic CO_2 assimilation rate (A_{net}), **B)** Leaf respiration in the darkness (R_{dark}), **C)** Leaf respiration in the light (R_{light}) and **D)** Photorespiration (P_R). The boxes indicate the interquartile range and median (black solid lines) for each treatment. Red points are mean values and open circles are outliers (observations outside whiskers). The boxes in light grey indicate low-P treatment (natural soil phosphorous availability) and in dark grey, high-P treatment (phosphorous addition). Text at the bottom right of each plot indicates the number of repetitions (n) for each treatment.

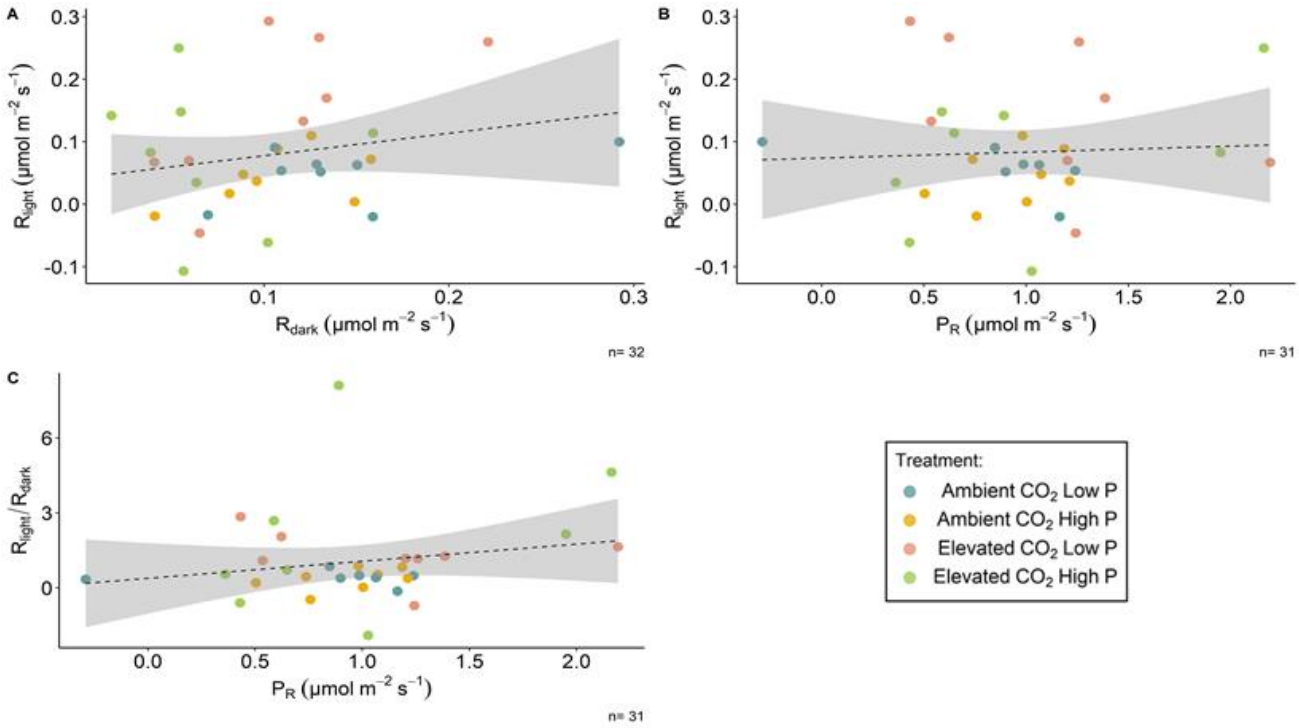

Figure 2 Pairwise relationships between physiological variables. **A)** Leaf respiration in the light (R_{light}) and leaf respiration in the darkness (R_{dark}), **B)** R_{light} and photorespiration (P_R) and **C)** Ratio R_{light}:R_{dark} and P_R. The number of points are equal to the number of observations (n = 31-32). Colored points indicate different treatments as specified in the legend. Black dashed lines indicate lack of a significant effect between the variables. Grey shaded regions indicate the standard error (SE) of the statistical model used (linear regression).

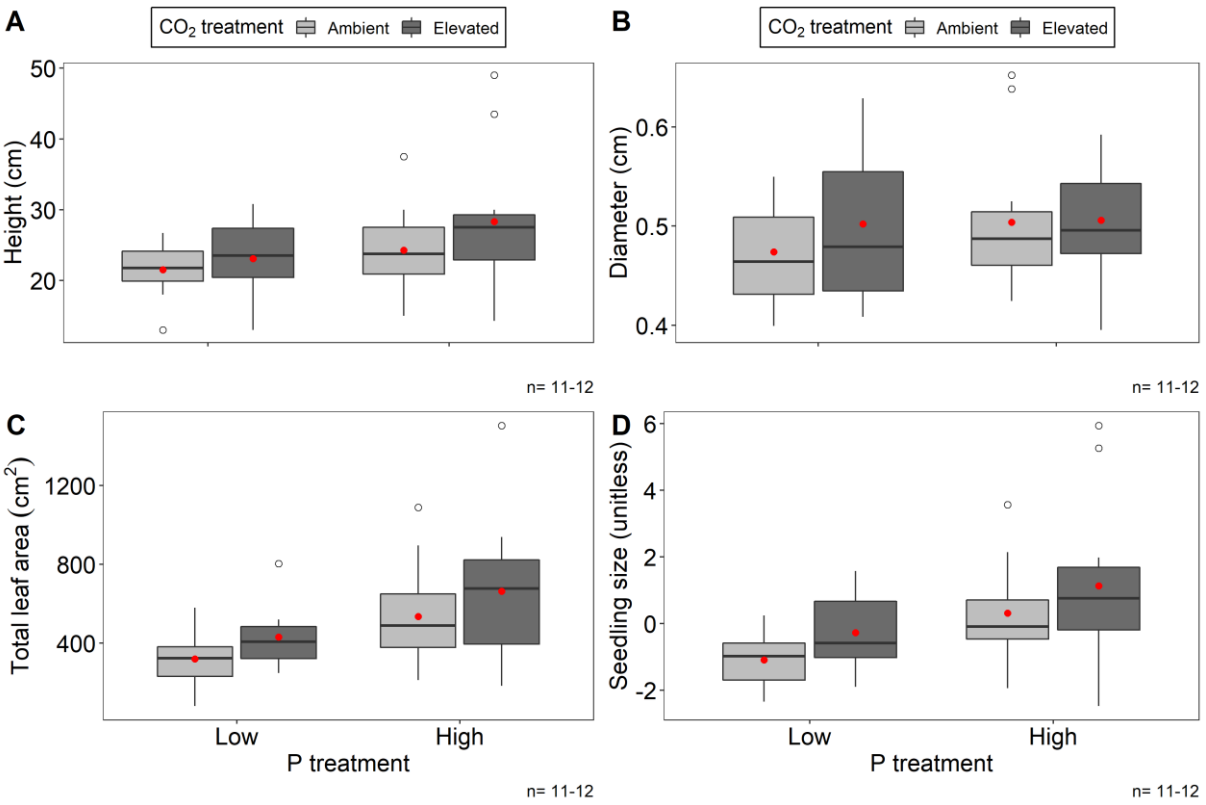

Figure 3 Boxplots showing the responses of aboveground organs (stem and leaves) and aboveground size for CO_2 and P treatments. Variables shown are: **A)** Height, **B)** Diameter, **C)** Total leaf area and **D)** Seedling size. The boxes indicate the interquartile range and median (black solid lines) for each treatment. Red points are mean values and open points are outliers (observations outside whiskers). The light grey boxes indicate low-P treatment (natural soil phosphorous availability) and dark grey, high-P treatment (phosphorous addition). The text at the bottom right of each plot indicates the number of repetitions (n) for each treatment.

Table 1 Environmental data of each open-top chamber (OTC), according to CO_2 treatment. Values shown in columns are means ± 1sd. Symbols next to the OTC identification indicate pairwise connected OTCs. (ambient and elevated CO_2).'TSRT' stands for Total Solar Radiation Transmitted and 'VWC', Volumetric Water Content. TSRT and VWC were averaged from measurements made on the six pots (n = 6) inside each OTC (expect for OTC 1, where n = 4).

Open-top chamber (OTC) ID	CO_2 treatment	Geographical coordinates (latitude/ longitude)	VWC (% volume)		TSRT (mol m^{-2} day^{-1})	
			Mean	SD	Mean	SD
1¶	Ambient	2º 35'44.12''S	13.81	4.81	2.37	0.51
2¶	Elevated	2º 35'43.92''S	13.80	3.43	1.68	0.63
X§	Ambient	2º 35'41.41''S	20.70	3.16	7.91	1.94
5§	Elevated	2º 35'41.77''S	21.73	5.18	6.22	5.28
4£	Elevated	2º 35'43.27''S	19.37	1.63	3.17	0.86
8£	Ambient	2º 35'45.86''S	18.34	1.67	2.00	0.68
Y¥	Elevated	2º 35'46.74''S	18.86	3.36	4.44	1.80
9¥	Ambient	2º 35'47.14''S	17.87	1.65	3.84	0.95

Table 2 Variable responses of each combination of CO_2 and P treatment. Values shown in columns are means ± 1 sd. Values in column '%Δ' are the percentage change of response relative to the control treatment (Ambient CO_2/Low-P). 'N' stands for the number of repetitions for each measurement. For variable abbreviations, see Material and Methods section.

Response variable	Ambient CO_2			Elevated CO_2				N
	Low P	High P	%Δ	Low P	%Δ	High P	%Δ	
A_{sat} (µmol $m^{-2}s^{-1}$)	4.486 ± 0.667	4.541 ± 0.588	1.23	5.154 ± 1.013	14.93	4.995 ± 0.596	11.40	8
g_{sw} at A_{sat} (µmol $m^{-2}s^{-1}$)	0.150 ± 0.046	0.144 ± 0.030	-3.67	0.132± 0.031	-11.8	0.113 ± 0.043	-24.84	8
R_{dark} (µmol $m^{-2}s^{-1}$)	0.143 ± 0.066	0.106 ± 0.038	-26.06	0.109 ± 0.057	-23.71	0.068 ± 0.044	-52.33	8
R_{light} (µmol $m^{-2}s^{-1}$)	0.048 ± 0.045	0.045 ± 0.044	-7.49	0.152 ± 0.119	213.69	0.075 ± 0.117	56.07	8
P_R (µmol $m^{-2}s^{-1}$)	0.844 ± 0.519	0.932 ± 0.246	10.37	1.109 ± 0.576	31.40	1.007 ± 0.686	19.32	8
R_{dark}/A_{sat}	0.031 ± 0.009	0.024 ± 0.009	-23.78	0.021 ± 0.010	-30.87	0.013 ± 0.007	-57.11	8
R_{light}/R_{dark}	0.330 ± 0.356	0.357 ± 0.437	8.25	1.322 ± 1.014	300.43	2.043 ± 3.169	518.99	8
Height (cm)	21.018 ± 4.622	25.691 ± 6.137	22.23	22.175 ± 4.388	5.50	27.933 ± 9.348	32.9	11-12
Diameter (cm)	0.455 ± 0.05	0.504 ± 0.068	10.91	0.493 ± 0.060	8.37	0.53± 0.06	16.34	11-12
H:D ratio	46.846 ± 12.047	50.821 ± 10.119	8.48	45.042 ± 7.621	-3.85	53.277 ± 18.856	13.73	11-12
Crown height (cm)	9.045 ± 3.382	14.655 ± 6.406	62.01	9.125 ± 3.276	0.88	14.208 ± 8.729	57.08	11-12
Crown diameter (cm)	24.136 ± 6.233	31.727 ± 5	31.45	32.542 ± 4.746	34.82	34.75 ± 9.087	43.97	11-12
CH:CD ratio	0.395 ± 0.168	0.468 ± 0.199	18.44	0.280 ± 0.094	-29.01	0.405 ± 0.189	2.56	11-12
Number of leaves (#)	6.00 ±1.265	8.000 ± 1.949	32.31	8.083 ± 2.392	26.92	9.000 ± 2.629	46.66	11-12
Mean leaf area (cm^2)	53 ± 21.915	61. 115 ± 19	15.30	55.574 ± 9.498	4.85	78.076 ± 23.474	47.3	11-12
Total leaf area (cm^2)	308.174 ± 151.615	498.127 ± 269.662	60.64	418.672 ± 114.985	35.86	697.605 ± 343.14	126.37	11-12
Leaf thickness (mm)	0.113 ± 0.006	0.111 ± 0.004	-1.48	0.116 ± 0.008	2.87	0.112 ± 0.005	-0.93	11-12
Relative growth rate (cm day^{-1})	0.064 ± 0.011	0.073 ± 0.017	22.78	0.069 ± 0.016	5.04	0.084 ± 0.03	32.36	11-12

Table 3 Statistical significance of predictors used in the models. Significant p-values ($p \leq 0.05$) are indicated in bold. '-' indicates interaction and/or covariates not included in the selected model. All models consider the same random effects structure (OTCs and parent tree). 'R^2_{fixed}' stands for the coefficient of determination of fixed effects and 'R^2_{random}' stands for the coefficient of determination of random effects. 'R^2_{model}' stands for the coefficient of determination including fixed and random effects. 'TSRT' stands for Total Solar Radiation Transmitted (mol m^{-2} day^{-1}) and 'VWC', Volumetric Water Content (%). For other variable abbreviations, see Material and Methods section.

Response variable	Interaction	Main effects		Covariates		R^2_{fixed}	R^2_{random}	R^2_{model}	Probability distribution	Link function
		CO_2	P	TSRT	WVC					
A_{sat} (µmol m^{-2} s^{-1})	-	**0.005**	0.926	-	**0.014**	0.301	0.007	0.308	Gamma	log
g_{sw} at A_{sat} (µmol m^{-2} s^{-1})	-	0.074	0.297	-	**0.003**	0.311	0.051	0.362	Gamma	log
R_{dark} (µmol m^{-2} s^{-1})	-	**0.045**	0.067	**0.013**	-	0.393	0.155	0.548	Gamma	log
R_{light} (µmol m^{-2} s^{-1})	-	**0.024**	0.177	-	-	0.181	0.016	0.0197	Gaussian	identity
P_R (µmol m^{-2} s^{-1})	-	0.492	0.933	-	-	0.028	0.264	0.292	Gaussian	identity
R_{dark}/A_{sat}	-	**0.011**	**0.019**	**0.013**	-	0.465	0.221	0.686	Gamma	log
R_{light}/R_{dark}		**0.017**	0.501	-	-	0.1651	0.0001	0.1652	Gaussian	identity
Height (cm)	-	0.932	**0.004**	-	-	0.072	0.537	0.609	Gamma	log
Diameter (cm)	-	0.685	0.268	-	-	0.027	0.094	0.121	Gamma	log
Number of leaves (#)	-	0.073	0.060	-	-	0.124	0.009	0.133	Poisson	log
Total leaf area (cm^2)	-	0.0773	**<0.001**	-	-	0.347	0.296	0.643	Gamma	log
Seedling size (unitless)	-	0.311	**<0.001**	-	-	0.207	0.330	0.537	Gaussian	identity
Relative growth rate (cm day^{-1})	-	0.902	**0.004**	-	-	0.075	0.527	0.602	Gamma	log

Supporting Information

A

B

Figure S1 Photographs of the study area: **A)** Photograph of an OTC, showing the steel-polypropylene structure and distribution of pots inside one of the OTCs **B)** Photograph of a pot inside one of the open top chambers (OTCs), on day 160 of the experiment .

Notes S1 Estimation of light saturation point (LSP)

Gas exchange and chlorophyll fluorescence measurements

We made light response curves to estimate the light saturation point (LSP) on three seedlings in three of the four treatments (Ambient CO_2/Low P, Ambient CO_2/High P and Elevated CO_2/Low P), using an open gas exchange system (Li-Cor 6800; Li-Cor Inc., Lincoln, NE, USA) with a 6cm² integrated fluorescence chamber head (Li-Cor 6800 Leaf Chamber Fluorometer). All the measurements were carried out as described in the section 'Gas exchange and chlorophyll fluorescence measurements' of Material and Methods, with air flow rate of 700 $\mu molm^{-2}\ s^{-1}$ and ambient O_2 (21%O_2). We acclimated leaves under irradiance of 700 μmol photon $m^{-2}s^{-1}$ for at least 30min and decreased photosynthetically photon flux density (PPFD) stepwise (700, 600, 500, 400, 350, 300, 250, 200, 150, 100, 75, 50, 25, 0 μmol photon $m^{-2}s^{-1}$). For every light step (3-5 min), five parameters of stability (ΔCO_2, ΔH_2O, A, g_{sw} and F) were used. Steady-state fluorescence (Fs), light-adapted maximal fluorescence (Fm'), applying an instantaneous saturating light pulse (8000 μmol photon $m^{-2}\ s^{-1}$ for 0.5 s), and light-adapted minimal fluorescence (Fo'), applying a dark pulse, were measured simultaneously with GE measurements at every step of the curves. Dark-adapted maximal fluorescence (Fm) was also measured, covering the same leaves used in the curves with an aluminum paper for at least 30min. We then calculated the photochemical quenching (qP) as qP= (Fm'-Fs)/(Fm' – Fo'),

used as an estimate of the proportion of photosystem II (PSII) open centers, which indicates that light energy is being used to photochemistry. Non-photochemical quenching (NPQ) was calculated as NPQ = (Fm/Fm')-1, and used as an estimate of the apparent rate of constant heat loss from PSII or the dissipation of light energy that is being lost through heat (Baker 2008).

Methods used to estimate the light saturation point

We used three different methods to estimate LSP. First, we estimated it visually as the light point where the relationship between net CO_2 assimilation (A) and photosynthetic photon flux density (PPFD) stops being linear and becomes curvilinear, where there are not significant changes in A (Figure S1A). Second, we estimated LSP by plotting the photochemical quenching (qP) against the non-photochemical quenching (NPQ), where LSP is the PPFD point in which qP and NPQ curves cross (Pimentel et al. 2011; Figure S1B). At last, we used the nine Microsoft Excel spreadsheets provided by Lobo et al. (2013) to fit A-PPFD curves. Each spreadsheet is linked to a mathematical model to estimate LSP. Therefore we chose the model that yielded the smallest error (Sum of the Squares of the Errors; SSE) as the most appropriate to describe our data (Non-Rectangular Hyperbola). Since LSP estimates varied little between methods (Table S1), we chose the highest value found using qP x NPQ plots (250 $\mu mol\ mol^{-1}\ s^{-2}$).

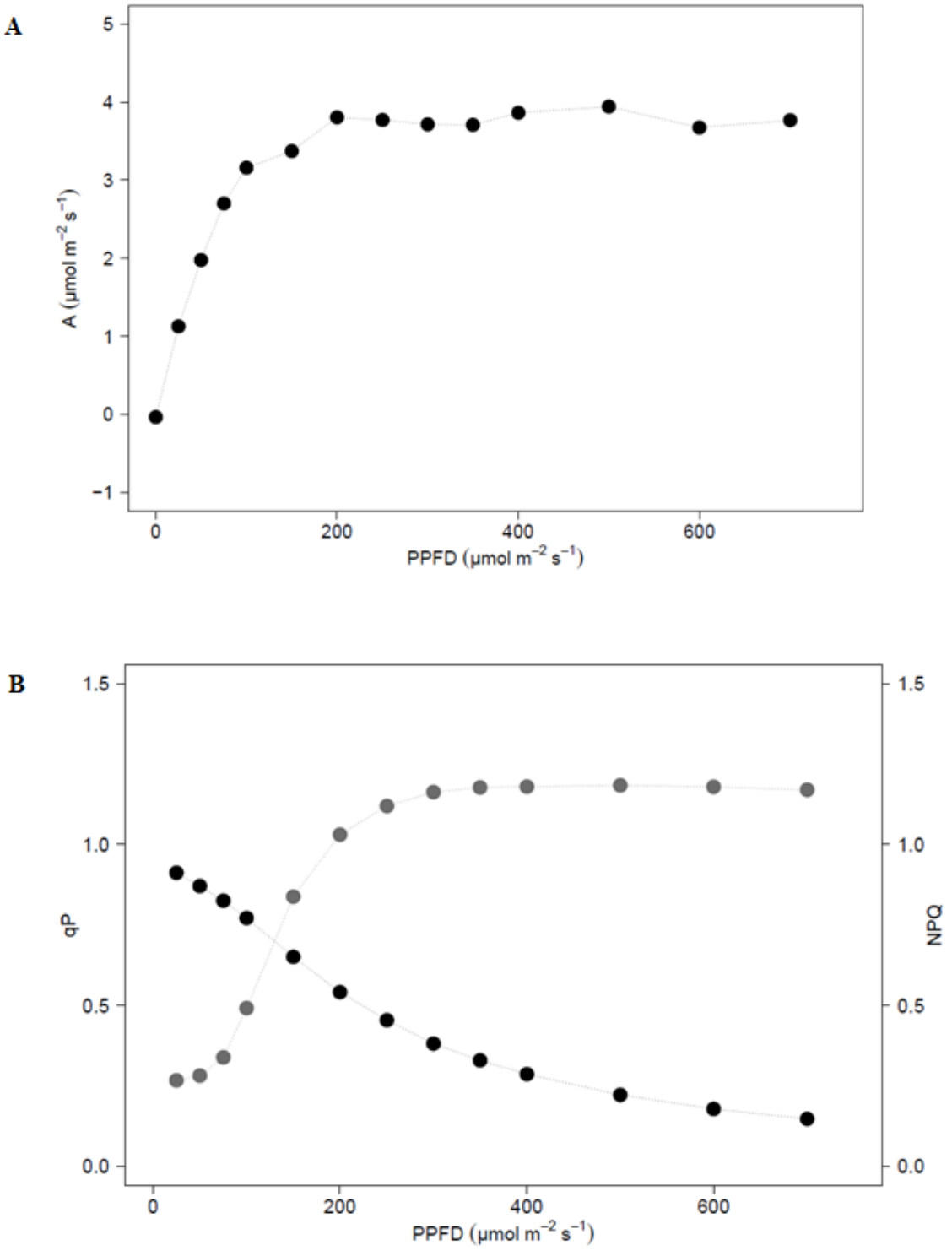

Figure S2. Representative plots of **A)** Net CO_2 assimilation (A, µmolCO_2 mol^{-1} s^{-2}) versus photosynthetically photon flux density (PPFD, µmol photon mol^{-1} s^{-2}). **B)** Photochemical quenching (qP) versus non-photochemical quenching (NPQ) versus PPFD (µmol photon mol^{-1} s^{-2}). Black points indicate qP values and grey points, NPQ values. Data are from one seedling, exposed to Ambient CO_2 and low-P supply (Control).

Table S1. Comparison of the three different methods to estimate light saturation point (LSP, μmol photon mol^{-1} s^{-2}). Light response curves were made on three seedlings of three treatments as described in the column 'Treatment'

Treatment	Pot	Light curve shape	qP x NPQ (Pimentel *et al*. 2013)	Non-Rectangular Hyperbola (Lobo *et al*. 2013)
Ambient CO_2 / Low-P (Control)	17	100-200	170	237
	31	100-200	170	159
	35	100-200	190	230
Ambient CO_2 / High-P	18	100-200	170	137
	32	100-200	150	181
	36	100-200	200	208
Elevated CO_2 / Low-P	7	100-250	250	315
	19	100-250	150	232
	21	100-250	150	220

Notes S2 Decision on the air flow rate for leaf respiration measurements

Considering that respiratory fluxes are at least two to six times lower than assimilatory ones (Taiz et al. 2006), we reduced the air flow rate inside the chamber to increase ΔCO_2 between reference and leaf sample. We made light curves using the same parameters described on section 'Gas exchange and chlorophyll fluorescence measurements' of Material and Methods, using ambient O_2 (21%O_2) and changing air flow rate (200, 300 and 400 μmolm^{-2} s^{-1}) (Figure S2). We acclimated leaves under irradiance of 80 μmol $m^{-2}s^{-1}$ PPFD for at least 30min and decreased photosynthetically photon flux density (PPFD) stepwise (80, 70, 60, 50, 40, 30, 25, 20, 15, 10, 5 μmol photon $m^{-2}s^{-1}$). At every light step, we allowed the stability parameters to reach steady-state (2-3min) and also measured steady-state fluorescence (Fs) and light-adapted maximal fluorescence (Fm') as we applied a saturating light pulse (pulse (8000 μmol photon $m^{-2}s^{-1}$ for 0.5 s). We then calculated PSII electron (e^-) transport efficiency (Φ_{PSII}) as $\Delta F/Fm' = (Fm'-Fs)/Fm'$ (Genty *et al.* 1989) and plotted the linear regression of A against (PPFD * $\Phi_{PSII}/4$) (Yin et al. 2009, 2011). These curve tests were made on the same seedlings used on the tests to estimate LSP. Our decision on the best air flow rate relied on comparison of the coefficients of determination (R^2), residual standard errors (SE) and percentage of leakage (averaged from all points of each curve) (Table S2). We chose the flow rate that yielded the highest R^2, lowest SE and lowest leakage (300 μmolm^{-2} s^{-1}).

Table S2. Values of coefficient of determination (R^2), Residual Standard Error (SE) and mean leak percentage (%) of the linear regressions obtained by plotting A against (PPFD * Φ_{PSII}/4) (Yin et al. 2009, 2011).

Treatment	Pot	Flow rate ($\mu mol\ m^{-2}\ s^{-1}$)	R^2	SE	Mean leak percentage (%)
Ambient CO_2 / Low-P (Control)	17	200	0.99	0.05	11
		300	0.99	0.07	7
		400	0.91	0.2	0
	31	200	0.99	0.05	12.6
		300	0.99	0.06	1
		400	0.99	0.07	0
	35	200	0.98	0.15	5
		300	0.99	0.06	0
		400	0.99	0.08	0
Ambient CO_2 / High-P (Control)	18	200	0.99	0.07	10
		300	0.99	0.08	4
		400	0.98	0.12	0
	32	200	0.99	0.08	7.3
		300	0.99	0.07	0
		400	0.99	0.07	0
	36	200	0.99	0.05	22
		300	0.99	0.03	0
		400	0.98	0.08	0
Elevated CO_2 / Low-P	7	200	0.98	0.09	6.85
		300	0.99	0.08	0
		400	0.99	0.14	0
	19	200	0.99	0.05	15
		300	0.98	0.14	8
		400	0.99	0.09	2
	21	200	0.99	0.06	5.01
		300	0.98	0.1	0
		400	0.98	0.13	0

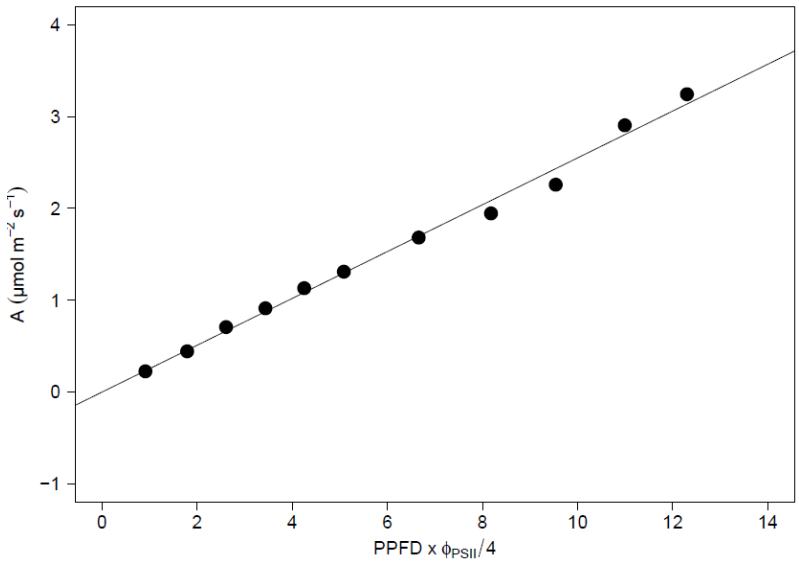

Figure S3 Representative plot of A against (PPFD * Φ_{PSII}/4). Data are from one seedling, exposed to Ambient CO_2 and low-P supply (Control).

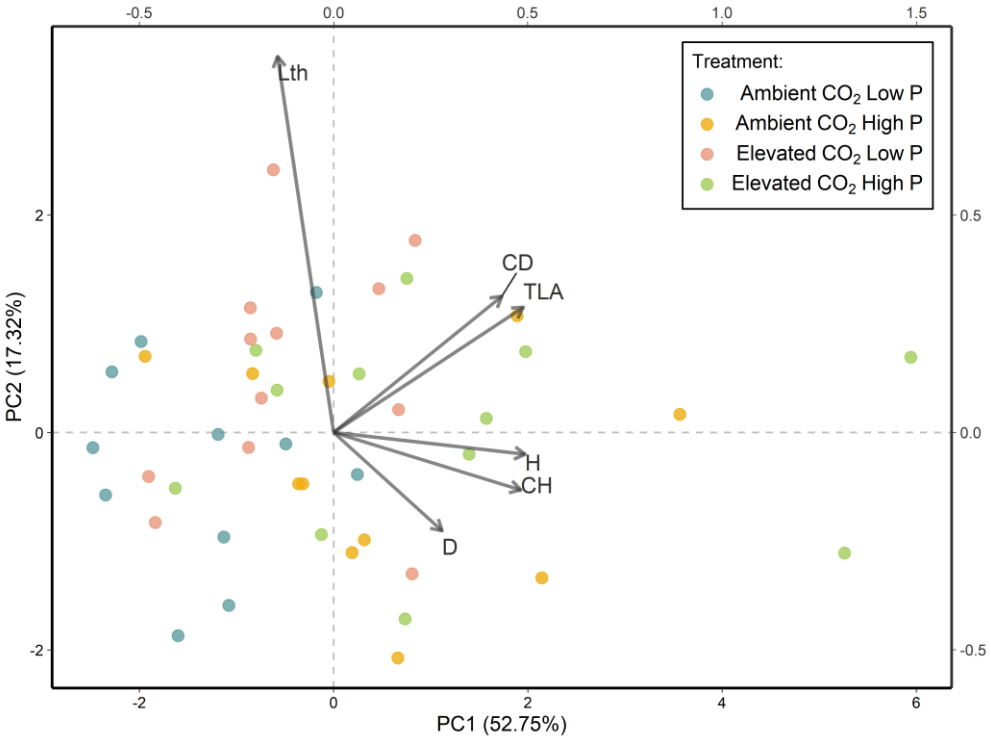

Figure S4 Principal Component Analyses (PCA) of allometric variables linked to carbon allocation. Variables used are: height (H), diameter (D), crown height (CH), crown diameter (CD), total leaf area (TLA) and leaf thickness (Lth). Colored points indicate different treatments as specified in the legend.

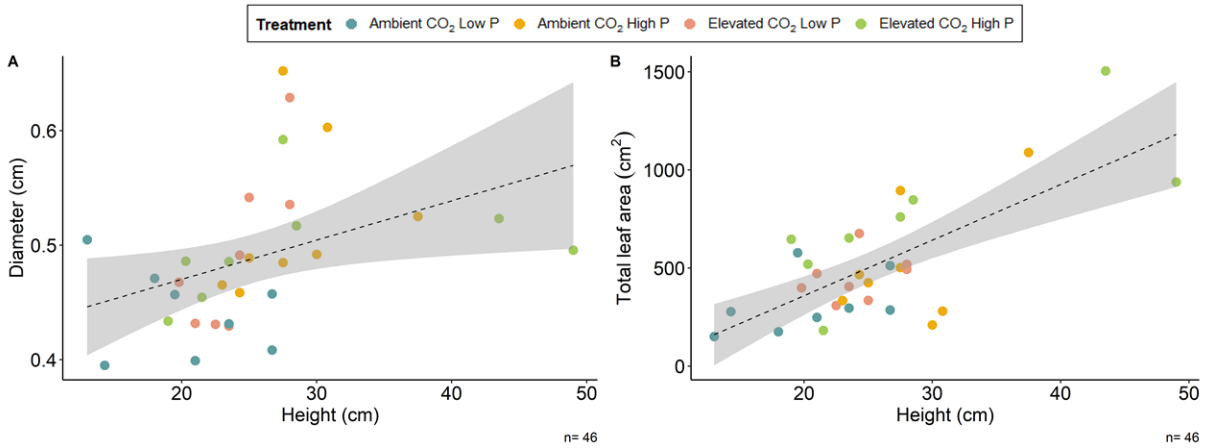

Figure S5. Pairwise relationships between allometric variables. **A)** Diameter (D) and height (H) **B)** Total leaf area (TLA) and H. The number of points are equal to the number of observations (n = 46). Colored points indicate different treatments as specified in the legend. Black solid lines indicate a significant effect between the variables. Grey shaded regions indicate the standard error (SE) of the statistical model used (linear regression).

CONCLUSÃO

Nossos resultados indicam que a adição de P impacta as respostas de desenvolvimento acima do solo em condições de eCO_2, mas não afeta as respostas fisiológicas, que são afetadas por eCO_2 independentemente de P. Em suma, esse estudo adiciona evidência experimental à hipótese de que, sem a quantidade necessária de P disponível no solo, espécies podem não responder a eCO_2 como esperado pelos atuais modelos. Se tais padrões se repetirem em outras espécies da mesma região, isso poderia representar grandes mudanças na ciclagem global de carbono, com impactos no sequestro de carbono pela região. Assim, estudos mais abrangentes que levam em consideração tanto a diversidade de espécies quanto de ambientes na floresta Amazônia merecem maior atenção.

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