



**UNIVERSIDADE ESTADUAL DE SANTA CRUZ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PÓS-GRADUAÇÃO EM ECOLOGIA E CONSERVAÇÃO DA BIODIVERSIDADE**

CAROLINA REIS DE BRITO

**TERMOTOLERÂNCIA E CONSERVAÇÃO DE PLANTAS LENHOSAS TROPICAIS
NUM CENÁRIO DE MUDANÇAS CLIMÁTICAS**

ILHÉUS - BAHIA

2024

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Tese apresentada à Universidade Estadual de Santa Cruz,
como parte das exigências para obtenção do título de
Doutor em Ecologia e Conservação da Biodiversidade.
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Ilhéus, 23 de fevereiro de 2024.

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"Science is the poetry of reality".

Richard Dawkins

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Lembro que quando entrei na graduação eu sonhava muito com o título de doutorado. Eu olhava para meus colegas doutores ou doutorandos e admirava tanto o quanto eles eram incríveis! Na época eu mal tinha ideia de verdade o que era ser um doutor, não sabia o que era a pesquisa. Mas foi assim que fui descobrindo o mundo da pesquisa, das perguntas, das discussões acadêmicas, e foi assim que eu me apaixonei pelo que faço hoje. Agora estou encerrando minha formação e nem tão movida pelo título, mas sim pelo gosto na pesquisa. Ainda assim, chega a ser estranho estar agora nessa “fase final”. Foi um longo percurso, e por isso quero agradecer muito a tanta gente por ter me ajudado nessa trajetória. Para quem não sabe, eu passei o ano de 2019 namorando despreziosamente vários laboratórios, fazendo várias campanhas de campo e cursos a fim de descobrir a onde eu faria o meu tão sonhado doutorado. No Rio de Janeiro, cidade onde eu nasci e triei o início da minha carreira acadêmica, eu sentia que já tinha conhecido um pouco de tudo, por isso eu queria ir além dos limites do estado. Foi assim que eu parei em setembro do mesmo ano em Ilhéus, na Bahia e me apaixonei pelo grupo de pesquisa e pelas árvores nativas. E foi assim que começou meu doutorado.

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Figure 2. Relationship between leaf traits and DLI in four different species. Gray - *Aspidosperma pyricollum*, red - *Goniorrhachis marginata* and yellow - *Handroanthus chrysotrichus*. (A) mesophyll area, (B) palisade thickness and (C) spongy thickness. Lines indicate the relationship according to analysis of covariance (ANCOVA, $p < 0.05$).

Figure 3. Relationship between leaf thermotolerance traits and DLI in four different species. Gray - *Aspidosperma pyricollum*, green - *Citharexylum myrianthum*, red - *Goniorrhachis marginata* and yellow - *Handroanthus chrysotrichus*. (A) T_{crit} ; (B) T_{50} . Lines indicate the relationship according to analysis of covariance (ANCOVA, $p < 0.05$).

Figure 4. Relationship between leaf traits and light transmission in *A. pyricollum* at two areas. Green: agroforestry (AGF) and red: restoration area (RA). (A) Leaf thickness and (B) T_{crit} . Lines indicate the relationship with p -value < 0.05 .

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The GM values, ranging from 0 to 5, denote varying degrees of shade intensity. Red line represents the T_{50} value, while the orange line, the T_{crit} within the graph.

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LISTA DE SIGLAS

AF – Atlantic Forest (Mata Atlântica)

A_n - Net photosynthetic rate (taxa de assimilação de CO₂)

$C_i:C_a$ – Atmospheric carbon ratio (taxa de carbono atmosférico)

$\delta^{13}\text{C}$ - Carbon isotopic ratio, ¹³C + ¹²C (razão de carbono isotópico)

F_v/F_m – Quantum efficiency of photosystem 2 (eficiência quântica máxima do fotossistema 2)

g_s – Stomatal conductance (condutância estomática)

J_{\max} - Electron transport rate (taxa de transporte de elétrons)

LMA – Leaf mass area (massa específica foliar)

PPFD - Photosynthetic photon flux density (fluxo de densidade de fótons fotossinteticamente ativos)

PSII - Photosystem 2 (fotossistema 2)

R_d – Dark respiration (taxa de respiração no escuro)

RR - Response ratio of means (razão de resposta das medias)

RSNo – Rosenthal's safe-number (número de segurança de Rosenthal)

T_{50} – temperature of decrease of 50% of F_v/F_m (temperatura de perda de 50% de F_v/F_m)

T_{crit} - temperature of the critical decline of F_v/F_m (temperatura do declínio crítico de F_v/F_m)

V_{cmax} – Rubisco carboxylation rate (velocidade de carboxilação da Rubisco)

RESUMO

A temperatura influencia o metabolismo das plantas, afetando sua fotossíntese, crescimento, germinação, floração, frutificação e compostos de defesa. Isso a torna um fator crucial na distribuição das espécies vegetais. As mudanças climáticas globais, impulsionadas pelo aumento do carbono atmosférico, já elevaram a temperatura média global em 1,1°C, com projeções de até 4,4°C em 2100. Essas mudanças resultam em eventos climáticos extremos, como ondas de calor que impactam as espécies de forma intensa e afetam seu metabolismo. A avaliação da resposta do balanço de carbono e a termotolerância foliar, medida pela queda na eficiência quântica máxima do fotossistema II (F_v/F_m) são ferramentas úteis para prever as respostas de espécies vegetais em relação as mudanças climáticas globais. Sendo assim, esta tese está composta por quatro capítulos: (1) uma metanálise do efeito da temperatura na fotossíntese em indivíduos jovens; (2) um experimento de termotolerância em espécies latino-americanas que ocorrem na MA; (3) avaliação da termotolerância em adultos de espécies de restinga; e (4) termotolerância em espécies brasileiras sob diferentes níveis de luz. Houve uma queda no balanço de carbono em resposta ao aumento da temperatura e uma baixa relação entre os atributos foliares e os índices de termotolerância. Além disso, foi demonstrado que a termotolerância de folhas tem uma relação positiva com o aumento da sombra, o que foi observado em experimento de viveiro e de campo. A plasticidade da termotolerância foliar foi notavelmente menor em comparação com outros atributos foliares e a termotolerância foi espécie-específica. Os nossos resultados sugerem que a termotolerância foliar é um critério interessante para a conservação das florestas tropicais no contexto das alterações climáticas globais

Palavras-chave: Eventos climáticos extremos; ondas de calor, sucessão ecológica, atributos foliares, espécies tropicais.

ABSTRACT

Temperature influences the metabolism of plants, affecting their photosynthesis, growth, germination, flowering, fruiting and defense compounds. This makes it a crucial factor in the distribution of plant species. Global climate change, driven by an increase in atmospheric carbon, has already raised the average global temperature by 1.1°C, with projections of up to 4.4°C by 2100. These changes result in extreme climatic events, such as heat waves, which have an intense impact on species and affect their metabolism. Evaluating the carbon balance response and leaf thermotolerance, measured by the drop in maximum photosystem II quantum efficiency (F_v/F_m), are useful tools for predicting the responses of plant species to global climate change. Therefore, this thesis is composed of four chapters: (1) a meta-analysis of the effect of temperature on photosynthesis in young individuals; (2) a thermotolerance experiment in Latin American species that occur in MA; (3) evaluation of thermotolerance in adults of restinga species; and (4) thermotolerance in Brazilian species under different light levels. There was a drop in carbon balance in response to the increase in temperature and a low relationship between leaf attributes and thermotolerance indices. In addition, leaf thermotolerance was shown to have a positive relationship with increasing shade, which was observed in nursery and field experiments. The plasticity of leaf thermotolerance was notably lower compared to other leaf attributes and thermotolerance was species-specific. Our results suggest that leaf thermotolerance is an interesting criterion for the conservation of tropical forests in the context of global climate change.

Keywords: Extreme weather events; heat waves, ecological succession, leaf attributes, tropical species.

INTRODUÇÃO GERAL

A atividade humana tem impactado profundamente a superfície terrestre, modificando ecossistemas naturais de forma rápida (KEYS et al., 2019; LEWIS; MASLIN, 2015). Procedimentos como o desenvolvimento do processo Haber–Bosch e o uso de combustíveis fósseis têm acarretado mudança drástica dos ciclos do nitrogênio e do carbono (LEWIS; MASLIN, 2015). De forma semelhante, a introdução de espécies exóticas, além da produção de híbridos e organismos geneticamente modificados, têm afetado o funcionamento de ecossistemas naturais (PENA RODRIGUES; LIRA, 2019). Esses impactos que alteram até mesmo ciclos biogeoquímicos e o processo evolutivo, alteram drasticamente ecossistemas, fazendo com que algumas espécies sejam mais favorecidas do que outras. Nesse sentido muitas populações de espécies nativas já diminuíram ou estão fadadas ao declínio (PENA RODRIGUES; LIRA, 2019).

Tal mudança no planeta é tão significativa que gerou o termo “Antropoceno”, a época geológica na qual o ser humano é uma força dominante de mudanças no planeta Terra (KEYS et al., 2019). Ainda existe muita discussão acerca de quando esta era começou, havendo defensores de que tenha sido desde o Pleistoceno, com a extinção da megafauna e o advento da agricultura, até tempos mais recentes, com a chegada de colonizadores nas Américas e a queda de CO₂ em 1600s, ou a produção de compostos persistentes como cimento e plástico, na década de 60 (LEWIS; MASLIN, 2015). O fato é que tem ocorrido uma mudança significativa no planeta Terra através da exploração de recursos, a qual tem alterado profundamente a maioria dos ecossistemas terrestres e marinhos. Nesse contexto, a restauração ecológica desempenha um papel fundamental para a reparação desses ecossistemas deteriorados (COOKE et al., 2018).

Atualmente, entre as mudanças globais ocasionadas pelos seres humanos, aquela que traz maior preocupação, sem dúvida, são as mudanças climáticas. Essas mudanças são consequência do aumento da concentração de carbono na atmosfera e já resultaram no aumento de mais de 1°C na temperatura média do planeta (IPCC, 2023). Estudos de modelagem preveem um aumento da temperatura média global entre 1,5 a 4,4°C até o ano 2100 (IPCC, 2020, 2021, 2023). No entanto, já podemos identificar algumas das principais consequências dessas mudanças, como eventos climáticos extremos, que incluem ondas de calor, geadas, inundações e secas extremas (BROWNE et al., 2021;

NIU et al., 2014). Esses eventos são caracterizados pela sua raridade e intensidade, impactando comunidades de espécies em locais específicos (NIU et al., 2014). Nos últimos anos, estes eventos extremos tornaram-se cada vez mais intensas e marcantes, como observado pelo último El Niño, que gerou a seca de 2015 e 2016 (BROWNE et al., 2021), e a onda de calor de 2023, que registou as temperaturas mais elevadas já registradas na Europa (COPERNICUS CLIMATE CHANGE SERVICE, 2023). A curta duração desses eventos dificulta a capacidade de aclimação das espécies às novas condições, pois representam situações de estresse ambiente intenso e de curta duração (JAGADISH; WAY; SHARKEY, 2021; NIU et al., 2014).

Esta preocupação é particularmente relevante para as florestas tropicais, uma vez que estes ecossistemas estão localizados em ambientes mais estáveis e com menor flutuação térmica em comparação com as florestas temperadas e boreais (WRIGHT; MULLER-LANDAU; SCHIPPER, 2009). Essa característica de florestas tropicais se dá devido a sua localização geográfica, que acarreta a menor amplitude térmica sazonal mínima como de 1-2°C na Amazônia (FISCH; MARENGO; NOBRE, 2024) e até 50°C em regiões do Canadá (GOVERNMENT OF CANADA, 2024). Devido à baixa flutuação térmica, as espécies tropicais são altamente especializadas e já estão em temperaturas próximas ao seu ótimo térmico (MULLER; ALBACH; ZOTZ, 2017; SENTINELLA et al., 2020). Esta hipótese, inicialmente proposta na década de 1960 por Janzen (1967), destaca a vulnerabilidade das espécies tropicais às mudanças climáticas. Conhecida como "hipótese da variabilidade térmica", argumenta que as espécies tropicais têm menor capacidade de aclimação às mudanças de temperatura devido às menores flutuações térmicas anuais em comparação com espécies de climas temperados (CUNNINGHAM; READ, 2002; NIEVOLA et al., 2017; VALLADARES et al., 2014). A vulnerabilidade das plantas de florestas tropicais às alterações climáticas globais deve ser tratada como uma preocupação global uma vez que florestas tropicais estão entre os biomas com maior biodiversidade do planeta, abrigando mais de dois terços da biomassa total de vegetais (WRIGHT; MULLER-LANDAU; SCHIPPER, 2009). Além disso, desempenham papéis cruciais na preservação da cultura das comunidades tradicionais, na manutenção dos recursos hídricos e na regulação do clima global. No entanto, apesar da sua importância, ainda são necessários estudos para prever padrões de resposta às alterações climáticas, tanto a nível individual como a nível do ecossistema

(CAVALERI et al., 2014; FEELEY; REHM; MACHOVINA, 2012; FEELEY; STROUD; PEREZ, 2017).

As mudanças globais, em particular, o aumento da temperatura impactam drasticamente na manutenção das florestas tropicais. A temperatura exerce influência significativa no metabolismo das plantas, afetando diversos aspectos de sua fisiologia (HATFIELD; PRUEGER, 2015; SLOT; WINTER, 2016). Entre os impactos mais notáveis, destaca-se a taxa fotossintética que já foi observada em diversos estudos (HAVAUX; TARDY, 1996; PEREZ; FEELEY, 2020; RICHARDSON et al., 2018; YAMORI; HIKOSAKA; WAY, 2014). Além disso, a temperatura também afeta a taxa de crescimento (FONTANA et al., 2018; HATFIELD; PRUEGER, 2015), a capacidade de absorção e uso de água (GODOY; DE LEMOS-FILHO; VALLADARES, 2011; RAN; ZHANG; ZHANG, 2013), a germinação de sementes (MATEKAIRE; MAROYI, 2007; TIETZE et al., 2019) e processos como a floração e frutificação (BRADLEY et al., 1999; CHEN; WANG; INOUE, 2017). Esses fatores fazem da temperatura um dos maiores determinantes da distribuição das espécies vegetais, podendo permitir ou restringir a presença de uma espécie em determinado local. Dessa forma, qualquer mudança brusca na temperatura pode impactar a permanência das espécies e a estabilidade das comunidades vegetais.

A Mata Atlântica consiste em uma floresta tropical muito impactada pela ação antrópica, que atualmente contém apenas 28% de sua área original (REZENDE et al., 2018). A Mata Atlântica é um bioma composto por florestas tropicais e subtropicais, englobando diferentes formações vegetais, como florestas ombrófilas, florestas estacionais, manguezais, restingas, campos de altitude, entre outras (SCARANO, 2009). É considerada um dos principais *hotspots* de biodiversidade do mundo, abrigando um grande número de espécies de árvores endêmicas (REZENDE et al., 2018). Este bioma ocupa aproximadamente 10-15% do território brasileiro e estende-se ao longo de uma extensa faixa litorânea, tanto ao norte quanto ao sul do trópico de Capricórnio. Atualmente, a Mata Atlântica enfrenta sérias ameaças devido às mudanças no uso da terra e mudanças climáticas. De acordo com Bellard et al., (2014), juntamente com a Província Florística do Cabo (África do Sul) e as Ilhas da Polinésia e Micronésia, a Mata Atlântica é considerada um dos três *hotspots* mundiais mais vulneráveis às mudanças globais.

A região sul do estado da Bahia está inserida no Corredor Central da Mata Atlântica e é considerada um dos cinco centros de endemismo desse bioma, tornando-se, assim, uma área prioritária para a conservação da biodiversidade (WAYT THOMAS et al., 1998). Além disso, essa região possui uma das maiores riquezas de espécies arbóreas do mundo (AMORIM et al., 2012; MARTINI et al., 2007; SAMBUICHI, 2009;). A diversidade de espécies de plantas encontradas nessa região é resultado de diversos fatores, incluindo a teoria dos refúgios florestais do Pleistoceno e a influência da ocupação humana que tem alterado a paisagem ao longo de milênios (FIASCHI; PIRANI, 2009). Além disso, as condições climáticas únicas, caracterizadas pela distribuição homogênea das chuvas e da radiação luminosa disponível ao longo do ano, bem como pelas temperaturas amenas e baixa demanda evaporativa da atmosfera, contribuem para a elevada resiliência dos ecossistemas florestais associados à Mata Atlântica do sul da Bahia.

Frente ao cenário de mudanças climáticas as espécies podem ser submetidas a quatro processos: aclimação por meio da plasticidade, adaptação ao longo da evolução, dispersão para áreas mais adequadas ou desaparecer por extinção. (FEELEY; REHM; MACHOVINA, 2012). O resultado depende da capacidade da espécie em se aclimatar, adaptar e dispersar. Além das questões intrínsecas à espécie, características do estresse, como intensidade, duração e frequência são fundamentais para determinar a resposta do indivíduo. Nesse sentido, os eventos climáticos extremos são muito problemáticos (JAGADISH; WAY; SHARKEY, 2021). Eventos climáticos extremos, tais como as ondas de calor, são eventos intensos e de curta duração, o que pode se tornar um problema se a temperatura alcançada provocar estresse excessivo ao indivíduo. Devido ao seu curto intervalo de tempo, o estresse acarretado por um evento extremo brando acarreta um “estresse de curto prazo” no qual a planta é capaz de superar o estresse por meio de mecanismos de adaptação, aclimação ou reparação, e sobreviver. Contudo, em se tratando da intensidade do evento, o estresse causado por ondas de calor pode levar à danos agudos e impedir a restituição da planta ao longo do tempo. Dessa forma os eventos extremos podem provocar um acúmulo de danos irreversíveis ao indivíduo com consequências a longo prazo (JAGADISH; WAY; SHARKEY, 2021; LAMBERS; CHAPIN; PONS, 2008; MOSA; ISMAIL; HELMY, 2017). É importante destacar que o nicho térmico, ou a faixa de temperatura tolerável, varia consideravelmente entre espécies, sendo mais amplo para aquelas capazes de tolerar uma faixa maior de

temperaturas (LAMBERS; CHAPIN; PONS, 2008; MOSA; ISMAIL; HELMY, 2017). Portanto é importante compreender quais os limites dessa faixa no cenário de eventos extremos para identificar espécies mais vulneráveis.

Os eventos climáticos extremos estão previstos para ocorrer com maior frequência e intensidade nos próximos anos (NIU et al., 2014; PERKINS-KIRKPATRICK; LEWIS, 2020). Portanto, é fundamental avaliar a capacidade das espécies em tolerar condições adversas de alta intensidade e curta duração (SLOT; WINTER, 2018). Nesse contexto, a termotolerância, avaliada por meio da performance fisiológica das plantas, surge como uma metodologia relevante para a melhor compreensão dos impactos de ondas de calor na performance ecológica das espécies vegetais. A termotolerância representa o limite térmico, tanto alto quanto baixo, no qual um organismo apresenta danos substanciais ou duradouros (GEANGE et al., 2021). Essa métrica também pode ser interpretada como a temperatura a partir da qual a planta inicia a ativação de mecanismos de proteção. Importante destacar que a termotolerância pode ser medida de diversas maneiras, algumas das quais não são diretamente comparáveis, contudo, todas válidas. Destaca-se o uso da termotolerância da clorofila *a*, medida por meio de alterações da emissão de fluorescência, como uma das mais utilizadas (GEANGE et al., 2021).

A fotossíntese, o metabolismo central das plantas, é um processo metabólico complexo e muito sensível às flutuações na temperatura ambiente. Dentre os parâmetros da fotossíntese mais comuns de serem mensurados, temos a taxa de assimilação de CO₂ (A_n), a taxa de respiração no escuro (R_d), a taxa de transporte de elétrons (J_{max}), a velocidade de carboxilação (V_c), e a eficiência quântica máxima do fotossistema 2 (F_v/F_m). Todos esses parâmetros mudam de forma relativamente rápida com o aumento da temperatura, devido a mudanças na velocidade do funcionamento de enzimas e na permeabilidade de membranas (MARIAS; MEINZER; STILL, 2017). Dentro de uma determinada faixa de temperatura, o aumento das temperaturas acarreta um aumento de A_n por meio da aceleração de atividade enzimática e as reações metabólicas, representadas também pela V_c e J_{max} (SLOT; WINTER, 2016). No entanto, as altas temperaturas podem ser prejudiciais ao afetar a estrutura proteica e a permeabilidade de membranas, prejudicando a função enzimática, aumentando a fotorrespiração e elevando a R_d . Dentro da cadeia de transporte de elétrons da fotossíntese, o fotossistema 2 (*PSII*) é o componente mais termosensível. Quando a temperatura crítica é excedida, ocorre o comprometimento permanente da função do *PSII* e a capacidade fotossintética

pode ser severamente limitada (MÜNCHINGER et al., 2023). Por isso é possível mensurar a sensibilidade do *PSII* à temperatura através da queda da sua eficiência quântica máxima (F_v/F_m) ao aumento da temperatura. A partir da razão F_v/F_m pode-se calcular os parâmetros de termotolerância T_{crit} e T_{50} (CHAVES; LEAL; LEMOS-FILHO, 2018; FEELEY et al., 2020; MARIAS; MEINZER; STILL, 2017; SASTRY; GUHA; BARUA, 2018). O T_{50} marca a temperatura em que ocorre uma queda de 50% do F_v/F_m inicial (SLOT et al., 2021). Já o T_{crit} é observado antes, e é marcado pela temperatura onde vemos uma queda abrupta no F_v/F_m , indicando danos que podem ser irreversíveis ao maquinário fotossintético (GEANGE et al., 2021).

Para prever e minimizar as consequências das mudanças climáticas, e definir estratégias tais como o plantio de mudas em ações de restauração, é necessário compreender os processos ecológicos e as respostas específicas que determinam como a temperatura influencia a performance fisiológica das espécies que compõe esses ecossistemas (COOKE et al., 2018; ROSENFELD; MÜLLER, 2020). As folhas são os principais órgãos responsáveis pelas trocas gasosas entre a planta e a atmosfera, sendo fundamentais para o funcionamento de ecossistemas terrestres. Por serem órgãos de crescimento determinado, as folhas apresentam grande plasticidade em relação aos atributos morfológicos e fisiológicos (POORTER et al., 2009). A partir delas podemos mensurar os atributos funcionais foliares, ou características funcionais, que são características mensuráveis anatômicas, morfológicas, bioquímicas ou fisiológicas, e que possuem relação com o estado do indivíduo. Estas características são excelentes ferramentas, capazes de relacionar o estado do organismo à condição ambiental a qual ele foi exposto (MCGILL et al., 2006; VIOLLE et al., 2007). Compreender como os atributos foliares estão relacionados à tolerância ao aumento súbito da temperatura é importante para prever as respostas das espécies às ameaças do aquecimento global e eventos climáticos extremos.

Nesse sentido, compreender como espécies nativas e exóticas invasoras respondem ao aumento da temperatura é de extrema importância para incorporar dados a modelos de predição, para estimar as espécies com maior risco de extinção, assim como para planejar áreas de conservação e organizar estratégias de manejo (CAVALERI et al., 2014; NICOTRA et al., 2011; RAGHUNATHAN, 2018). Tendo isto em mente, esta tese possui quatro capítulos: (1) uma metanálise com intuito de verificar o efeito da temperatura na fotossíntese de plantas jovens em escala mundial, (2) um experimento de

aquecimento para mensurar a termotolerância foliar de indivíduos jovens de espécies de árvores latino-americanas, (3) verificar a termotolerância foliar de indivíduos adultos de espécies de restinga nativas e invasoras; e (4) testar a termotolerância foliar em diferentes condições de luz de espécies de árvores brasileiras, visando sua aplicação em projetos de restauração por meio do enriquecimento.

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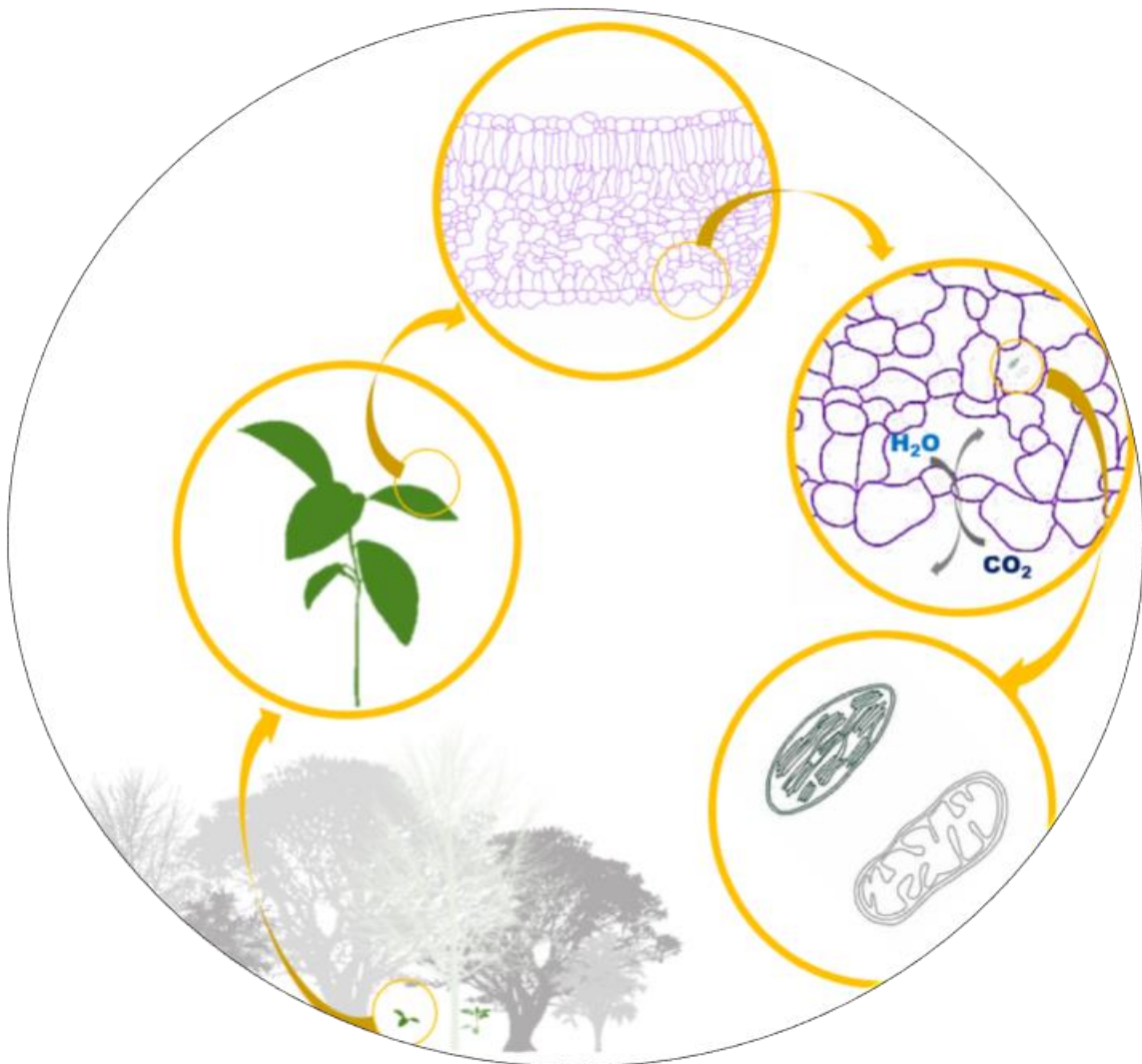
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CAPÍTULO 1 - A meta-analysis on the impacts of warming on leaf carbon balance of young woody plants: implications to forest regeneration

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A systematic review and meta-analysis on the impacts of warming on leaf carbon balance of young woody plants: implications to forest regeneration

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Abstract. Tree seedlings play a crucial role in maintaining and regenerating local diversity, are essential for the functioning of forest ecosystems, and are more vulnerable to environmental changes due to their smaller metabolic reserve and photosynthetic production capacity than the adult trees. To address the responses of young plants (seedlings, saplings, juveniles, and young) from different geographic origins to temperature increase, we conducted a systematic review and meta-analysis of temperature increase experiments with leaf carbon balance traits of young woody plants. We investigated the effects of increasing temperatures on seven leaf traits related to carbon balance (g_s , $C_i:C_a$, F_v/F_m , J_{max} , V_{cmax} , A_n , R_d and LMA) on 62 species at 39 papers. There was consistent acclimation pattern in A_n response to warming and an increase in R_d and photorespiration, disrupting carbon balance and potentially reducing young tree growth and survival. Elevated temperatures intensify photorespiration, influencing growth and defense in the low light conditions of the forest understory, and posing challenges to forest regeneration in a warming world.

Keywords: forest regeneration, photosynthesis, trees, seedlings and saplings.

Introduction

Regeneration and maintenance of forest diversity depends on the recruitment and growth of seedlings and saplings (Stefanski et al. 2020). These still-young individuals will make up the forest and fulfill their ecological function as they mature (Kitajima 1996). Young individuals, in turn, are particularly vulnerable because they are highly dependent on their smaller energy reserves and have lower net photosynthetic

production compared to adult trees (Stefanski et al. 2020). In addition, these life stages have smaller roots that make water uptake more difficult during periods of heat and drought. Although temperatures in the canopy are higher than in the understory, the proximity to the ground limits temperature loss through convection (Jagadish et al. 2021). Young plants often live in a low-light environment under the dense forest canopy, further limits their light interception and net photosynthesis (Valladares et al. 2012). Those particular circumstances make them more sensitive to environmental changes when their plasticity and reserves are more necessary, such as temperature increase and drought, observed in the context of climate change (Browne et al. 2021). Given future climate projections, this sensitivity could negatively impact global forest diversity, as seedling and sapling recruitment depends on favorable environmental conditions to survive.

In the context of climate change, there has been an increase in hot extreme events and rising mean temperatures (Niu et al. 2014). This year of 2023, record-breaking heatwaves have swept across various regions, with temperature anomalies reaching unprecedented levels (Copernicus Climate Change Service 2023). From the Pacific Northwest in the United States to parts of Europe, Asia, and the Middle East, these extreme heat events have led to soaring temperatures, prolonged droughts, and adverse health effects (Copernicus Climate Change Service 2023). As temperatures have increased in recent years, the impacts of rising temperatures on plant communities are of concern. Current projections predict around to 4°C increase in average temperature on Earth by 2100 (IPCC 2023). This temperature increase is expected to have significant impacts on species competition, community composition, and ecosystem (Olsen et al. 2016; Slot and Winter 2016). As it is predicted further environmental changes, it is also expected a reduction in suitable periods for seedling recruitment, particularly impacting woody species with slower growth rates. This poses a significant threat to the regenerative capacity of forests, which heavily relies on the responses of seedlings and saplings to environmental changes.

This concern is particularly relevant for tropical forests, as these ecosystems are located in more stable environments with less thermal fluctuation than temperate and boreal forests (Wright et al. 2009). This characteristic of tropical forests is due to their geographical location, which leads to the lowest possible annual temperature fluctuation. Due to the low thermal fluctuation, tropical species are highly specialized

and already stay at temperatures close to their thermal optimum (Muller et al. 2017; Sentinella et al. 2020). This hypothesis, which was first proposed by Janzen (1967) in the 1960s, highlights the vulnerability of tropical species to climate change. It is known as the "thermal variability hypothesis" and states that tropical species have a lower capacity to acclimatize to temperature changes due to lower annual temperature fluctuations compared to species from temperate climates (Cunningham and Read 2002; Valladares et al. 2014; Nievola et al. 2017). The vulnerability of tropical forest plants to global climate change should be treated as a worldwide concern. Tropical forests are among the most biodiverse biomes on planet Earth, containing more than two-thirds of the total biomass of living terrestrial plants, yet there are still few studies on temperature increase in relation to their diversity (Cavaleri et al. 2014; Feeley et al. 2017). In addition to harboring a large number of living organisms, tropical forests are of great importance for the preservation of the culture of traditional communities, for maintaining water resources (Netzer et al. 2019) and for maintaining the global climate (Wright et al. 2009). However, despite the importance of tropical forests worldwide, there is still a need for studies are still needed to predict patterns of response to climate change, both at an individual and ecosystem level (Wright et al. 2009; Feeley et al. 2012; Cavaleri et al. 2014).

Changes in temperature are in particular of great importance to plants because temperature plays a critical role in the spread and fitness of tree species (Nievola et al. 2017; Geange et al. 2021) (Fig. 1). It affects plant metabolism and impacts various aspects of their physiology, such as growth rate (Kumarathunge et al. 2019), water loss (Marchin et al. 2016), seed germination (Tietze et al. 2019), and processes such as flowering and fruiting (Bradley et al. 1999; Chen et al. 2017) and secondary metabolite production (Wani et al. 2017). In particular, photosynthesis is one of the most important processes in plant function. It is largely temperature dependent, and any change could translate into an increase in membrane permeability or a decrease in enzyme function.

Photosynthesis is a fundamental biological process responsible for the carbon balance in plants and is affected by temperature (Mathur et al. 2014; Dusenge et al. 2019; Perez and Feeley 2020). Within a given temperature range, increasing temperatures can enhance the rate of photosynthesis (A_n) by accelerating enzyme activity and metabolic reactions and reflects in increases in net maximum Rubisco carboxylation rate (V_{cmax}), electron transport rate (J_{max}), and atmospheric carbon ratio (C_i/C_a). However, high

temperatures can be detrimental by disrupting protein structure, impairing enzyme function, increasing photorespiration and dark respiration rate (R_d), leading to a consequent decrease in the net photosynthetic rate (A_n). Therefore, the effect of temperature on net photosynthesis is a critical determinant of ecosystem productivity and plant growth because it shapes the overall carbon balance of terrestrial ecosystems and affects distribution patterns. Therefore, assessing the photosynthetic response of young woody plants to a rise in temperature is important for evaluating the risk of species extinction and for planning protected areas and forest restoration strategies (Nicotra et al. 2011; Raghunathan et al. 2014).

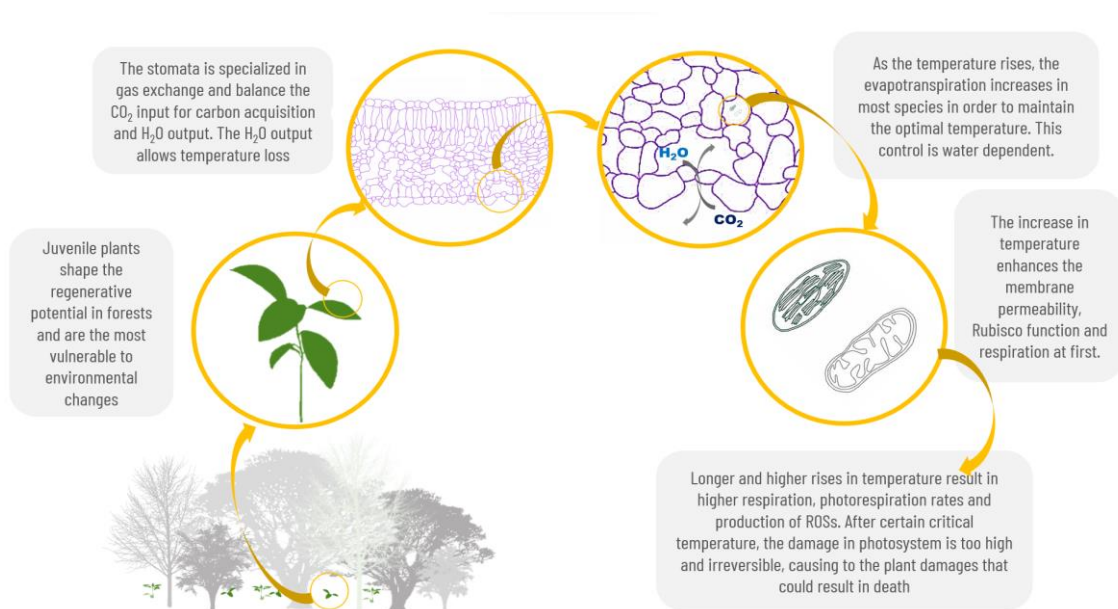


Figure 1. Schematic illustration of the temperature increase influencing the photosynthetic performance in juvenile plants.

In order to assess how the forest regeneration will be affected by warming, we conducted a systematic review followed by meta-analysis and meta-regression to answer if there is a pattern in temperature response in young woody plants of three climate regions: boreal, temperate and tropical. Our hypothesis is that, despite fewer studies, young woody plants in the tropics are more susceptible to temperature increases than in other regions and we therefore expect a greater decline in the carbon balance of tropical plants.

Materials and Methods

Systematic Review: To answer how young woody plants respond to the temperature increase we conducted a systematic review and later a meta-analysis. The systematic review followed the PRISMA methodology. We searched the terms "warming" AND "experiment" AND " 'plant' OR 'tree' " in the Scopus and the Web of Science database for all published articles up to January of 2021 (Figure 1). Only studies with young (seedlings, saplings, juveniles, and young) woody (tree, shrub, and lianas) were selected. Studies with aquatic plants or with natural gradients were discarded to avoid other edaphoclimatic effects, as elevation, rainfall, soil, along with others. In studies in where more than one environmental factor was tested, only the temperature treatment and control were selected. In experiments with more than one warming temperature, the different temperatures were compared as independent treatments. The average ambient temperature was calculated for the control for outdoor experiments. In the case of temperature fluctuation, (cold → hot → cold) only the first warming was sampled. When the treatment presented variation in morning/night warming, the average temperature was used for the control and warming temperature. In heatshock or temperature curve experiments, the first temperature was used as a control and two others (one intermediate and the last) as treatment, or, when possible, at the respective control and treatment temperatures. All data collected was sampled in the morning. Only studies in where it was possible to estimate the standard deviation were selected. We classified the methodology into eight types: infrared warming, growth chamber, soil warming, IRGA cuvette, water warming, heat shock, open top chamber, and greenhouse. Additionally, we considered the duration of warming exposure, magnitude of warming (intensity), and photosynthetic photon flux density (PPFD) as reported in the studies. We estimated the maximum height, average leaf mass per area (LMA), shade tolerance, and deciduousness of each species. Subsequently, we utilized these categories as moderators for the meta-analysis.

Carbon balance leaf traits: The leaf traits chosen for this meta-analysis reflect the gain in carbon acquisition with photosynthesis, the carbon loss in the respiration and the carbon allocation in the leaf per area. The net photosynthetic rate (A_n), the dark respiration rate (R_d) and the Rubisco maximum carboxylation rate (V_{cmax}) correspond to biochemical process and express the balance of carbon acquisition and cost in the plant and the maximum biochemical efficiency. The quantum efficiency of photosystem II (F_v/F_m) is a variable that indicates stress or impairment of the electron transport system.

Along with F_v/F_m , the maximum electron transport rate (J_{max}) represents the photochemical step of photosynthesis, related to electron transport in photosystem II. Finally, the internal and atmospheric carbon ratio (C_i/C_a) and stomatal conductance (g_s) reflect the diffusive phase of gas exchange. The photosynthesis and respiration changes according to temperature due to membrane permeability and enzymes function alterations, what makes this reaction a good framework to investigate how plants will respond to future climate changes. The widely used morphological trait leaf mass area (LMA) were collected in addition to the carbon balance triats. The LMA indicates biomass allocation in an area for light interception for photosynthesis and is a common trait in plant ecology, agronomy and physiology studies (Poorter et al. 2009).

Meta-analysis: A meta-analysis was conducted to investigate the influence of temperature increase on carbon balance leaf traits. Meta-analysis is a well-established statistical method that facilitates the integration of results from multiple studies. By compiling data from different sources, this approach allows the calculation of treatment effects (or effect sizes) and thus a more accurate estimation of the collective effect size compared to individual studies conducted in isolation (Borenstein et al. 2009). There are various ways to measure the effect size, and we choose the log response ratio of means (RR) for the experiments according to the number of samples, variance and mean values, following Borenstein et al. (2009) as there is many different methodologies. The global effect was calculated using a random model, as the heterogeneity was part of the studies, and its confidence tested by maximum-likelihood ($p < 0.05$). The global effect was measured for all species data, and additionally in subgroups according to plant geographical origin: (a) boreal, (b) temperate, and (c) tropical. Bias was tested through an asymmetry analysis with a regression test for funnel plot asymmetry with 95% confidence. Outliers were excluded from the data when observed. We choose moderators related to the methodology (exposure time, warming magnitude, methodology and photosynthetic photon flux density - $PPFD$) and to the plant functional life story (maximum height, average LMA , shade tolerance and deciduous) to test how those variables influenced the result. Warming magnitude correspond as the difference at the initial temperature and the warming temperature. The moderators were tested individually by mixed-effects meta-regression model with restricted maximum-likelihood estimator. The “safe-number” ($RSNo$) was be estimated by Rosenthal's method, to calculate the number of articles with a null result, which would be necessary to add to our data to decrease the

significance level obtained. The results were considered consistent when the safe number was superior to $5N \times 10$, where N is the original number of studies (Yuan et al. 2018). All analyzes were performed using the R 4.1.0 program and the metafor package (Ripley 2001).

Results

Systematic review

A total of 38 studies comprising 62 species were included in the analysis (Tab. 1). However, only a limited number of papers focused on tropical species, so additional sources had to be included to facilitate the meta-analysis of tropical origin. Although most studies on tropical trees examined multiple species, *Ochroma pyramidale* and *Ficus insipida* were the most frequently studied species, appearing in six papers each. This was followed by *Acer rubrum*, which was studied in five papers, and *Betula papyrifera*, *Quercus rubra* and *Pinus sylvestris*, which were each found in four papers. Although no time frame was specified for the literature search, no work was found that was dated before 1999. Notably, most publications were published after 2015, reflecting a consistent trend observed during the screening: Studies focusing on temperature were mainly from the 2010s. The predominant methods were growth chambers, which were used in 10 studies, followed by greenhouses (9 studies) and infrared heating (8 studies). The most commonly assessed leaf trait was An, which was documented for 35 species, followed by leaf mass area (*LMA*), which was reported for half of all species in the study, although some studies only provided *LMA* data.

Table 1: Reference table of all studies, species, methodology and traits used for meta-analysis.

Species	Methods	Traits	References	Species	Methods	Traits	References
<i>Abies balsamea</i>	IR	A_n, J_{max} and V_{cmax}	27, 31	<i>Picea glauca</i>	IR and OTC	A_n, J_{max} and V_{cmax}	3, 27, 31
<i>Acacia aneura</i>	GC	<i>LMA</i>	38	<i>Picea Rubens</i>	Gh	A_n, g_s	1
<i>Acacia melanoxylon</i>	GC	<i>LMA</i>	38	<i>Pinus banksiana</i>	IR	A_n, J_{max}, V_{cmax}	27, 31
<i>Acer rubrum</i>	Cuv, IR and SH	$A_n, C_i: C_a, R_d, J_{max}, V_{cmax}$ and <i>LMA</i>	9, 16, 20, 27, 31	<i>Pinus flexilis</i>	IR	A_n	11
<i>Acer saccharum</i>	Cuv and IR	$A_n, C_i: C_a, J_{max}$ and V_{cmax}	9, 27, 31	<i>Pinus halepensis</i>	GC	F_v/F_m	15
<i>Adenantherapavonina</i>	GC	<i>LMA</i>	35	<i>Pinus nigra</i>	GC	F_v/F_m	15
<i>Baccharis uncinella</i>	OTC	R_d, LMA	22	<i>Pinus pinaster</i>	GC	F_v/F_m	15
<i>Betula lenta</i>	IR	<i>LMA</i>	20	<i>Pinus strobus</i>	IR	A_n, J_{max}, V_{cmax}	27, 31
<i>Betula papyrifera</i>	Cuv, IR and SH	$A_n, C_i: C_a, R_d, J_{max}, V_{cmax}$ and <i>LMA</i>	9, 16, 27, 31	<i>Pinus sylvestris</i>	Gh, GC, IR and WH	A_n, g_s and F_v/F_m	15, 17, 19, 23
<i>Calophyllum longifolium</i>	Cuv, GC and HS	$A_n, R_d, F_v/F_m, J_{max}, V_{cmax}$ and <i>LMA</i>	8, 21, 37	<i>Pinus uncinata</i>	GC	F_v/F_m	15
<i>Castanea sativa</i>	WH	g_s	17	<i>Populus deltoides</i>	Gh	$A_n, R_d, J_{max}, V_{cmax}$	29
<i>Cecropia insignis</i>	GC	A_n and <i>LMA</i>	37	<i>Populus grandidentata</i>	IR	<i>LMA</i>	20
<i>Citharexylum sp.</i>	GC	<i>LMA</i>	35	<i>Populus tremula</i>	IR	$A_n, g_s, C_i: C_a$ and <i>LMA</i>	10, 17
<i>Clusia pratensis</i>	GC	<i>LMA</i>	37	<i>Populus tremuloides</i>	Cuv, IR and SH	$A_n, C_i: C_a, R_d$ and <i>LMA</i>	9, 16, 31
<i>Cunninghamia lanceolata</i>	OTC	$A_n, g_s, C_i: C_a$ and R_d	13	<i>Prunus serotina</i>	IR	<i>LMA</i>	20
<i>Eucalyptus delegatensis</i>	GC	<i>LMA</i>	38	<i>Quercus alba</i>	GC	R_d	2
<i>Eucalyptus dumosa</i>	GC	<i>LMA</i>	38	<i>Quercus macrocarpa</i>	Cuv and IR	$A_n, C_i: C_a, J_{max}$ and V_{cmax}	9, 27, 31
<i>Eucalyptus globulus</i>	Gh	A_n, g_s	12	<i>Quercus pubescens</i>	OTC	A_n, g_s, J_{max} and V_{cmax}	5
<i>Eucalyptus grandis</i>	GC	A_n, g_s	26	<i>Quercus rubra</i>	Cuv and IR	$A_n, C_i: C_a, J_{max}, V_{cmax}$ and <i>LMA</i>	9, 20, 27, 31
<i>Eucalyptus pauciflora</i>	Gh	F_v/F_m	32	<i>Quercus sp</i>	WH	g_s	17
<i>Eucalyptus platyphylla</i>	GC	A_n, g_s	26	<i>Rhamnus catártica</i>	IR	A_n	31
<i>Eucalyptus resinifera</i>	GC	A_n, g_s	26	<i>Salix matsudana</i>	Gh	A_n and $C_i: C_a$	30
<i>Eucalyptus</i>	Gh	A_n, R_d, g_s	24	<i>Schima superba</i>	Gh	A_n	33

<i>sideroxylon</i>							
<i>Ficus insipida</i>	Cuv, GC and HS	$A_n, R_d, F_v/F_m, J_{max}, V_{cmax}$ and LMA	6, 8, 21, 35, 36, 37	<i>Tabebuia guayacan</i>	GC	LMA	36
<i>Garcinia intermedia</i>	GC	LMA	36	<i>Tabebuia rósea</i>	GC	A_n, R_d e LMA	21
<i>Lacistema aggregatum</i>	GC	LMA	36	<i>Terminalia catappa</i>	OTC	LMA	25
<i>Lantana camara</i>	Gh	$A_n, g_s, F_v/F_m$ and LMA	7	<i>Theobroma cacao</i>	GC	LMA	36
<i>Luehea seemannii</i>	GC	A_n and LMA	21, 36	<i>Tsuga canadenses</i>	GC	A_n, g_s and R_d	14
<i>Ochroma pyramidale</i>	GC and OTC	$A_n, R_d, J_{max}, V_{cmax}$ and LMA	6, 21, 34, 35, 36, 37	<i>Ulmus americana</i>	IR	LMA	20, 28
<i>Ormosia macrocalyx</i>	groth chamber	A_n, R_d, LMA	21, 35, 36	<i>Virola sebifera</i>	OTC	F_v/F_m	4
<i>Picea abies</i>	Gh, IR	A_n, g_s and F_v/F_m	19, 23	<i>Virola surinamensis</i>	GC	R_d, LMA	37

IR - infra red warming; GC - growth chamber; SH - soil warming; Cuv - Cuvette; WH - water warming; HS - heat shock; OTC - open top chamber; and Gh - greenhouse. References: 1 - Day 2000; 2 - Bolstad et al. 2003; 3 - Danby and Hik 2007; 4 - Krause et al. 2010; 5 - Contran et al. 2013; 6 - Cheesman and Winter 2013a; 7 - Zhang et al. 2014; 8 - Krause et al. 2015; 9 - Sendall et al. 2015; 10 - Randriamanana et al. 2015; 11 - Moyes et al. 2015; 12 - Quentin et al. 2015; 13 - Yu et al. 2016; 14 - Wilder and Boyd 2016; 15 - Matías et al. 2017; 16 - Wei et al. 2017; 17 - Nissinen et al. 2017; 18 - Martínez-Sancho et al. 2017; 19 - Kivimäenpää et al. 2017; 20 - Rodgers et al. 2018; 21 - Slot and Winter 2017; 22 - Dechoum et al. 2018; 23 - Kurepin et al. 2018; 24 - Duan et al. 2018; 25 - Tietze et al. 2019; 26 - Apgaua et al. 2019; 27 - Stefanski et al. 2020; 28 - Smith et al. 2021; 29 - Turnbull et al. 2002; 30 - He and Dong 2003; 31 - Reich et al. 2015; 32 - Loveys et al. 2006; 33 - Sheu and Lin 1999; 34 - Cheesman and Winter 2013a; 35 - Cheesman and Winter 2013b; 36 - Slot and Kitajima 2015; 37 - Slot and Winter 2017b; 38 - Loveys et al. 2003.

Metanalysis

The global estimation on how the temperature increase affects the stomatal conductance in young woody plants showed low reliability, with a $RSNo$ of 38 (Fig. 2a). The only moderator related to the g_s response was the methodology (Tab. 2), implying also that the differences in methodology also contributed for the high heterogeneity and probably for the unsure response. For $C_i:C_a$, the global model response was much more robust ($RSNo:814$; $Q:84.80$; $I^2: 0.79$), demonstrating a general global trend of increase in internal carbon concentration when species are subjected to a temperature increase ($RR: 1.12$; Fig. 2b). All moderators, besides average LMA and maximum height, presented high relationship with the response of $C_i:C_a$ to the warming, ranging between 40% and 99% of possible explanation. Unfortunately, no data was found to analyze the response of young woody tropical species for this trait.

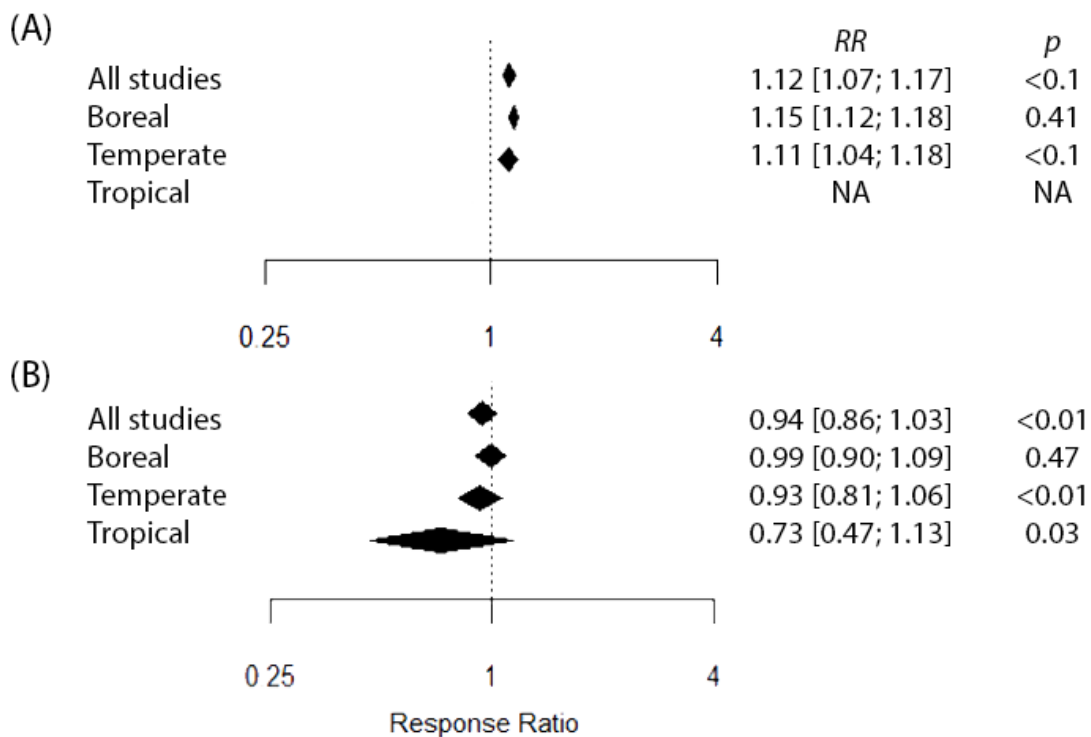


Figure 2. Global effect of (A) $C_i:C_a$ and (B) g_s , of juvenile woody species to warming. Each data point represents the mean $\pm 95\%$ CI of all species (all studies) and the subgroup effects (boreal, temperate and tropical).

Table 2: Meta-regression analysis for each carbon balance traits and functional moderators. Values represent R^2 . Bold values signif. $p < 0.05$. n.s: non-significant.

	G_s	$C_i:C_a$	F_v/F_m	J_{max}	A_n	V_{cmax}	R_d	LMA
Exposure time	0.00	0.83	0.04	0.04	0.00	0.01	0.06	0.05
Warming magnitude	0.22	0.40	0.44	0.02	0.23	0.24	0.06	0.21
Methodology	0.25	0.99	0.55	0.22	0.12	0.26	0.40	0.00
<i>PPFD</i>	0.00	0.99	0.00	0.20	0.00	0.00	0.00	0.00
<i>LMA</i>	0.00	0.14	0.09	0.00	0.00	0.03	0.34	0.00
Shade tolerance	0.00	0.26	0.00	0.04	0.00	0.00	0.03	0.01
Deciduity	0.00	0.99	0.00	0.00	0.00	0.00	0.05	0.18
Maximum height	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00

The Rubisco maximum carboxylation rate (V_{cmax}) is affected by temperature as the temperature influences in the Rubisco activity. In our data, V_{cmax} reduced for most subgroups when temperature was increased (RR : 0.82; Fig. 3c).. Rubisco's carboxylation rate affects the net photosynthetic rate (A_n), but the results differed for the two traits (Fig. 3a). A_n did not change when provided with an temperature increase in all species, which demonstrates a real global pattern (RR : 0.97) of null response. Both traits had the same moderators influence of the warming magnitude and the methodology used for warming. The influence of the traits was around 25% for the V_{cmax} , and much less for the A_n response (Tab. S2). For dark respiration (R_d) response to the temperature increase was null for temperate and tropical species, and not significant for boreal species (Fig. 3b). The carbon acquisition and plant production are based on the balance between assimilation (A_n) and emission (R_d).

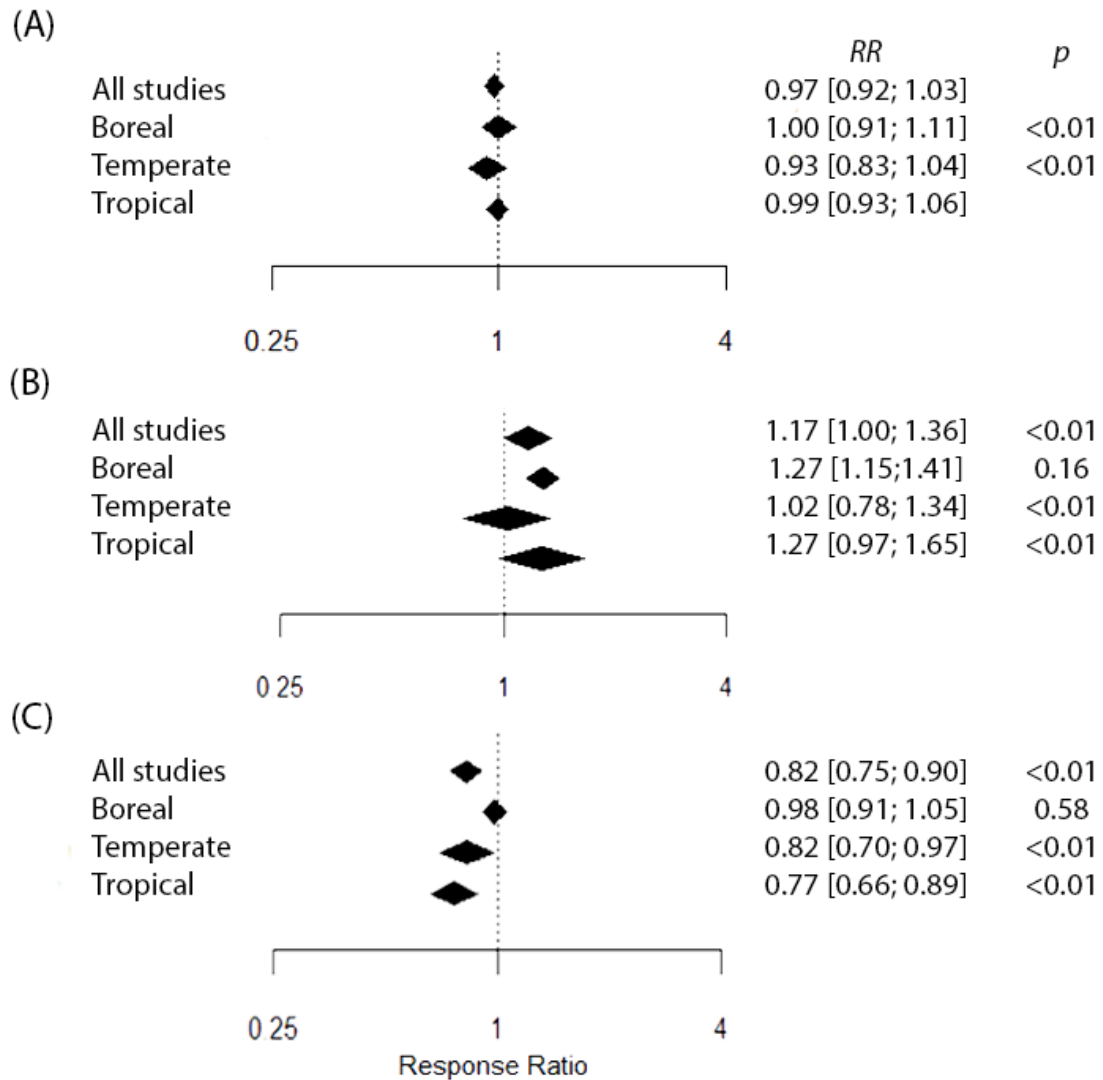


Figure 3. Global effect of (A) A_n , (B) R_d , and (C) V_{max} of juvenile woody species to warming. Each data point represents the mean \pm 95% CI of all species (all studies) and the subgroup effects (boreal, temperate and tropical).

It was not observed change in mostly photochemical traits. The F_v/F_m of boreal and temperate species (RR : 1.00 and RR : 1.01, respectively), the warming effect was null (Fig. 4a). The global response was positive (RR : 1.20) due to the response of tropical species (RR : 1.44), which pulled the tendency to positive side. However, both the global response and the response of tropical species present a high variance and heterogeneity (Q : 1230.02; I^2 :1000 and Q : 678.39; I^2 :1000, respectively, Tab. S2), which indicates that this result is not so well represented. The methodological moderators influenced more in the response of F_v/F_m , being possible to explain the results by more than 50% by the

methodology used for warming, and 40% for the magnitude of warming used in this methodology (Tab. 2). In relation to the other photochemical response, J_{\max} , the results were dubious (Fig. 4b). As well as the stomatal conductance response, J_{\max} obtained a very low safe number value ($RSNo$: 60), which makes the null result unreliable so far. The only moderator found significant for the J_{\max} was the PPFD with 20% as expected.

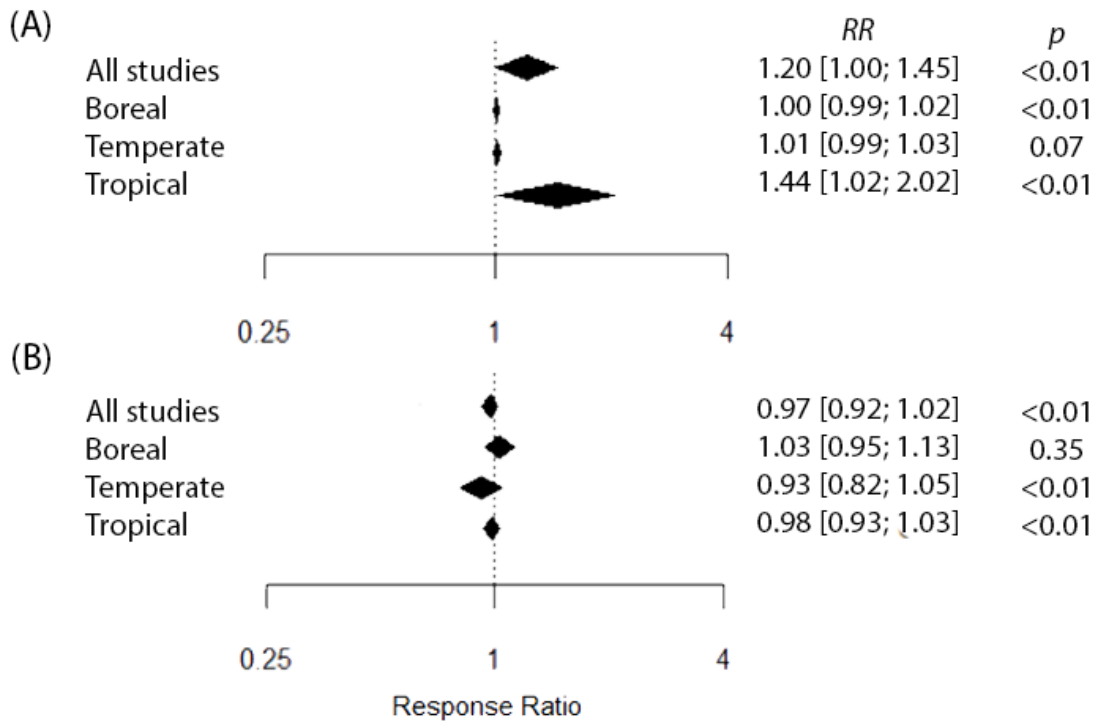


Figure 4: Global effect of (A) F_v/F_m and (B) J_{\max} of juvenile woody species to warming. Each data point represents the mean \pm 95% CI of all species (all studies) and the subgroup effects (boreal, temperate and tropical).

For LMA , warming provided an increase in this index (RR : 1.11; Fig. 5). Thus, the temperature increase resulted in the production of denser leaves, with the exception of temperate species, where the effect was null (RR : 1.05). The time of exposure and magnitude also affects the response of LMA . Interesting, the difference between the leaf longevity (deciduous and evergreen) also influenced in the response of LMA in juvenile woody plants submitted to warming (Tab. 2).

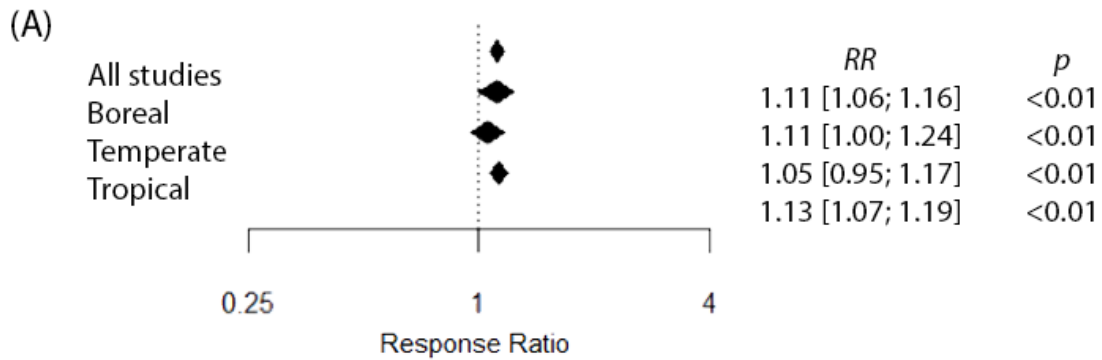


Figure 5: Global effect of (A) *LMA* of juvenile woody species to warming. Each data point represents the mean $\pm 95\%$ CI of all species (all studies) and the subgroup effects (boreal, temperate and tropical).

Discussion

Systematic review

In accordance with our first hypothesis there was few papers in tropical species, although most of those worked with three to twelve species in total. Tropical forests, hosts over two-thirds of Earth's terrestrial plant biomass, are highly biodiverse. Yet, studies on temperature increase and their diversity are limited (Cavaleri et al., 2014; Feeley et al., 2017). Despite the recent increase in thermal response of tropical plant studies following the global trend, tropical organisms make up a small percentage of studies in the area and the majority of climate change and conservation research focuses on temperate rather than tropical systems which are conservation priorities (Feeley et al. 2017). However, it is important to notice that most tropical focused papers include several species, from one to ten species. This trend suggests that studies with tropical species often aim to capture the diversity of the region.

Unsurprisingly, the majority of papers originated from North America and Europe, with Panama, particularly associated with the Smithsonian Tropical Research Institute, being the most commonly studied tropical region. This result underscores the institutional bias and financial resources favoring higher-income countries and institutes associations. The increase in publications on thermal response probably has relation with the scientific and public concern over global warming in the last decades Geange et al.

(2021) further supported this observation by demonstrating an increase in studies on thermotolerance since the 2000s, confirming our findings.

The later input of tropical species papers has led to an increase in tropical species, specifically *Ochroma pyramidale* and *Ficus insipida*. The two are early-successional species highly used in ecophysiology studies (Slot et al. 2016). *Acer rubrum*, also known as red maple, was also seen in many studies. It is one of the most widely distributed eastern temperate tree species, being widely dominating in forests mainly due to anthropogenic changes (Wheeler et al. 2017). *Betula papyrifera* (white birch) and *Quercus rubra* (red oak) are deciduous broad leaf species with higher biomass accumulation commonly found in North America, while *Pinus sylvestris* (Scots pine) is a pine tree largely found in Northern Hemisphere (Reich et al. 2015; Sivadasan et al. 2018). By those means, the most used species are also most common species.

The methodologies varied in most papers and were needed to simplify in order to assemble in different groups. Growth chamber was the most used one and is a great methodology to achieve environmental control and are widely used in plant research (Nievola et al. 2017). This method has a size limitation, although this is not a problem working with young individuals, such as seedling and saplings, accessed in this review. They are more sophisticated and need a higher investment and running costs but allows a higher environmental control (Poorter et al. 2012). With less environmental control than growth chambers, but more control than common gardens, there are greenhouses, which offer a better control over water uptake and temperature (Poorter et al. 2012; Dongsansuk et al. 2013). Typically, greenhouses present higher temperatures compared to outdoor conditions, which contribute to working with this methodology in warming experiments. Two unique methodologies were seen in Wei et al. (2017) with belowground warming made by soil heating cables and in Martínez-Sancho et al. (2017) with a water heating system, which was connected gridding each seedling. Those two methodologies underscore how warming can be accomplished with different forms, but also how each methodology should acknowledge its own bias.

Most used leaf trait were two of the most common traits related to the leaf economic spectrum: net assimilation (A_n) and leaf mass area (*LMA*). A_n is a direct measure of photosynthesis for a given species, quantified by leaf area and its relationship with the leaf surface area for light interception and gas exchange (Wright et al. 2004; Lobo et al.

2013). Another common metric is photosynthesis per unit leaf mass (A_{mass}), which also considers the leaf biomass investment for this parameter (Donovan et al. 2011). Both measures assess carbon uptake within a defined time period and determined area and mass, essential for evaluating carbon balance. As for *LMA*, it is a mass-based leaf trait, easily measured since need only a scale, balance and oven. This low-cost soft trait is much-used leaf trait in the context of ecology and regional distribution, and could be related to herbivory, leaf lifespan, light interception, xeromorphy (Wright et al. 2004; Poorter et al. 2009a). Data on internal and atmospheric carbon rates ($C_i:C_a$) are scarce across all regions, particularly for tropical species where no data was found. This trait is challenging to obtain, requiring not only an Infrared Gas Analyzer (IRGA) but also the measurement of a carbon concentration curve (A/C_i) to derive the parameter (Randriamanana et al. 2015). This difficulty likely contributes to the limited data available on this subject. However, given the projected increase in carbon emissions and atmospheric concentrations in future, it is crucial to prioritize the measurement of such trait.

Metanalysis

Contrary to our hypothesis, we did not find young woody tropical plants particularly more susceptible to temperature increases compared to other regions. Although, there is still concern over the temperature response on young woody plants, specially taking account on the environment conditions that this stage of life is found. In the forest understory, the availability of light is severely limited, so young trees are in a constant state of stress with limited growth due to limited light exposure (Valladares et al. 2002). This situation is particularly exacerbated in dense tropical forests where, less than 2% of photosynthetically active radiation can reach the forest floor (Valladares et al. 2002, 2012). In addition, the proximity of young trees to the shaded forest floor increases their susceptibility to structural damage from herbivory, pathogens, or impact from falling debris (Kitajima 1994; Nabeshima et al. 2001; Valladares et al. 2016). Consequently, survival of these young trees in the forest understory depends on two key strategies: an increase in biomass production and/or investment in defensive structures and metabolites (Kitajima 1994; Leakey et al. 2002a; Kitajima and Poorter 2010). These strategies are used by young trees to try to accelerate their ascent to higher strata or to defend themselves against harsh environmental conditions in the lower layers of the forest. This increment in plant biomass and defense depends on the plant carbon

balance, which involves the dynamic equilibrium between carbon uptake through photosynthesis (A_n) and carbon loss through respiration (R_d) and growth processes (Lobo et al. 2013; Kalaji et al. 2017; Dusenge et al. 2019).

As the carbon balance is result of the photosynthesis, it is a highly temperature-dependent biochemical process (Mathur et al. 2014). Within a certain range, increasing temperatures can increase net photosynthesis, but beyond the optimal temperature range, the net rate decreases (Sendall et al. 2015). However, in our meta-analysis, we found no positive or negative pattern in A_n due to warming. This result demonstrates the acclimation capacity of net photosynthetic rate to warming despite its climatic origin. Since we see no difference in A_n with respect to climatic origin in our data, this suggests that the acclimation of photosynthetic output is correlated with the new temperature and not with the original climatic temperature (Kumarathunge et al. 2019). In this sense, the response of net photosynthetic rate seems to be mainly related to plant acclimation potential and ecological plant history.

The carbon balance can be optimized by most plants to acclimate to changing environmental conditions, including temperature fluctuations (Kurepin et al. 2018). Under favorable conditions, when carbon uptake exceeds loss, the carbon excess is used for growth, reproduction, and storage, increasing plant biomass. Conversely, under unfavorable conditions, carbon allocation prioritizes maintenance and survival over growth. In environments where plants are already at the limit of positive carbon balance, such as low light-conditions, the loss of carbon gain should be avoided. As such, understory species present decreased dark respiration rates as a strategy of shade tolerance in order to compensate the low photosynthetic rates (Leakey et al. 2002b; Zaragoza-Castells et al. 2008). However, we see in response to warming an increase in R_d in the global trend despite the acclimation of A_n to temperature rise. This increase in dark respiration is generally related to the higher cost of cell maintenance, increase in membrane permeability, and enzymatic activity due to warming (Slot and Kitajima 2015). Because dark respiration has a temperature optimum that is above the optimal A_n temperature, the A_n temperature decreases above a certain temperature while the R_d increases (Slot and Winter 2016). Previous studies have also shown that R_d appears to be increasingly responsive in the context of warming plants around the world (Bolstad et al. 2003; Campbell et al. 2007; Heskell et al. 2016), leading to an imbalance in carbon uptake and a decline in plant biomass production and growth. The decline can be

significant for young individuals because they are highly dependent of the metabolic reserves (Kitajima 1994, 1996).

According to our results, it appears that young plants exposed to warming could exhibit photorespiration as there was a rapid increase in $C_i:C_a$ ratio and a decrease in V_{cmax} with increasing warming. Photorespiration is a metabolic process in plants that occurs when the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) binds oxygen instead of carbon dioxide during photosynthesis. This process becomes particularly significant in warmer environments as it intensifies with increasing temperatures. Higher temperatures increase the oxygenase activity of RuBisCO and decrease solubility of O_2 less than the CO_2 inside the cell, leading to increased photorespiration and carbon loss (Peterhansel and Maurino 2011). Photorespiration not only decreases photosynthetic efficiency, but also consumes energy and generates toxic byproducts (Walker et al. 2016; Dusenge et al. 2019). Despite the cost of photorespiration, there are several evidence that photorespiration is a mechanism for coping with stress in light, temperature, and drought (Peñuelas and Llusà 2002; Quentin et al. 2015; Dusenge et al. 2019). At high temperatures, photorespiration not only helps maintain electron flow, thereby preventing photooxidation by reactive oxygen species (ROS), but also serves to provide a substrate for the production of isoprene, a vital volatile organic compound essential for protecting photosynthesis from thermal stress (Slot and Winter 2016). This dual function underscores the adaptive importance of photorespiration in plant resilience to elevated temperatures.

In the perspective of defense strategy of juveniles in understory, we observed an increase in leaf mass per area (*LMA*) in response to increasing temperatures, which could potentially increase the resistance of young trees leaves exposed to warming scenarios. The increase in *LMA* is associated with an enhance in structural defense, diminishing herbivory and pathogens (Nabeshima et al. 2001; Poorter et al. 2009b). Despite the contribution in structural defense of *LMA*, it is important to note that while this may provide some benefits, it does not fully offset the negative consequences of reduced carbon uptake related to the increase in dark respiration and photorespiration under certain strain conditions.

The reduction of carbon uptake by photorespiration can have a significant impact on the allocation of resources for growth and defense mechanisms, especially in the difficult,

low-light conditions of the understory (Zaragoza-Castells et al. 2008). Models for the future suggest that photorespiration will decline as atmospheric CO₂ concentrations are expected to increase (Peterhansel and Maurino 2011; Walker et al. 2016), but in scenarios of temperature increase, such as heat waves, photorespiration will play an important role in the survival and growth of young trees. As we experience temperature records being broken and temperatures exceeding 25°C, photorespiration reduces A_n and becomes energetically expensive (Busch 2020). As photorespiration is temperature dependent, the future projections of temperature increases and extreme heat events could significantly disrupt forest regeneration. However, further research on photorespiration in woody species is required to investigate the actual effects of this metabolism on seedling establishment, growth, mortality, and carbon balance in young plants at different forests.

Conclusions

Photosynthesis is a temperature-sensitive biochemical process, with net rates increasing within a certain temperature range but declining beyond it. However, our meta-analysis revealed a consistent pattern in net photosynthesis (A_n) in response to warming, highlighting the capacity for acclimation to new optimal temperatures (T_{opt}). This acclimation allows for the maintenance of photosynthetic performance despite the climate origin. Warming also tends to increase R_d , contributing to an imbalance in carbon uptake, potentially leading to reduced plant biomass production, particularly in young plants. Photorespiration, which intensifies with rising temperatures, appears to become a primary response in young plants coping with warming, affecting their growth and defense mechanisms.

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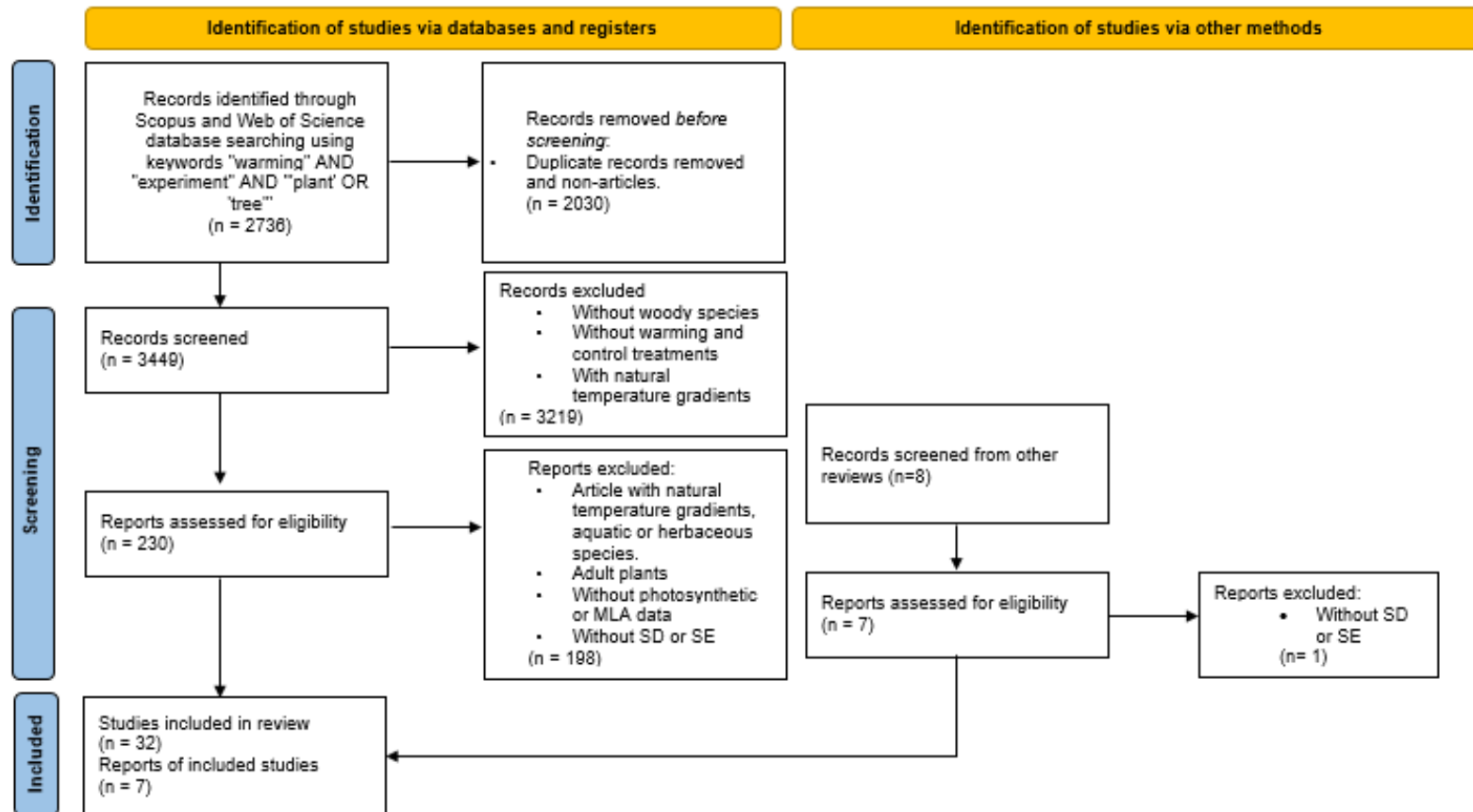
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Appendix

Figure S1: PRISMA framework flow diagram describing the process used for selection of studies for the systematic review meta-analysis

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, registers and other sources



*Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers).

**If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021;372:n71. doi: 10.1136/bmj.n71. For more information, visit: <http://www.prisma-statement.org/>

Table S2: Statistical results of meta-analysis results.

		Q	df	i^2	t^2
g_s	all	70.43	26	67.30%	0.03
	boreal	8.63	9	14.30%	0
	temperate	49.85	12	77.80%	0.04
	tropical	8.62	3	64.90%	0.13
$C_i:C_a$	all	84.8	17	78.90%	0.01
	boreal	5.07	5	10.60%	0
	temperate	72.74	11	80.30%	0.01
	tropical	NA	NA	NA	NA
F_v/F_m	all	1230.02	18	100.00%	0.17
	boreal	546.22	6	98.50%	0
	temperate	3.36	1	70.30%	0
	tropical	678.39	9	100.00%	0.29
J_{max}	all	140.79	25	81.40%	0.01
	boreal	4.4	4	27.50%	0
	temperate	53.42	8	80.80%	0.02
	tropical	33.86	11	73.00%	0.01
V_{cmax}	all	338.98	25	92.70%	0.05
	boreal	2.89	4	0.00%	0
	temperate	117.87	8	92.40%	0.05
	tropical	161.55	22	94.60%	0.06
A_n	all				0.0555

	boreal	224.47	25	93.40%	0.06
	temperate	2308.29	26	98.60%	0.08
	tropical				0.0183
	all	630.26	30	95.30%	0.17
<i>R_d</i>	boreal	9.18	9.18	36.80%	0.01
	temperate	416.62	12	97.50%	0.24
	tropical	122.69	10	93.70%	0.18
	all	1056.87	84	95.80%	0.04
<i>LMA</i>	boreal	64.55	5	91.30%	0.04
	temperate	254.66	20	87.60%	0.05
	tropical	735.05	57	96.70%	0.04
	all				

**CAPÍTULO 2 - Leaf thermotolerance of endemic and non-endemic tree species in
a hotspot threatened by global climate change**

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Leaf thermotolerance of endemic and non-endemic tree species in a hotspot threatened by global climate change

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Abstract. Climate change is affecting global biodiversity through an increase in Earth surface temperature and the intensity of heat waves. The Brazilian Atlantic Forest (AF) is recognized as one of the most threatened biodiversity hotspots by global climate change. To predict the consequences of extreme temperatures in AF tree species regeneration and diversity, it is necessary to understand the species' limits to temperature stress. In this study, we examined the leaf traits and leaf thermotolerance of seedlings of nine species of endemic and non-endemic AF trees grown experimentally in order to foresee how extreme heat events will affect the forest regeneration and diversity. We found that endemic species did not present lower thermotolerance compared to intermediate and wide-range species. There was a low relationship between the leaf traits and thermotolerance indices. On the other hand, we identified some species-specific responses to high temperature stress that deserve attention for tropical tree conservation, as is the case of *Arapatiella psylophylla*, a vulnerable endemic species with a very restricted geographical distribution. Our results suggest that the species-specific leaf thermotolerance an important criterion to be considered for tropical tree forest conservation in the context of global climate change.

Keywords: global warming, extreme climate events, heat stress, *PSII* chlorophyll fluorescence, and Tropical Forest.

Key Message

Leaf thermotolerance to sudden increases in temperature seems to be more species-specific dependent than its geographic distribution.

Introduction

The impact of global climate change on biodiversity is undoubtedly one of the greatest concerns of scientists, governments, and environmental managers in the 21st century. This phenomenon is closely related to the emission of greenhouse gases by human activities and has led to a gradual increase in average temperature worldwide (Allan et al. 2021). Not only is the temperature rising, but the occurrence of extreme events such as heat waves and intense droughts has become more frequent (Niu et al. 2014; Browne et al. 2021). These events are predicted to increase in intensity and frequency in the coming years (Allan et al. 2021), which could affect the regeneration and establishment of seedlings of tree species in natural forest ecosystems and degraded restoration sites.

In particular, the impacts of climate change may be more intense in tropical forests, which have a more stable environment with less temperature seasonal variability than temperate and boreal zones (Wright et al. 2009; Perez and Feeley 2020). In the tropics, the Atlantic Forest (hereafter AF) is one of the three hotspots in the world most threatened by global change, together with the Cape Floristic Province (South Africa) and the Polynesian and Micronesian Islands (Bellard et al. 2014). Furthermore, Brazilian AF is considered one of the most important biodiversity hotspots and hosts many endemic tree species (Cezar et al. 2009). Highlighting the Central AF region, encompassing the south of Bahia and northern of Espírito Santo States, which is considered a hot-point (Martini et al. 2007), due the highest endemic diversity of this biome (Wayt Thomas et al 1998.; Amorim et al. 2012). Currently, the AF is also under severe threat due to land use change and global climate change (Martini et al. 2007; Cezar et al. 2009; Colombo and Joly 2010). In this sense, a better understanding of how the increase in heat extremes will affect the diversity and endemic rates of the entire AF including its central region is fundamental to conservation.

Endemic species are generally more vulnerable to climate change than non-endemic native and introduced species, being 60% of endemic tropical species are threatened with extinction in future climate scenarios (Manes et al. 2021). This risk is usually associated with a smaller population size, a higher risk of decline due to habitat loss (Sambuichi et al. 2008), a narrower range of distribution, and a small ecological niche (Staude et al. 2020). Endemism arose through selection for very specific environmental conditions and thus the thermal niche of endemic AF species is narrower than broad

species. This thermal niche is the Atlantic coastal area and has lower temperature variability and higher pluviocity than continental areas (Thomas and Barbosa 2008). In this sense, non-endemic species, which have a wider niche and distribution far from the costal, could be more thermotolerant and displace the endemic species in extreme events. If this scenario is confirmed, the diversity of endemic species in the hot-point of the Central AF will decline, with far-reaching consequences for tropical conservation.

Studies that seek to understand the limits of a species thermal stress may help decipher species vulnerability to temperature increases (Geange et al. 2021). In this sense, the thermotolerance test has been useful in identifying morphotypes and species that are more sensible to heat stress (Slot and Kitajima 2015; Slot et al. 2021b). Using this method, Godoy et al. (2011) demonstrated that invasive species are more thermotolerant than native Mediterranean species, while Slot et al. (2021) observed that thermotolerance decreases with elevation. Different approaches can be used for thermotolerance testing, such as visual damage, and changes in electrolyte loss, leaf gas exchange, and chlorophyll fluorescence. In particular, thermotolerance of chlorophyll a is one of the most commonly used thermotolerance techniques because it analyzes how temperature affects the photochemical efficiency of the photosystem II (*PSII*), which is the most heat-sensitive part of photosynthesis (Geange et al. 2021; Münchinger et al. 2023). The effect of heat stress on the photochemical efficiency of *PSII* can be measured using the F_v/F_m ratio to characterize the loss of photochemical efficiency as a function of temperature. This allows calculation of thermotolerance traits and thus comparison of different species, treatments, or individuals.

With this in mind, the present study was conducted with the objective of investigating the responses of nine endemic and non-endemic Atlantic Forest species to extreme heat events. Considering that the ecological niche of Atlantic Forest endemic species is less variable and more restricted in comparison with non-endemic species, we formulate the hypothesis that endemic species present here have lower heat thermotolerance than the other species with a broader geographic distribution. We also assume that leaf traits are positively correlated with thermotolerance, particularly leaf mass area (*LMA*). Given these assumptions, we seek to answer the following questions: i) Can seedlings of Atlantic Forest tree species with different geographic distributions cultivated under nursery conditions be differentiated with respect to thermotolerance? ii) Is there a

relationship between thermotolerance and species leaf traits, such as *LMA*, leaf thickness, and chlorophyll content? iii) How do leaf traits influence thermotolerance in the species studied? Using these questions, we will examine the relationship between thermotolerance, leaf characteristics, and geographic distribution of Atlantic Forest tree species.

Materials and methods

Tree species and growth conditions

In our study, we selected nine tropical tree species: *Arapatiella psilophylla* (Harms) Cowan, *Byrsonima stipulacea* hurb., *Cariniana legalis* (Mart.) Kuntze, *Cedrela odorata* Ruiz & Pav., *Copaifera lucens* Dwyer, *Eschweilera ovata* (Cambess.) Mart. ex Miers, *Gallesia integrifolia* (Spreng.) Harms, *Garcinia gardneriana* (Planch. & Triana) Zappi and, *Trema micrantha* (L.) Blume. The seeds came from different matrix sources all from the AF central region, the only region of co-occurrence of all species, and were purchased from the Arboretum Institute nursery (Teixeira de Freitas, Brazil). This region is characterized as dense ombrophylous forest with climate hot, and humid, with no defined dry season being Af Köppen. Annual average temperature and precipitation of 25°C and 1,800 to 2,000 mm (Wayt Thomas et al. 1998; Reis et al. 2021). All tree species have ecological and silvicultural importance and occur naturally in tropical and seasonal rainforests (Thomas et al. 2009) and agroforestry systems associated with cocoa plantations in Brazil (Sambuichi et al. 2012). Also, three of the nine species in this study, *C. legalis*, *A. psilophylla*, and *C. odorata*, are vulnerable to extinction (IUCN 2022) (Tab. S1).

Based on available literature (BFG 2018), we classified the tree species into three distribution range categories: restricted, intermediate, and wide. We defined as restricted distribution, species that are endemic to the AF of southern Bahia and northern Espírito Santo (i.e., *A. psilophylla*, *C. legalis*, and *C. lucens*), while intermediate range species as those restricted to the Brazilian territory, occurring also in other biomes and states (i.e., *E. ovata*, and *G. integrifolia*). In addition, species that have a distribution along Latin America (i.e., *B. stipulacea*, *C. odorata*, *G. gardneriana*, and *T. micrantha*) were defined as wide-range species.

Ten seedlings per species were transferred to the nursery of Santa Cruz State University (UESC), located in AF central region in Ilhéus, Brazil, and transplanted into 1.7 L pots containing forest soil. All plants grew under the same light, soil, and water conditions to avoid environmental bias when comparing the different species. The nursery had a light attenuation of 60%, which is an intermediate light condition for all species. The seedlings were watered regularly.

After four months, we estimated three leaf traits and started the thermotolerance test of photosynthesis. On the day before the tests, the plants were irrigated abundantly to avoid any dehydration bias and at the beginning of the following morning, we selected one healthy, fully expanded and mature leave per plant for the evaluation of leaf traits and perform of thermotolerance tests.

Leaf traits

For each tree seedling, we evaluated the leaf mass per area (*LMA*), leaf thickness (*LT*) total chlorophyll content of one selected leaf (i.e., on leaf per individual, totaling 10 leaves per species). The chlorophyll concentration was measured with a SPAD-502 portable chlorophyll meter (Minolta, Japan) using the *SPAD index* that was later converted to $\mu\text{g cm}^{-2}$ (Coste et al. 2010). The leaf thickness (*LT*) was measured using an analogic micrometer (Zaas Precision, Brazil). The leaf or leaflet areas (*LA*) were estimated using the *ImageJ software* (Abràmoff et al. 2005; Brito-Rocha et al. 2017) from photos taken by a smartphone. The leaves and leaflets were placed on a standard white background sheet, which contained a scale used to calculate the *LA*. After the end of thermotolerance essays, the leaves and leaflets were dried in a forced air circulation oven at 60°C until constant mass to obtain the dry mass (*DM*). The *LMA* was estimated through the formula $LMA = DM/LA$.

Test of photosynthesis thermotolerance

Immediately after the evaluation of leaf attributes, the chlorophyll fluorescence emission of each leaf was measured using a Pocket PEA portable fluorometer (Hansatech Instruments, UK), to calculate the maximum quantum efficiency of photosystem II (F_v/F_m). This measurement corresponds to the initial F_v/F_m value at standard laboratory temperature (23°C). To avoid mechanical stress of cutting leaf discs that resulted in water loss problems in previous tests (unpublished data), we used entire

leaves or leaflets for all the thermotolerance tests. After measuring the chlorophyll fluorescence, the leaves or leaflets were placed in sealed plastic bags to avoid water loss and direct contact with hot water. Then, the bags were immersed in a preheated water bath using a Sous-Vide system for slow cooking (Huispark). In particular, the leaves or leaflets were exposed to seven categories of temperature (*i.e.*, 30, 35, 40, 45, 50, 55 e 60°C) whose irreversible damage is already visually observed. The water temperature was measured by the multi-purpose thermometer TM879 (Equitherm) and no differences were found between the preprogrammed values on the Sous-Vide water heater and the reference thermometer. After 15 minutes at each temperature, the leaves or leaflets were placed between two layers of wet paper towels for another 15 minutes in the dark at room temperature for F_v/F_m measurements. Then, the leaves or leaflets were heated again at more 5°C and the process was repeated until 60°C. The use of the same leaves occurred due to a limitation in the number of leaves available in seedlings and to simulate the gradual increase in temperature (Geange et al. 2021). The water temperature and F_v/F_m data were used to determine the maximum temperature that promotes the loss of 50% of the initial F_v/F_m (referred as T_{50}) and the temperature at which F_v/F_m decreases dramatically (referred as T_{Crit}) (Marias et al. 2017; Kumarathunge et al. 2019; Feeley et al. 2020). We used the equations described in Slot et al. (2021) to estimate the T_{50} and T_{Crit} using the formula:

$$F_v/F_m = (F_v/F_{m,Max}) / (1 + e^{b \cdot (T_{leaf} - T_{50})}),$$

where $F_v/F_{m,Max}$ is the upper horizontal asymptote representing F_v/F_m associated with healthy, non-stressed leaves, b is the steepness of the decrease in F_v/F_m around T_{50} , and T_{Leaf} is the incubation temperature (°C) of the leaf. During the thermotolerance tests, the temperature of the laboratory's air conditioning was set at 23°C.

Statistical analysis

The thermotolerance curves were fitted with the “*nlsLM*” function from the *minpack.lm* package in *R* version 4.2.1 (Elzhov et al. 2022). From the curves, we estimated individual values of the of T_{50} and $F_v/F_{m,Max}$ for each plant individual and calculate the mean species' values with their standard errors. T_{Crit} was estimated as the temperature at which a line describing the slope of the F_v/F_m decline at T_{50} intersected with a horizontal line indicating $F_v/F_{m,Max}$ (Slot et al. 2021b). Individuals' outliers were excluded to

achieve a better adjustment of the curve models. Therefore, one individual was excluded from *A. psilophylla*, *C. legalis*, *C. odorata*, *E. ovata* and *G. gardneriana*. We had to exclude individuals of *C. lucens* with low initial F_v/F_m , maintaining only three individuals. All thermotolerance curves were compared using maximum likelihood for best fit and were selected according to the lowest Akaike information criterion corrected (AIC_c) for small samples (Berk 2018).

The thermotolerance parameters (T_{50} and T_{crit}), and leaf traits variables (LMA , LT , and total chlorophyll content), were compared between the species using Fisher's one-way ANOVA or Kruskal-Wallis test with *post-hoc* Tukey's Honest significant difference test or Bonferroni's test (respectively) in order to describe and compare the species thermotolerance.

Using generalized linear mixed models, we assessed the effects of leaf traits (LMA , LT , or chlorophyll content) on thermotolerance indexes (T_{50} or T_{crit}), included the distribution categories (restricted, intermediate, and wide-range) as fixed effects. To account the species effect, we also used the species as random effect in the model. The tweedie family distribution was selected for the model according to test of singularity, convergence, zero inflation and overdispersion. The model presented convergence but no singularity. All statistical analyses were performed using *R* statistical software with the *glmmTMB* function in the *glmmTMB* package (R Core Team 2022).

In order to test whether the species thermotolerance differed along different ranges groups, we compared the values do T_{50} and T_{crit} among the three distribution range categories (i.e., restricted, intermediate, and wide) using Kruskal-Wallis. All statistical analysis were done in *R* version 4.2.1.

Results

The nine tropical tree species presented an initial maximum photosystem efficiency F_v/F_m varying between 0.7 and 0.8, implying no photodamage or other stress (Fig. 1). In particular, high F_v/F_m values were maintained at room temperature of 23°C up to 30°C to most species. However, a sharp decline was observed above 40°C to 50°C in all species, with the T_{crit} from 45°C to 51°C. In our study we also detected that the T_{50} ranged from 49°C to 54°C. Only *G. gardneriana* maintained F_v/F_m above 0.2 at 60°C, while most tree species reached F_v/F_m value equal zero in this temperature (Fig. 1).

Among studied tree species, *G. gardneriana* and *G. integrifolia* achieved the highest mean values of T_{50} (Fig. S2), with respectively 54.84°C and 54.59°C. The lowest mean values of T_{50} were observed in *A. psilophylla*, *E. ovata*, *C. odorata*, and *T. micrantha*, ranging between 49.50°C and 50.71°C. The other three species, *B. stipulacea*, *C. legalis*, *C. lucens*, stayed in between those extremes.

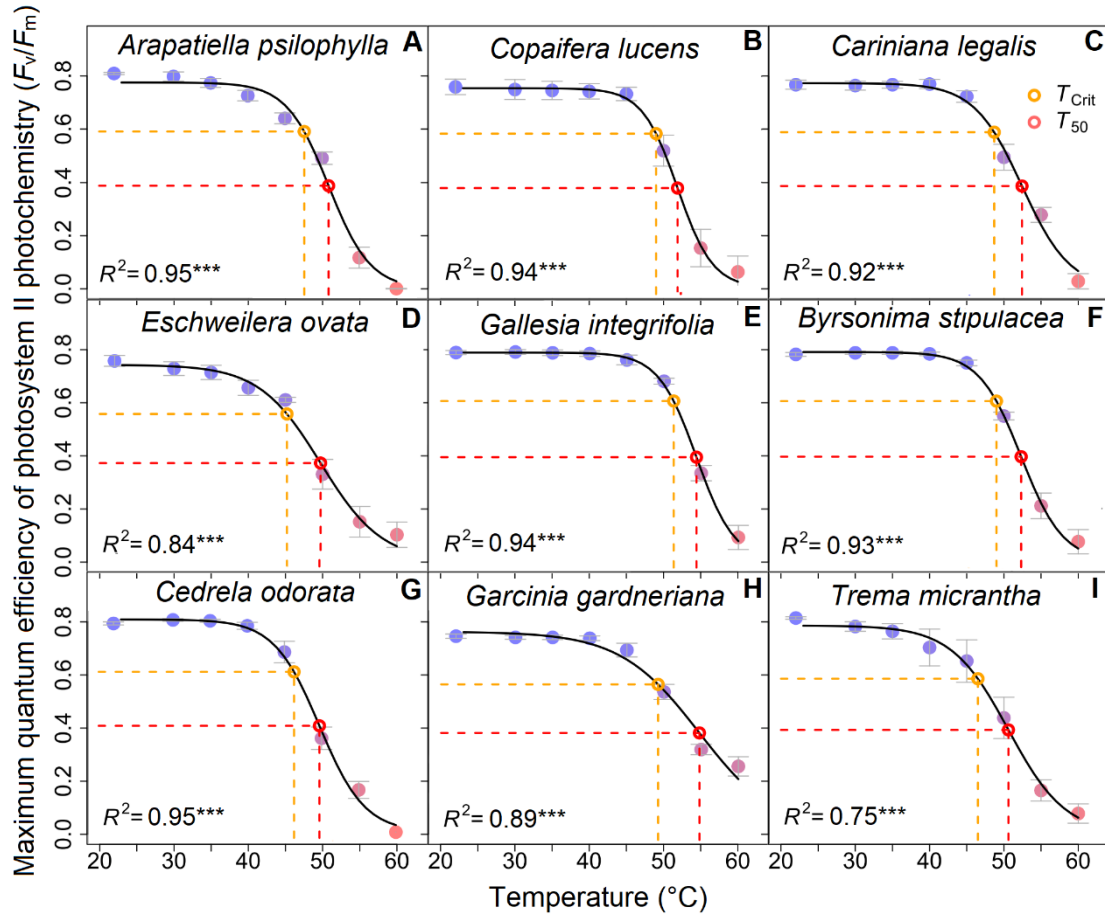


Figure 1. Thermotolerance curves of nine tropical tree species. A-C: restricted range (AF endemic species), D-E: intermediate range (Brazilian territory distribution), and F-I: wide-range (Latin America distribution) species. The red dashed lines represent the decrease of 50% of F_v/F_m (T_{50}) and the orange dashed lines and points indicate the temperature of the critical decline of F_v/F_m (T_{crit}). The orange and red points in each graphic represent the mean value and standard error of F_v/F_m in each temperature category. We also indicated the determination coefficient (R^2) of relationship between F_v/F_m and temperature for each tree species and its respective significance ($p > 0.001$ represented to ***).

According to the T_{crit} values, we have *G. integrifolia* and *B. stipulacea* the two higher values of T_{crit} , with 50.89°C and 49.42°C. The *G. gardneriana*, *C. legalis*, and *C. lucens* reached middle values (around 53°C), while *A. psilophylla*, *E. ovata*, *C. odorata*, and *T. micrantha* presented the lowest values ranging between 45.54°C and 47.38°C (Fig. 1 and Tab. S2).

Regarding leaf traits, we observed that mean *LMA* values ranged 40 to 160 g m⁻², *LT* ranged 0.15 to 0.35 mm, and plants' chlorophyll content ranged 28 to 61 µg cm⁻² (Fig. 2). Chlorophyll content was not related to species distribution. The lowest and highest values of leaf mass area and chlorophyll content were register in the wide-range species *C. odorata* and *G. gardneriana*, respectively (Fig. 2 and Tab. S2).

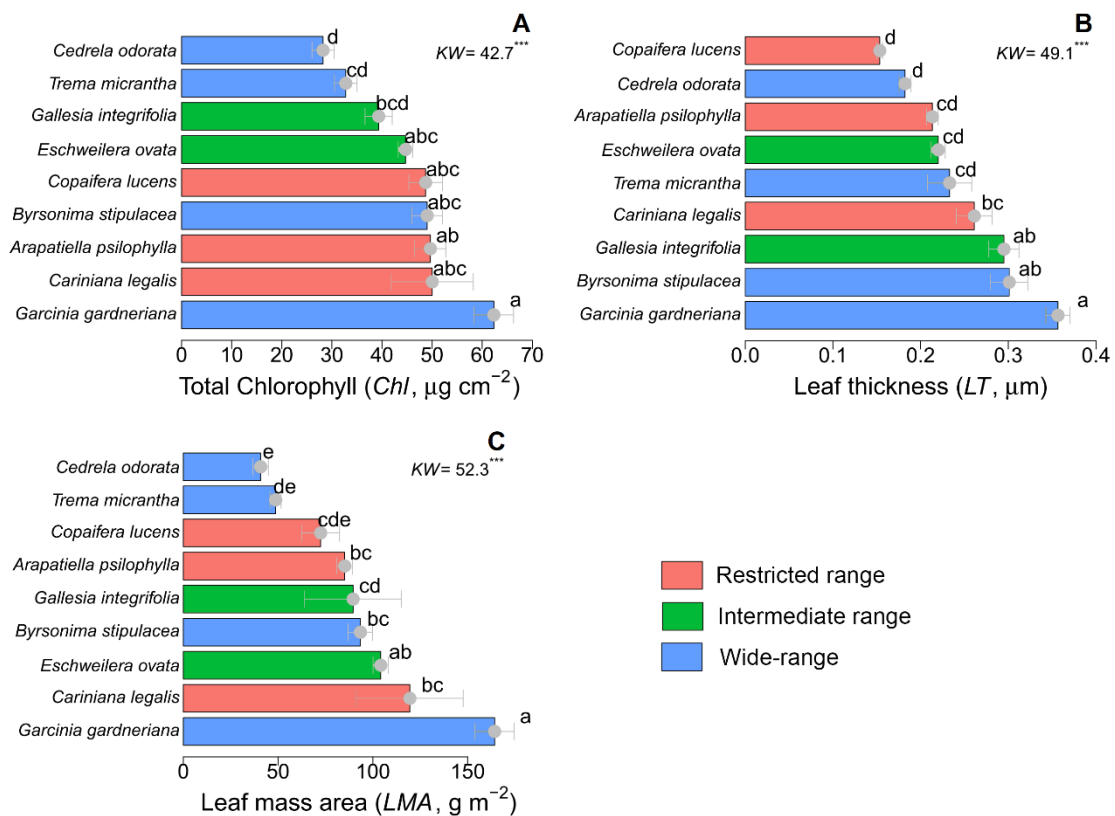


Figure 2. Leaf traits of nine tropical tree species grouped into three range distribution categories: endemic species to the AF (restricted range); restricted to the Brazilian territory (intermediate range); and distribution along Latin America (wide-range). (A) Total chlorophyll content; (B) Leaf thickness, and (C) Leaf mass area. Different letters indicate significant difference among species ($p < 0.05$; values: *mean ± standart error*)

Leaf traits showed little influence on thermotolerance. Since of the three leaf traits analyzed, only chlorophyll influenced the T_{50} and that leaf thickness positively effect of T_{Crit} (Fig. 3; Tab. S3).

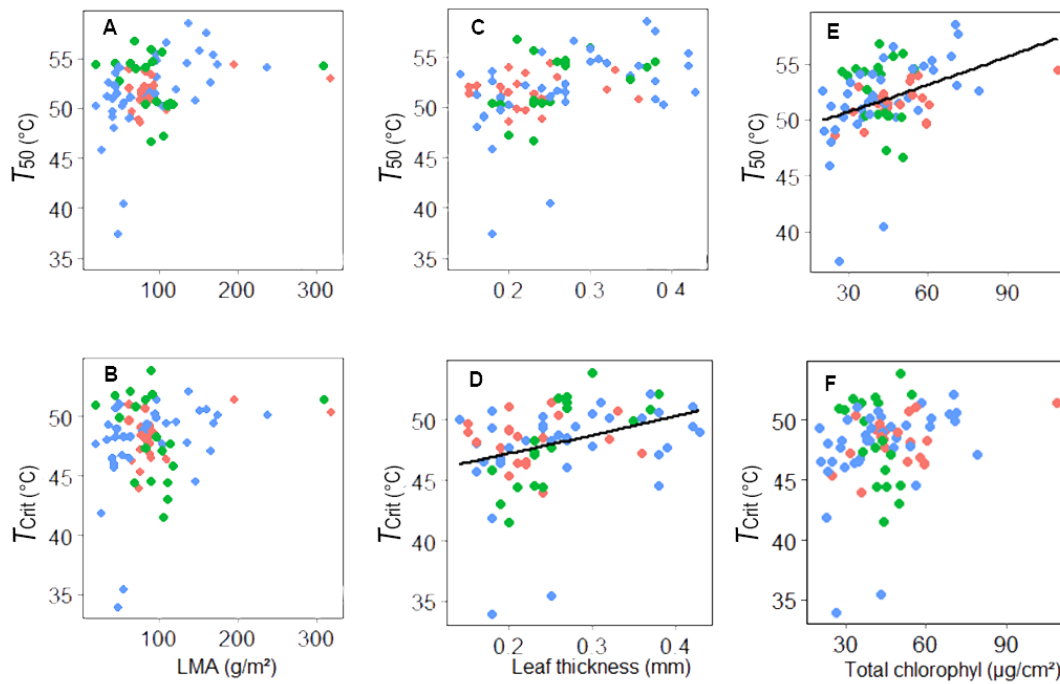


Figure 3. Relationship between thermotolerance indexes (T_{50} and T_{Crit}) of nine tropical tree species and leaf traits (leaf mass area - LMA , leaf thickness, and, chlorophyll content). Red, green and blue points represent respectively restricted, intermediate, and wide-range species. The black line represents the adjust of generalized linear mixed model.

Our species with wide distribution had distributions around 49 to 45°C of T_{Crit} and 49 to 54°C of T_{50} . For intermediate the T_{Crit} was 45 to 50°C and T_{50} of 50 to 54°C. Restricted species presented T_{50} of 45 to 50 and T_{Crit} of 50 to 52°C. Our findings indicated no significant differences of T_{50} and T_{Crit} among the three geographic distribution range categories in the species present in this study (Fig. 4).

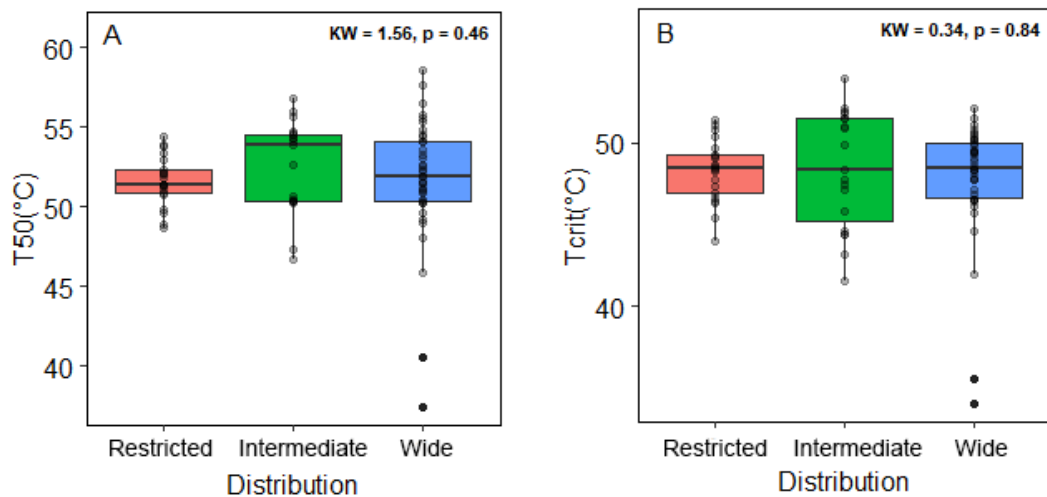


Figure 4. Thermotolerance indexes of the tree tropical species grouped in three distribution range categories: restricted range (red), intermediate range (green), and wide-range (blue). (A) T_{50} indicates the temperature that promotes the loss of 50% of the initial F_v/F_m , and (B) T_{crit} represents the temperature at which F_v/F_m decreases dramatically. In the upper right corner, we indicate the values of the Kruskal-Wallis test and the significance

Discussion

In our study, we compared nine species and found that the endemic species with stable habitat (low variability) were not particularly less thermotolerant than the other species found in other biomes and throughout Latin America. We expected that endemic AF species had less variability and thermotolerance than non-endemic species due the restricted ecological niche. This hypothesis was tested before, and no consensus was found yet. To bromelids for example, the endemic species *Vriesea minarum* did not exhibit lower thermotolerance than two widespread bromeliad species, *Vriesea bituminosa* and *Aechmea nudicaulis* (Chaves et al. 2018). For tropical trees there are suggestions of relationship, as there is a positive relationship between the estimated species optimum temperature (distribution) and thermotolerance (T_{50}) (Feeley et al. 2020), and a negative with the habitat elevation (also related to species distribution) and thermotolerance (Slot et al. 2021a). Thus, our results suggested that for our nine species, the thermotolerance is not related to species distribution and other factors such as species phylogeny can play a more important role for those species.

We found different thermotolerance responses through the species, agreeing that the response is species specific. The *A. psilophylla*, *E. ovata*, *C. odorata* and *T. micrantha*, are among the least thermotolerant species, indicating a high vulnerability to future extreme heat events. Specifically, *A. psilophylla* is on the Red List of Threatened Species (Sampayo-maldonado et al. 2019) and is endemic to the Central AF region. This species is used in forest restoration, and highly recommended since it attracts fauna and increases soil nutrient content (Sambuichi 2009). When the sensitivity to extreme heat events, small population, limited niche, and barochorous dispersal are added together in the context of *A. psilophylla*, the need for measures to protect this species is evident (Sambuichi 2009). Other iconic species is *C. odorata*, which is also a vulnerable species at the Red List. It has with anemochory dispersion and a wide distribution through Latin America (Mark and Rivers 2017). Despite the lower sensitivity when compared to *A. psilophylla*, *C. odorata* is emergent species with a large canopy, and acts as a keystone for biodiversity conservation by providing shelter, food, shade, moisture, and other resources to other species (Sampayo-maldonado et al. 2019). Thus, among the nine species studied, these two stand out for their higher risk of extinction, which is aggravated by exclusion due to competition or stress from climate change, emphasizing how fragile the diversity of the AF can be.

We did not detect any effects of *LMA* on thermotolerance indices, but we found that chlorophyll content and leaf thickness positively affected T_{50} and T_{Crit} . Functional traits such as seed weight, *LMA*, wood density, and tree height are used to reveal ecological differences among tropical tree species (Wright et al. 2009). In particular, *LMA* is an important, much-used leaf trait in the context of ecology and regional distribution of plant species (Poorter et al. 2009). Higher *LMA* is generally associated with less favorable environmental conditions, as seen in sclerophyllous and xeromorphic plants in warm or dry environments (Gratani and Bombelli 2000; Pariyar et al. 2017). In this sense, we expected *LMA* to be positively related to thermotolerance, as was observed in some previous studies (Sastry and Barua 2017; Slot et al. 2021a). However, there was no pattern in relation to *LMA*. Possibly, the number of species was not enough to see the relationship with this trait. Differences in leaf traits among species could also be related to ecophysiological traits associated with survival and growth of tree seedlings in the forest understory (Kitajima 1996; Lusk et al. 2008) or defense against herbivory (Nabeshima et al. 2001), rather than species-specific tolerance (Mielke et al. 2023). In

this sense, *G. gardneriana* had the highest values for *LMA*, *LT*, and chlorophyll content among the species studied and was among the highest thermotolerance indices, being the only species that did not reach 0 F_v/F_m at 60°C. The species in question occurs in a wide ecological niche, in ecosystems with different temperatures and water availability, such as the Amazon, Caatinga, Cerrado, and Atlantic Forest biomes (Muniz 2020). Therefore, in particular for *G. gardneriana*, although there is no relationship between the leaf traits and the thermotolerance, they could contribute to its establishment in different environments. Other factors such as heat shock proteins, chaperones or biochemical strategies could also contribute to the superior thermotolerance.

Similar to our results, tropical species generally have a photosynthetic thermal optimum around 30°C and further decline at 40°C and 50°C, corresponding to T_{crit} (Krause et al. 2015; Slot and Winter 2016; Sastry and Barua 2017; Kumarathunge et al. 2019). This temperature is only a few degrees above the temperature experienced by the leaves in the upper canopies of tropical trees, which exceed 40 °C at midday (Slot and Winter 2016; Ntawuhiganayo et al. 2019). These temperatures will be reached and exceeded more frequently as extreme events are expected to increase in frequency and intensity. These changes will affect populations, and it is important to take action to protect the most vulnerable species. Thermotolerance has proven to be a useful method for determining the limits of a species' heat stress and deciphering species' vulnerability to temperature increases, which can aid in conservation efforts (Geange et al. 2021). Thermotolerance indices, particularly the T_{50} more than the T_{crit} , could help improve criteria for classifying species according to their extinction risk in the context of physiological conservation (Wikelski and Cooke 2006). It is also recommended that conservation institutions retain genetic material from multiple populations of these species for germplasm banks, seedlings for botanical gardens, and seeds for seed banks and thus partially perpetuate the variability of the species.

Conclusions

Our study suggests that the endemic species studied are not particularly less thermotolerant than the non-endemic species. Although we did not find a significant relation, more resilient species appear to have greater thermotolerance. Comparing the thermotolerance indexes, the T_{50} has great potential to improve the criteria for classifying species according to their extinction risk in future climate scenarios

compared to T_{Crit} . This study contributes for the development of theoretical approaches and conservation practices related to shifts in species composition at the population and community levels in a tree diversity Hotpoint within one of the most vulnerable hotspots to global climate change.

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Appendix

Table S1: Conservation status, distribution, and morphological traits of nine tropical tree species used in our study.

Species	Distribution	Occurrence	Conservation status	Leaf type	Maximum height (m)
<i>Arapatiella psilophylla</i>	Restricted	Atlantic Forest restricted	Vulnerable	Compound	25
<i>Cariniana legalis</i>	Restricted	Atlantic Forest restricted	Vulnerable	Simple	60
<i>Copaifera lucens</i>	Restricted	Atlantic Forest restricted	Least Concern	Compound	35
<i>Eschweilera ovata</i>	Intermediate	Brazilian restricted	Least Concern	Simple	20
<i>Gallesia integrifolia</i>	Intermediate	Brazilian restricted	Least Concern	Simple	30
<i>Byrsonima stipulacea</i>	Wide	Latin-America	Least Concern	Simple	20
<i>Cedrela odorata</i>	Wide	Latin-America	Vulnerable	Compound	35
<i>Garcinia gardneriana</i>	Wide	Latin-America	Least Concern	Simple	15
<i>Trema micrantha</i>	Wide	Latin-America	Least Concern	Simple	20

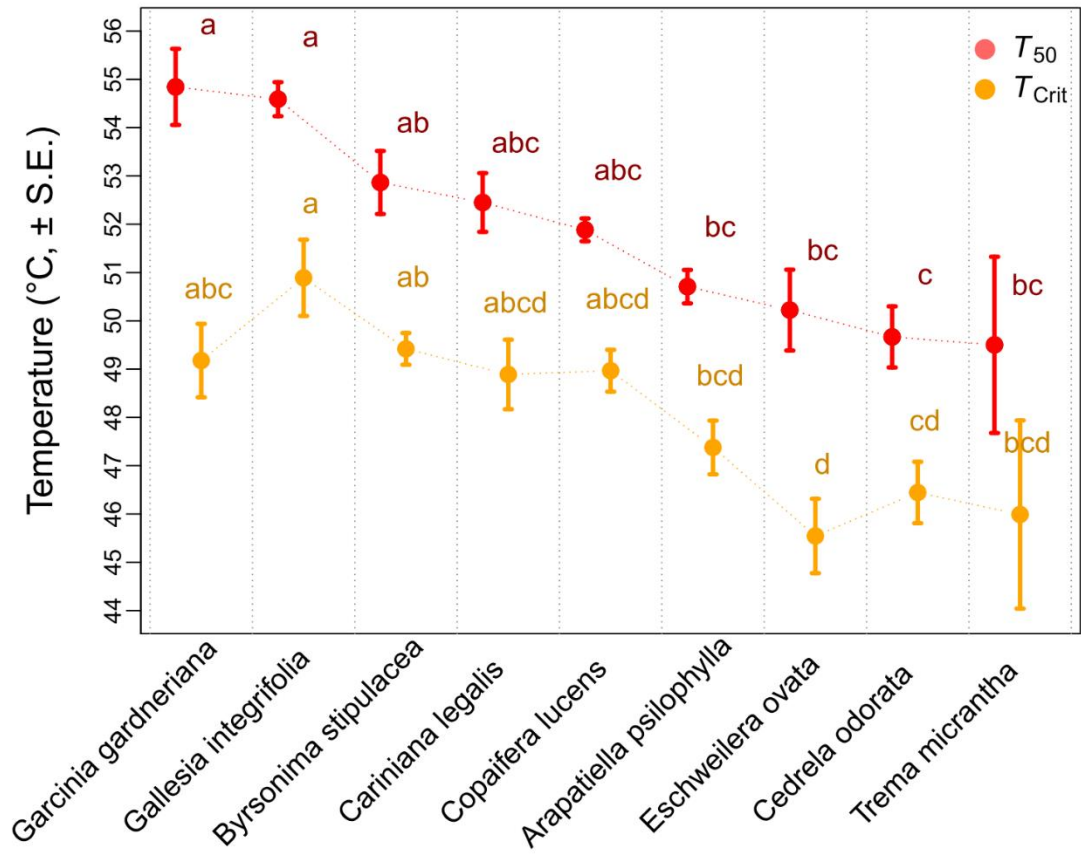


Figure S1. T_{50} and T_{crit} of nine tropical tree species. Different letters indicate significant difference ($p \leq 0.05$) among species. Values are represented by temperature mean and standard error.

Table S2: Distribution range, thermotolerance and leaf traits values (mean and standard error) of nine tropical tree species. Different letters indicate significant ($p \leq 0.05$) difference among species. *LMA*, *LT* and *n* indicate respectively leaf mass area, leaf thickness and number of individuals used in the analysis ($p < 0.05$; values = *mean* \pm *SE*).

	Distribution	T_{crit}	T_{50}	<i>LMA</i> (g m ⁻²)	<i>LT</i> (mm)	Chlorophyll ($\mu\text{g cm}^{-2}$)	<i>n</i>
<i>Arapatiella psilophylla</i>	Restricted	47.38 \pm 0.56 bcd	50.71 \pm 0.36 bc	85.09 \pm 4.06 bc	0.21 \pm 0.01 cd	49.63 \pm 3.13 ab	9
<i>Cariniana legalis</i>	Restricted	48.89 \pm 0.72 abcd	52.45 \pm 0.61 abc	119.45 \pm 28.20 bc	0.26 \pm 0.02 bc	50.01 \pm 8.18 abc	9
<i>Copaifera lucens</i>	Restricted	48.97 \pm 0.43 abcd	51.88 \pm 0.24 abc	72.39 \pm 8.17 cde	0.15 \pm 0.00 d	48.70 \pm 3.33 abc	3
<i>Eschweilera ovata</i>	Intermediate	45.54 \pm 0.77 d	50.22 \pm 0.84 bc	104.11 \pm 4.01 ab	0.22 \pm 0.01 cd	44.69 \pm 1.45 abc	9
<i>Gallesia integrifolia</i>	Intermediate	50.89 \pm 0.79 a	54.59 \pm 0.35 a	89.53 \pm 25.53 cd	0.30 \pm 0.02 ab	39.59 \pm 2.72 bcd	10
<i>Byrsonima stipulacea</i>	Wide-range	49.42 \pm 1.04 ab	52.86 \pm 0.65 ab	93.40 \pm 6.41 bc	0.30 \pm 0.02 ab	49.01 \pm 3.07 abc	10
<i>Cedrela odorata</i>	Wide-range	46.44 \pm 0.64 cd	49.67 \pm 0.63 c	40.79 \pm 4.12 e	0.18 \pm 0.01 d	28.22 \pm 2.21 d	9
<i>Garcinia gardneriana</i>	Wide-range	49.18 \pm 0.76 abc	54.84 \pm 0.79 a	160.14 \pm 10.61 a	0.35 \pm 0.01 a	61.23 \pm 3.88 a	9
<i>Trema micrantha</i>	Wide-range	45.99 \pm 2.05 bcd	49.50 \pm 1.92 bc	48.67 \pm 2.90 de	0.23 \pm 0.03 cd	32.77 \pm 2.37 cd	10

Table S3: Results of the generalized linear mixed models used to assess the influence leaf traits (leaf mass area, leaf thickness and chlorophyll) and distribution range categories (restricted, intermediate and wide-range) on thermotolerance indexes (T_{50} and T_{crit}). Values represent Estimate, standard error, and P -value. Significant values are indicated in bold.

	T_{50}			T_{crit}		
	Estimate	Std. Error	P -value	Estimate	Std. Error	P -value
Intercept	3.930	0.025	<0.001	3.857	0.268	<0.001
Leaf mass area	0.000	0.000	0.241	0.000	0.000	0.181
Intermediate	0.013	0.032	0.674	-0.001	0.033	0.980
Wide-range	-0.001	0.027	0.983	-0.011	0.028	0.696
Intercept	3.894	0.000	<0.001	3.812	0.033	<0.001
Leaf thickness	0.226	0.136	0.097	0.294	0.122	0.016
Intermediate	0.007	0.026	0.796	-0.011	0.257	0.668
Wide-range	-0.010	0.022	0.654	-0.025	0.022	0.263
Intercept	0.882	0.031	<0.001	3.832	0.035	<0.001
Chlorophyll content	0.001	0.001	0.013	0.001	0.001	0.104
Intermediate	0.024	0.028	0.379	0.005	0.032	0.858
Wide-range	0.009	0.024	0.706	-0.006	0.027	0.829

CAPÍTULO 3 – Heat stress tolerance of coastal plants in Brazilian Atlantic Forest

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Heat stress tolerance of coastal plants in Brazilian Atlantic Forest

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Abstract. The increase in frequency and intensity of climate extremes pose significant threats to ecosystems and biodiversity worldwide. As these events, such as heatwaves, become more frequent, prolonged, and severe, the species-specific response to temperature increases transforms ecosystem dynamics, favoring thermotolerant species while damaging others. We assessed leaf traits (leaf mass areas, leaf thickness and chlorophyll content) and leaf thermotolerance through chlorophyll *a* fluorescence in four coastal species, two native and two invasive. Despite observing no difference in leaf thermotolerance between species, there was notable differences in leaf mass area and thickness. The amplified thermotolerance observed in beach species underscores their resilience and adaptation to harsh coastal conditions, potentially attributed to acclimation or adaptation processes. Further investigations considering the influence of environmental factors on leaf thermotolerance are suggested to understand the thermotolerance responses.

Keyword: Thermotolerance, coastal areas, *Anacardium occidentale*, *Allagoptera arenaria*, *Acacia mangium*, and *Terminalia catappa*.

Introduction

Climate extremes are events that are rarer in frequency and distribution over time (Niu et al. 2014). Because they are climate anomalies, they shift ecosystem functions in a higher or lower state than usual and pose a threat to human society and biodiversity (Browne et al. 2021). These events, such as frosts, heat waves, floods, and droughts, are becoming more frequent, prolonged, and intense in recent decades (Perkins-Kirkpatrick and Lewis 2020; IPCC 2023). In particular, extreme temperature events, commonly referred to as heat waves, have increased on all continents, reaching unprecedented temperatures (Copernicus Climate

Change Service 2023). Future models suggest that these extreme events are likely to increase in the future if carbon emissions do not decline, threatening biodiversity conservation (Perkins-Kirkpatrick and Lewis 2020; Kitudom et al. 2022).

Because species responses to extreme events is species-specific, periods of temperature increase can lead to differences in competition and ecosystem dynamics (Niu et al. 2014; Olsen et al. 2016; Slot and Winter 2016). In this sense, species that are more tolerant to temperature increases, competitively benefit from warmer periods if they can maintain their functional metabolism at warmer temperatures, while less thermotolerant species already show some stress. Invasive species, in particular, are highly competitive and capable of invading new areas due to their high dispersal, rapid reproduction, and fast growth. They already pose a significant threat to biodiversity and ecosystem stability and require management and control efforts to mitigate their impacts (Bañuelas et al. 2019). As invasive species tend to have a higher competition traits (de Melo-Silva et al. 2014; Tietze et al. 2019), it is possible that they respond differently in the context of heat events. Previous studies show that high temperatures may favor some invasive species, such as *Schinus terebinthifolius* in the United States and eight invasive species of the Iberian Peninsula under water stress (Godoy et al. 2011; Osland and Feher 2020), which could interfere in those ecosystem balance.

Native species of the Atlantic Forest (AF) present a particular risk in the context of their conservation, as this habitat is one of the three hotspots in the world most threatened by climate change (Bellard et al. 2014). This vulnerability is the result of severe degradation and fragmentation, which has left about 28% of the original area of 150 million hectares intact (Rezende et al. 2018). Nevertheless, the AF has a great diversity resulting from its different forest formations, including the coastal area with mangroves, restingas (vegetation with marine influence) and herbaceous vegetation with fluvial-marine influence (Rezende et al. 2018). Those ecosystems compass marginal communities located in between the sea and the AF wet forest (Scarano 2009). The harsh conditions of high salinity, sandy soil with high permeability, solar radiation, thermal amplitude, act as a filter for most AF plant species, which makes only a small percentage of AF species to colonize this environment (Scarano 2009; Fernandes and De Queiroz 2015). Those communities are also exposed to strong human influence, as they are part of the coastal zone. Severe ecosystem impacts and its destruction for the building of resorts and houses is a result of urban expansion (Silva et al.

2015b). Coastal areas, characterized by a highly stressful environment, pose an elevated risk during extreme heat events due to the likelihood that plants already exist at the limit of their environmental tolerance (French et al. 2017). As heatwaves are expected to increase even more in South America (Wikelski and Cooke 2006; Simolo and Corti 2022), it is important to see how native and invasive coastal species tolerate to heat extremes.

One of the most used methods to describe the species tolerance to heatwaves is the, thermotolerance of chlorophyll a fluorescence through analyze of the temperature influence on photosystem II (*PSII*) photochemical efficiency (Münchinger et al. 2023). The *PSII* is the most heat-sensitive part of photosynthesis electron transport chain. If temperatures surpass critical thresholds, it leads to *PSII* damage. The impact of heat stress on *PSII*'s photochemical efficiency can be measured using the F_v/F_m ratio, aiding in the determination of thermal tolerance traits like T_5 , T_{50} , and T_{95} (Slot et al. 2021a, c)

In this paper we selected two AF native species and two invasive species to test their thermotolerance. Our main hypothesis is that the two native species present less thermotolerance than invasive species and exhibit a minor thermotolerance index (lower T_{50} and T_{crit}). We expect the heat thermotolerance of invasive species to be higher compared to native ones and heat extreme events would be extreme prejudicial to native environment conservation. Through this investigation, we aim to contribute to the understanding of how native species respond to the sudden heat extremes

Material and methods

Selected species and area

Two native species and two invasive species were selected to study the thermotolerance of photosynthesis of restinga species in southern Bahia, Brazil. The two native species were *Allagoptera arenaria* (Gomes) Kuntze (Arecaceae) and *Anacardium occidentale* L. (Anacardiaceae), and the invasive *Acacia mangium* Willd. (Fabaceae) and *Terminalia catappa* L. (Combretaceae). The small palm *A. arenaria* is an endemic species (BFG 2023) with a coastal distribution found in coastal dunes (Noblick 2019). The popularly known as cashew tree, *A. occidentale*, has wide distribution around Brazil (BFG 2023). It has economic importance since its fruits and nuts are used for consumption. The Australian acacia *A. mangium* can significantly affect the environment due to competition for allelopathy and high-water requirements (Leão et al. 2011; Attias et al. 2013). The second invasive species is *T.*

catappa, also known as beach almond, a species that occurs naturally in the East Indies, Indochina, Malaysia, Australia, the Philippines, and Taiwan (Leão et al. 2011). Both invasive species are native from tropical region. The Australian species, *A. mangium*, demonstrates allelopathy, impeding the germination of native species within its surrounds (Leão et al. 2011). It was initially introduced in Brazil to safeguard *Pinus* plantations against fire, and its continued presence is primarily driven by timber extraction and ornamental use (Attias et al. 2013). The other invasive species, *T. catappa*, capable of growing up to 30 meters tall, is widely used in urban forestry despite not being recommended (Fabricante et al. 2021; Vidal-Couto et al. 2023). The species' expansive distribution owes much to zoochory, predominantly facilitated by bats, enabling their spread across extensive areas (Sanches 2009; Guisande Rojas et al. 2012). Utilizing these diverse transportation methods, both species have invaded numerous coastal regions in Brazil, significantly impacting the local ecosystem (Sanches 2009; Guisande Rojas et al. 2012; Seki et al. 2022). Furthermore, their presence impedes the natural regeneration process by casting shade through their canopy, effectively excluding heliophilic species native to restinga habitats (Leão et al. 2011; Attias et al. 2013).

For the thermotolerance tests, leaves were collected from the coastal strip between the Cururupe and Acuípe rivers in the municipality of Ilhéus, southern Bahia, Brazil. All species occur spontaneously in the collection area. This area is composed of coastal barriers and lowlands (“tabuleiros”) inserted in the AF biome with moderate anthropogenic influence (Silva et al. 2015b, a). The area is composed specifically by evergreen ombrophilous forests with canopy trees reaching up to 50 m in height and an understorey of dense shrubs (Silva et al 2015a). Has continent-sea interaction systems, as mangroves, restingas and salt marshes (Silva et al. 2015a). The climate is Af Koppen, a tropical forest climate with no defined dry season and average annual average temperature and precipitation of 25°C and 1,800 to 2,000 mm (Reis et al. 2021). Six individuals of each species were selected, and six leaves were collected from each individual. Leaves were collected the day before the thermotolerance test, washed with fresh water to remove excess sea salt, and placed in labeled plastic bags that were placed in a thermal bag overnight. The next morning, the leaves were taken to the UESC Plant Physiology Laboratory where the leaf traits were measured and the thermotolerance test was performed. Some *A. arenaria* leaves presented a low initial F_v/F_m in the beginning of the thermotolerance test and other 2 individuals were added, each with six leaves. Leaves with initial F_v/F_m under 0.64 were excluded and at the end, 36 leaves of 8 individuals were used for *A. arenaria*.

Leaf functional traits and heat tolerance test

The leaf thermotolerance was determined by Chl *a* fluorescence using a heat bath and measuring the fluorescence along the temperatures. First, the leaves were measured for leaf thickness (*LT*), total chlorophyll content, and leaf mass area (*LMA*) of each selected leaf. Leaves were photographed to measure the leaf area (*LA*) using the software ImageJ (Abràmoff et al. 2005; Brito-Rocha et al. 2017). For each leaf the leaf thickness (*LT*) was measured using an analogic micrometer (Zaas Precision, Brazil) and leaf's chlorophyll *a* and *b* content was measured using a portable chlorophyll meter, ClorofiLog (Falker, Brazil). Finally, after the thermotolerance test, the leaves were dried in a forced air circulation oven at 60°C until constant mass to obtain the dry mass (*DM*) The *MLA* was estimated through the formula $MLA = DM/LA$.

Leaf thermotolerance test

After the measurement of the previous leaf traits the leaves were placed in a wet paper towel for dark-adapt for 15 minutes at controlled 23°C room temperature and measured the initial chlorophyll *a* fluorescence by the portable fluorimeter Pocket PEA (Hansatech Instruments, UK). The F_v/F_m , which represents the maximum quantum yield of photosystem II, were calculated by the equation $F_v/F_m = (F_m - F_o)/F_m$, where F_m and F_o are, respectively, the maximum and basal fluorescence yield for dark-adapted leaves. Later, the leaves were placed in a sealed plastic lock bag and immersed in a pre-heated water bath warmed by Sous Vide Slow Cooker with Thermal Immersion Circulatory (Huispark). The plastic bag was used to avoid water loss and direct contact with hot water. The temperatures set were 30, 35, 40, 45, 55, 55, and 60°C. After 15 minutes at each temperature, the same leaf was placed in a wet paper towel for 15 min in dark-adapted, and the F_v/F_m was measured again. The period of 15 min is usually commonly used in thermotolerance tests and mimetites better the extreme peak temperatures that are usually short in duration in the natural environment (Krause 2015). The samples were submitted to each temperature and measured following the heat and dark adapt step. The water temperature and F_v/F_m data were used to determine the maximum temperature that promotes the loss of 50% of the initial F_v/F_m (referred as T_{50}) and the temperature at which F_v/F_m decreases dramatically (referred as T_{Crit}) (Marias et al. 2017; Kumarathunge et al. 2019; Feeley et al. 2020). We used the equations described in (Slot et al. 2021) to estimate the T_{50} and T_{Crit} by fitting the data with Equation (1) as:

$$F_v/F_m = (F_v/F_{m,Max})/(1 + e^{b \cdot (T_{leaf} - T_{50})}) \text{ (Eq. 1),}$$

where $F_v/F_{m,Max}$ is the upper horizontal asymptote representing F_v/F_m associated with healthy, non-stressed leaves, b is the steepness of the decrease in F_v/F_m around T_{50} , and T_{Leaf} is the incubation temperature (°C) of the leaf. During the thermotolerance tests, the temperature of the laboratory's air conditioning was set at 23°C.

Statistical analysis

The chlorophyll content, LMA , LT , T_{50} , T_{crit} were compared between the species using Fisher's one-way ANOVA or Kruskal-Wallis test with *post-hoc* Tukey's Honest significant difference test or Bonferroni's test, respectively, in order to describe and compare the species thermotolerance.

Results

The four species differed in some extent throughout the leaf traits. The LMA ranged from 43 up to 214 g/m² in all species, but the native *A. arenaria* and *A. occidentale* presented lower values of LMA than the invasive *T. catappa* and *A. mangium* (Figure 1A). The thickest leaves were found in *A. arenaria*, with mean values around 0.49 mm, followed by the *A. mangium* with 0.35 mm. The two native species presented lower and statistically similar values of LT , being *T. catappa* and *A. occidentale* thickness of 0.25 and 0.21 mm respectively (Figure 1B).

For the photosynthetic pigments, *A. occidentale* presented the lowest values of chlorophyll a and b indexes among the four species, with mean values around 23.27 and 10.87 respectively (Figure 1C-D). The chlorophyll a did not differ in *T. catappa* to *A. arenaria* and *A. mangium*, with values of 20.5 to 32. The invasive species, *T. catappa* and *A. mangium* presented similar values of chlorophyll b content with values ranging between 8 to 27.

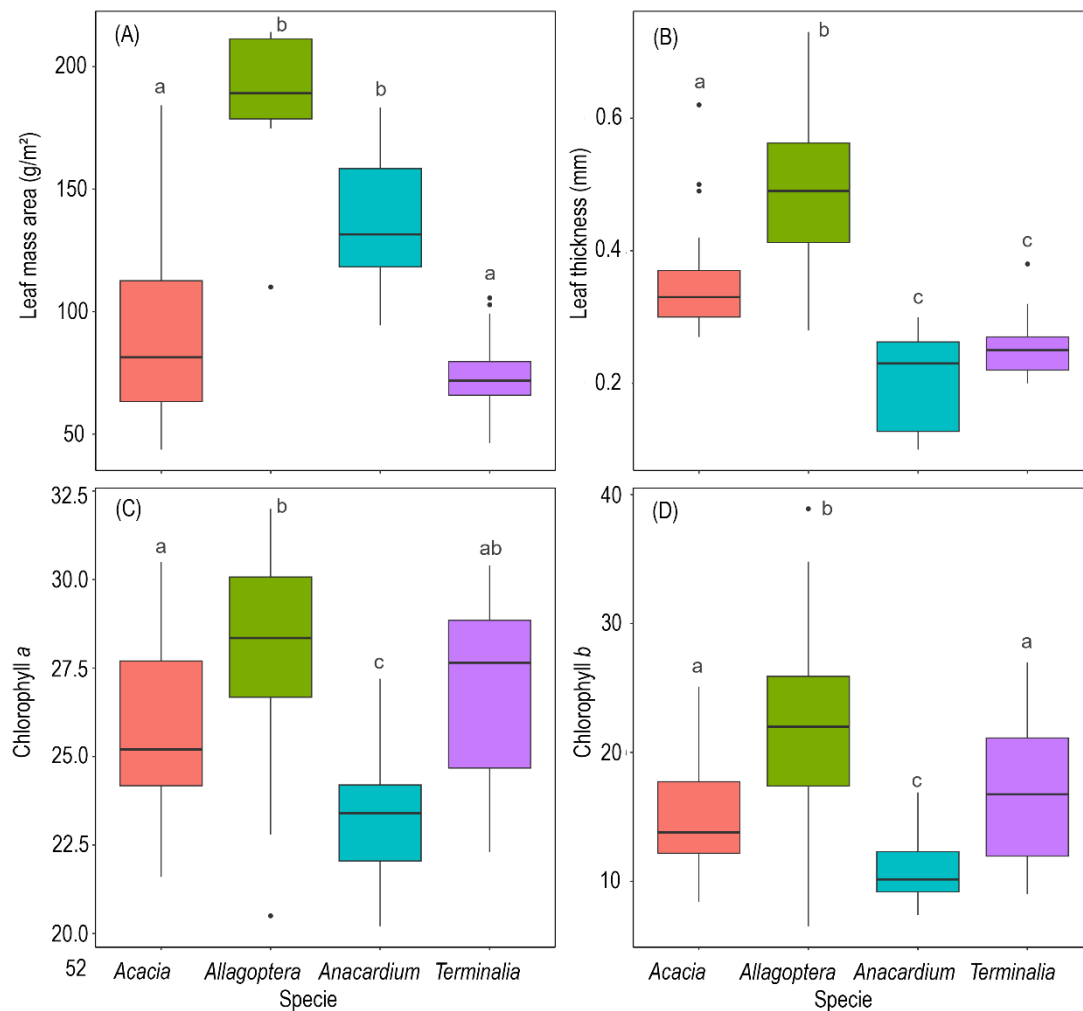


Figure 1. Leaf traits of four tropical shrubs species (A) Leaf mass area; (B) Leaf thickness, (C) Chlorophyll *a* index, (D) Chlorophyll *b* index. Different letters indicate significant difference among species ($p < 0.05$; values: *mean* \pm *standart error*)

The increase in temperature made the F_v/F_m decrease along reaching close to zero in almost all plants at 60°C (Figure 2A-D). The most substantial decrease in F_v/F_m occurred after 35°C. For the leaf thermotolerance traits, there was little difference between the species. The T_{crit} ranged between 37 to 51°C for all species. The mean T_{crit} of *A. occidentale* was of 43.3°C, while for *A. arenaria* was of 45.3°C, for *T. catappa* was of 46°C, and *A. mangium* was 47.4°C. The T_{50} for the four species was around 45 to 55°C (Figure 3A-B). The mean values of T_{50} for *A. occidentale* was of 48.2°C, while for *A. arenaria* was of 50.6°C. The invasive species presented higher mean values as the T_{50} of *A. mangium* was 50.7°C and for *T. catappa* was of 51.5°C. Although this difference in mean values, both thermotolerance traits

(T_{crit} and T_{50}) did not present significant difference along the species, with all species presenting similar thermotolerance.

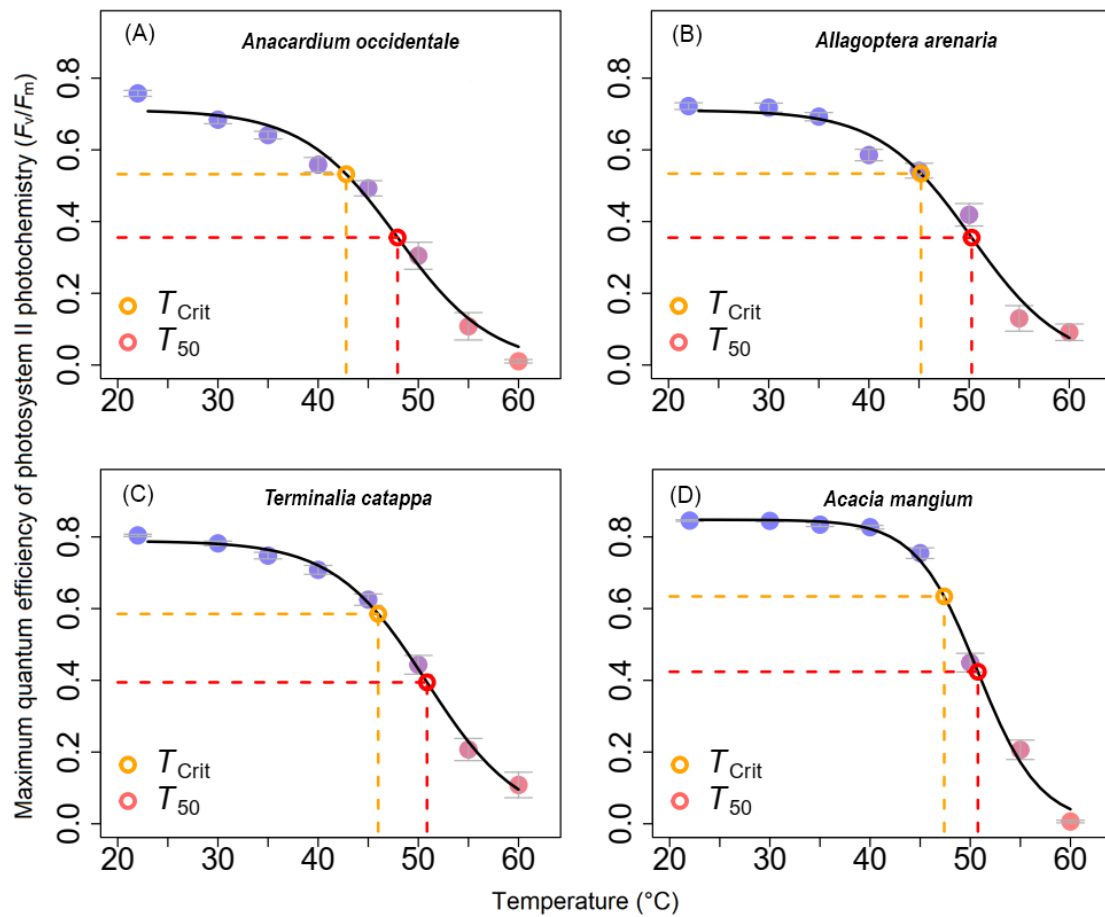


Figure 2. Maximum quantum efficiency of photosystem II photochemistry (F_v/F_m) in response to temperature increase in four coastal woody plants. (A) *A. occidentale*, (B) *A. arenaria*, (C) *T. catappa*, and (D) *A. mangium*. Red dashed lines represent the decrease of 50% of F_v/F_m (T_{50}) and the orange dashed lines indicate the temperature of the critical decline of F_v/F_m (T_{crit}). The orange and red points in each graphic represent the mean value and standard error of F_v/F_m in each temperature category.

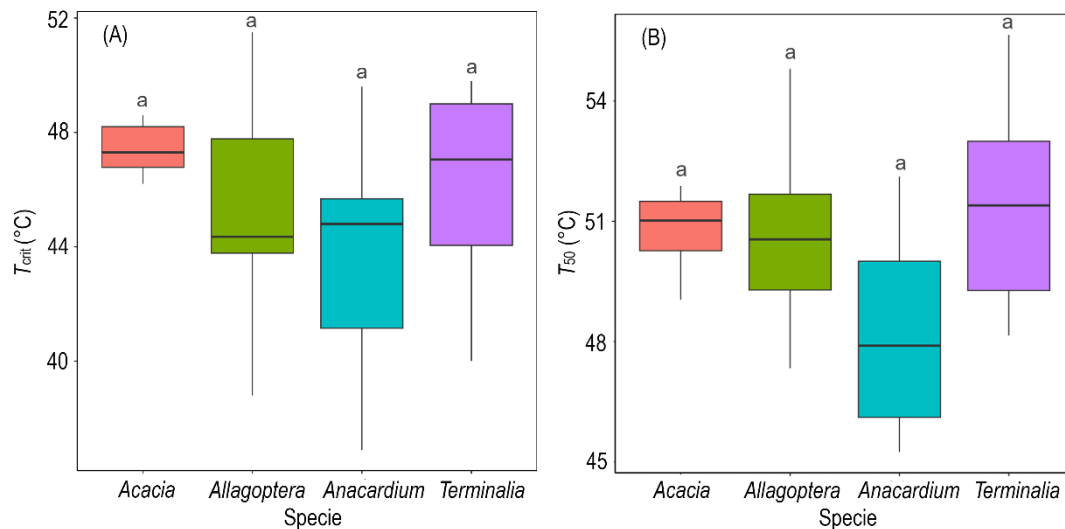


Figure 3. Leaf thermotolerance traits of four coastal woody plants: (A) temperature of the critical decline of F_v/F_m (T_{crit}), and (B) the temperature of 50% loss in F_v/F_m (T_{50}). Different letters indicate significant difference among species ($p < 0.05$; values: *mean* \pm *standart error*)

Discussion

Despite variations in global distribution and competitive abilities among the studied species, their leaf thermotolerance, measured as T_{50} and T_{crit} , exhibited similarity. However, distinctions in biomass allocation, specifically in leaf mass area and leaf thickness, were observed. Although there was no difference in leaf thermotolerance among the four coastal woody plants, the climate origin of invasive species is also warm, and the competitive ability of the invasive species is still at an advantage over the native species (Crone 2011; de Melo-Silva et al. 2014; Bañuelas et al. 2019). Invasive species commonly possess traits conducive to invasion success, including fast growth rates, high reproductive and dispersal capacities, and adaptability to diverse environmental conditions (Bhatt et al. 2023). The lower LMA observed in the invasive *A. mangium* and *T. catappa* correspond to fast growth strategies frequently associated with various alien invasive species (Leishman et al. 2007; Poorter et al. 2009), which could help the success of those species in the area. Although we found no difference in thermotolerance of chlorophyll a, earlier studies with *T. catappa* demonstrated an increase in seed germination and seedling growth in 1.6°C above the average (Tietze et al. 2019), which could improve the occurrence the open sandy areas (Sanches 2009; Suryawan et al. 2014) and at the edges of restinga forests (Sanches et al. 2007; Sanches 2009; Guisande Rojas et al. 2012).

The native palm tree, *A. arenaria*, exhibited a notably low photochemical yield within the collection area, suggesting potential stress within its natural environment. Generally, palm trees could present different physiological response and ecological function since they belong to monocots group, which can make it difficult to compare with other phylogenetically distant species. Nonetheless, previous study by Gessler et al. (2008) found evidence of acute photoinhibition in *A. arenaria*, indicating by a mean F_v/F_m of 0.70 and further confirming the presence of local environmental stress. In our sampling some *A. arenaria* leaves presented F_v/F_m below 0.60, which denotes considerable photochemical compromise. Additionally, prior studies have highlighted that elevated temperatures correlate with a decline in biomass growth in *A. arenaria* (Zani et al. 2023). Despite its vulnerability, *A. arenaria* holds significant ecological importance as one of the few pioneer species capable of colonizing bare sand soil, serving as a keystone species crucial for initial vegetation succession (Gessler et al. 2008; Scarano 2009). Consequently, the projected increase in heat waves may pose detrimental effects on *A. arenaria*. As only a few AF species are able to colonize coastal sandy open areas, the conservation of this particular species is very important to maintain the ecological richness and diversity within the AF ecosystem.

Coastal sand environment it is hostile conditions for diverse organisms (Moon and Ali 2022). Environmental stressors, such as salinity, nutrient-poor soil, well-drained sandy soil, are present in sandy coastal areas, as restingas, beaches and mangroves (Rosado and De Mattos 2010). These elements collectively act as an environmental filter, permitting only a limited number of AF species adapted to such stressors (Scarano 2009). Temperature fluctuations in these areas, influenced by sandy soil characteristics and proximity to the sea, can exhibit a mean daily coefficient of variation exceeding 15% (Gripp et al. 2023). In some cases, the midday air temperature reaches up to 33-40°C on the sand (Mantovani 2002; Gessler et al. 2008), which is above the optimal temperature for many tropical species (Slot and Winter 2016). Consequently, species residing in sandy coastal environments have developed significant thermotolerance, an adaptation to the demanding coastal conditions (Scarano 2009). The invasive tree *T. catappa*, occurs naturally at coastal areas of eastern India, Indochina, Malaysia, Australia, the Philippines and Taiwan (Leão et al. 2011), while the other invasive species, *A. mangium*, are particularly common in arid, semi-arid and dry subtropical regions of Australia (Attias et al. 2013). Hence, both species originate from habitats that are similar to the Restinga ecosystem and have developed adaptations to the numerous environmental stressors that characterize coastal regions. This adaptation not only contributes

to their successful invasion, but also indicates a likely high thermotolerance. This enhanced thermotolerance emphasizes the resistance of beach species to environmental stressors, indicating their ability to withstand fluctuating temperatures and stressful conditions. As the thermotolerance of these species could be related to environmental factors, it would be useful to test the effects of other environmental factors such as light, salt and water to see if thermotolerance changes with these factors.

Conclusion

The study revealed comparable leaf thermotolerance of T50 and Tcrit, among different coastal species with distinct world distributions and competitive capacities. However, contrasting biomass allocation, as evidenced by leaf mass area and thickness, diverged between these species. Despite similar thermotolerance, invasive species have a competitive advantage over native species, with fast growth rates, high reproductive and dispersal capacities, and adaptability to varied environmental conditions. The native species *A. arenaria* showed lower photochemical yield in its habitat, suggesting existing stress in its natural habitat. Furthermore, could be more susceptible to temperature increases. The high thermotolerance of coastal species underscores their ability to endure harsh coastal conditions and could be related to acclimation or adaptation. Further studies in environmental factors on thermotolerance are needed.

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CAPÍTULO 4 – Influence of light availability on leaf traits and thermotolerance plasticity in four tropical tree species used in forest enrichment plantations

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Influence of light availability on leaf traits and thermotolerance plasticity in four tropical tree species used in forest enrichment plantations

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Abstract. Human activities have profoundly altered the Earth's surface through deforestation, pollution, and resource overexploitation, leading to climate change and extreme climate events that favoring only certain species. To mitigate these effects and preserve biodiversity, forest conservation and restoration are needed, including the reintroduction of native species through enrichment plantings. Given the increasing risk of seedlings loss under extreme heat events, we investigated the response of leaf traits and thermotolerance of four species used in forest enrichment plantations to varying light gradients, under both nursery and field conditions. The leaf traits accessed were: leaf mass per area (*LMA*), leaf thickness (*LT*), chlorophyll index, leaf anatomy, and leaf thermotolerance parameters of 50% and critical loss of F_v/F_m (T_{50} and T_{crit}). Leaf thermotolerance has been shown to have a positive relationship with increased shade, which was observed in the nursery and field experiments, and suggests the effectiveness of forest enrichment in mitigating the impacts of extreme heat waves. Although light significantly influenced most leaf traits studied, its impact on leaf thermotolerance was notably lower compared to other leaf traits.

Keyword.: Heat waves, extreme events, chlorophyll *a* fluorescence, seedlings, restoration.

Introduction

Humans have directly influenced the earth's surface through deforestation, pollution and overexploitation of natural resources. This impact has drastically changed the Earth's surface and altered ecosystems in such a way that some species are more favored than others. Such changes are even leading to new evolutionary processes with the introduction of hybrids, alien species and genetically modified organisms that have led to the decline of many native

species populations (Pena Rodrigues and Lira, 2019). The most worrying human impact at global scale is climate change, which has already led to an increase in the Earth's average temperature by more than 1°C (IPCC, 2023). We can already see some of the major consequences of these changes, such as extreme weather events, which include extreme heatwaves, frosts, floods and droughts (Browne et al., 2021; IPCC, 2023; Perkins-Kirkpatrick and Lewis, 2020). These events are characterized by their rarity and intensity, which affect species communities in specific locations (Niu et al., 2014; Reyer et al., 2013). The short duration of these events overcome the ability of species to acclimatize to new conditions, as they represent intense and short-lived stress situations.

To effectively mitigate the effects of climate change and preserve biodiversity, forest conservation and restoration is necessary as it plays a crucial role in repairing the extensive damage that many forest areas have suffered (Bastin et al., 2019). Restoration consists of a targeted intervention aimed at restoring ecosystem function and returning to its initial functional state (SER, 2004; Standish et al., 2013). In especially severely affected areas where natural regeneration is insufficient, restoration often involves the deliberate reintroduction of native species, e.g. through tree planting (Laughlin and Clarkson, 2018). When the reintroduction of native species is necessary, the plantation of trees require careful planning in management and species selection in accordance with the target area (Liu et al., 2019). This strategic approach is essential to prevent the loss of seedlings, maximize the effectiveness of restoration investments, and guarantee the successful restoration (Cooke et al., 2018; Mangueira et al., 2019).

As restoration aims to return the ecosystem to its original state before human impact, it also aims to restore the original biodiversity of an area (SER, 2004). In this sense, many of the restoration efforts consist of increasing the biodiversity in the area and accelerating the succession path. This is the main purpose of enrichment plantings, which usually consist of planting native species of different successional stages that would not spontaneously regrow in the area, to increase biodiversity and ecosystem recovery in degraded landscapes (Liu et al., 2019; Mangueira et al., 2019). These actions are fundamental to enhance the structure, function and diversity of degraded areas and to diversify the forest understory (Laughlin and Clarkson, 2018; Mangueira et al., 2019). As this action usually targets degraded forests, the seedlings are planted in the understory of the forests for enrichment (Forbes et al., 2020; Laughlin and Clarkson, 2018). Herewith it is important to acknowledge the light conditions

in the understory to plan the enrichment action and the species selection in enrichment restoration actions (Forbes et al., 2020).

In addition to shaping the success of restoration initiatives, light also plays an important role in the plasticity of important leaf traits, such as leaf mass per area, leaf thickness, and chlorophyll content (Cerqueira et al., 2018; Mantovani et al., 2018), but it still unknown if leaf thermotolerance also have great or low plasticity in response to light availability. Previous work has tested whether shade leaves of mature tropical trees have lower heat tolerance than sun leaves and this hypothesis was partially supported (Slot et al., 2019). In two species (*Calophyllum inophyllum* and *Inga spectabilis*), thermotolerance was lower for shade leaves, but in one species (*Ormosia macrocalyx*) there was no difference in heat tolerance between shade and sun leaves. Thus, similar to Krause et al. (2015), who found a positive effect of light availability on the heat tolerance of sun leaves of tropical *Ficus insipida* and *Calophyllum longifolium* seedlings. Those relates together support the idea that thermotolerance is species-specific as seen in previous studies (Brito et al. 2023; Slot et al., 2019) but also the response of leaf thermotolerance to shade could be positive for most species. The intensity of this response could be measure through phenotypic plasticity, as for most leaf traits, but the plasticity of thermotolerance in light gradient is still not known.

In the context of climate change, phenotypic plasticity emerges as a potential mechanism to cope with environment conditions variation (Nicotra et al., 2010). A comprehensive evaluation of phenotypic plasticity requires the projection of an environmental gradient that reflects the conditions experienced by species in their natural habitat, providing a comprehensive understanding of the relevant range of the response norm (Gianoli and Valladares, 2012). In the specific context of studying phenotypic plasticity for forest enrichment, it is important to maintain a light gradient that approaches deep shade and resembles the natural environment for tropical seedlings (Valladares et al., 2016). In the broader context of forest enrichment under climate change, it is also particularly important to investigate how these species respond to temperature. Given the significant impact of temperature on plant metabolism and various physiological aspects, this stress has the potential to be lethal (Hatfield and Prueger, 2015; Slot and Winter, 2016).

As extreme heat events are expected to increase in duration, frequency and intensity due to climate change, there is an increased risk of heat stress for seedlings worldwide (Hatfield and Prueger, 2015; Perkins-Kirkpatrick and Lewis, 2020). This increased risk amplifies the

potential for increased seedling mortality, a concern already evident in restoration efforts (Mangueira et al., 2019; Stolarski et al., 2018). To address these challenges, evidence-based data for species selection is crucial to implement more successful restoration actions (Cooke et al., 2018). Furthermore, ecophysiology is proving to be an important tool in this context, especially with leaf traits studies (Rosenfield and Müller, 2020). By measuring leaf traits and leaf thermotolerance of four species used in enrichment plantings under both nursery and field conditions, we systematically tested the following hypotheses:

- 1- Although seedlings typically exhibit species-specific thermotolerance, and based on the previous reports of Krause et al. (2015) and Slot et al. (2019), we hypothesize a reduction in leaf thermotolerance under simulated conditions of dense shade.
- 2- As the leaf traits present a great and species-specific plasticity to the light environment, we hypothesized that the leaf thermotolerance also will present a great plasticity in a wide range of light availability.
- 3- Leaf traits, including thermotolerance, in an experimental forest enrichment plantation reflects the results obtained in the simulated dense shade conditions obtained in a nursery experiment.

We believe that our approach will help in the selection of species to be used in forest enrichment plantations in future extreme events context and better understand aspects of the leaf thermotolerance of tropical tree seedlings.

Material and methods

Species and growth conditions

Seedlings of four tropical species with contrasting light niche were selected for this study of leaf thermotolerance and light availability, they were: *Aspidosperma pyricollum*, *Cytharexylum myrianthum*, *Goniorrhachis marginata* and *Handroanthus chrysotrichus*. All four species are natural in Atlantic Forest (AF) used in reforestation, urban forestry and restoration enrichment (Table 1).

Table 1.: Species description

	<i>Aspidosperma pyricollum</i>	<i>Citharexylum myrianthum</i>	<i>Goniorrhachis marginata</i>	<i>Handroanthus chrysotrichus</i>
Family	Apocynaceae	Verbenaceae	Fabaceae	Bignoniaceae
Endemism and conservation	Endemic (LC)	Non endemic (NE)	Endemic (LC)	Non endemic (NE)
Biomes and vegetation	Occurs in AF and reports on Savannas and Caatinga	Broad nationally. Occurs in AF with in Riparian or Gallery, Seasonal Semideciduous, Ombrophylous (Pluvial Forest), and Mixed Ombrophylous Forests.	Restricted to AF northeast, center and associated Caatinga. In Seasonal, Seasonal Semideciduous, Ombrophylous (Pluvial Forest).	Broad nationally. AF, Pampa and Cerrado (lato sensu). Ombrophylous forest (Rainforest), Restinga, and Rocky Outcrops.
Heights	3-10m	15-25m	15 - 30m	12 - 25m
Ecological aspects	Early to late secondary species, or light-demanding climax, without preferences to soil conditions. Occurs both at the bottom of the valleys, on the mid-slope, and at the top. Tolerates low temperatures.	Pioneer with excellent natural regeneration in several stages of succession. Produces many flowers and fruit annually. Preferentially inhabits humid places.	Keystone as an evergreen species in deciduous forests. Dominates disjunct patches of seasonally dry tropical forests, arboreal caatinga or evergreen deciduous forests. Preferentially inhabits open areas, riche soils and along temporary rivers.	Commonly found in the AF and coastal sand areas. Preferentially in open, shrubby forests, as on hilltops or disturbed areas. Can adapt well to different climates, light intensities, soil, and water availability.
Economical use	Medicinal use as anti-inflammatory and one of the best species for in vitro activity against malaria	Restoration of riparian forests, urban afforestation and mainly attracting fauna.	Has a dark and heavy wood used for woodwork.	Ornamental use, reforestation and wood exploitation.

(Acra et al., 2012; Amaral et al., 2013; BFG, 2023; Carvalho, 2006; Do Carmo et al., 2015; Prenner and Cardoso, 2017; Rocca and Sazima, 2006; Rocha et al., 2014; Silva and Neves, 2014; Vital, 2018)

Thirty seedlings of each species were acquired from Programa Arboretum nursery (Teixeira de Freitas, Bahia, Brazil). All of the Arboretum Program's mother trees are located near Teixeira de Freitas, in the Atlantic Forest of southern Bahia. The seedlings were transferred to the nursery of Santa Cruz State University (UESC), located in Ilhéus, Bahia, Brazil. The UESC's nursery measures 20 × 15 m and is covered with a shade cloth that allows the passage of about 60% of light radiation at full sun. All seedlings were transplanted into 1.7 L pots containing forest soil.

Immediately after the transplant the seedlings were divided into six groups of five individuals per species. Each group was distributed into six light intensity treatments: five shade houses covered in black shade cloth were used for light attenuation and one in nursery light condition (i.e. 60% of full sunlight). Of the five shade houses, two presented a low light attenuation, and three a high light attenuation. The shade houses were distributed 1m above the ground on slatted benches, allowing air circulation. During the experiment, photosynthetic photon flux density (PPFD) in each light environment was monitored by light radiation sensors S-LIA-M003, coupled to a meteorological station Hobo Micro Station Data Logger (Onset, USA). The data logger was programmed to obtain readings at 1 sec interval and to store readings every 10 minutes. Based on the instantaneous values of PPFD ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$), we calculated the daily light integral (DLI) values ($\text{mol photons m}^{-2} \text{day}^{-1}$). The DLI for each treatment was $18.2 \text{ mol photons m}^{-2} \text{day}^{-1}$ in nursery condition, 9.2 and $8.0 \text{ mol photons m}^{-2} \text{day}^{-1}$ in low shade treatment, and 0.7, 2.9 and $1.4 \text{ mol photons m}^{-2} \text{day}^{-1}$ at deep shade treatment. Temperature data was provided by the meteorological station of the Cocoa Research Center (CEPEC) of the Executive Committee of the Cocoa Farming Plan (CEPLAC). Mean temperature over the experiment was of 23.54°C and the mean relative humidity was of 81.81. Maximum and minimum temperature at the site in the experiment period was 32.2°C and 15.0°C (Table S1). Small difference in the temperature between the different shade houses and the nursery was registered in previous works (Feijó et al., 2009; Santos et al., 2012).

The seedlings grew with abundant daily irrigation. The seedlings were rotated weekly in each treatment to avoid any local bias in the shade houses. After approximately 10 months, we started the leaf thermotolerance test. On the day before the test, the plants were irrigated abundantly and at the beginning of the following morning, we selected two healthy, fully expanded, and mature leaves of two individuals per tree species for the evaluation of leaf traits and perform of thermotolerance test.

Field experiment and conditions

Sixty seedlings of *A. pyricollum*, *C. myrianthum* and *H. chrysotrichus* were planted in two areas in Serra Bonita Private Heritage Reserve (Camacan, Bahia, Brazil) and studied to compare leaf thermotolerance under natural and nursery conditions. Seedlings were also obtained from the Programa Arboretum nursery (Teixeira de Freitas, Bahia, Brazil), transported, and planted in the areas. The planting areas consist of two cacao agroforestry

areas, one abandoned regeneration area (RA) and one active agroforestry (AGF), with a total of ten 1.5 x 1.5m plots per area, and twenty plots in total. The plots have varying degrees of shade and are spaced approximately 250 m apart. In each plot, nine seedlings were planted maintaining a minimum space of 60 cm between each seedling.

Over four months, plot temperatures were monitored using Hobo Pro V2 Data Logger stations (Onset Computer, Massachusetts, USA). The data was collected for a minimum period of one month in each plot. Data of three plots of the AGF were discarded due to data logger system fail. Light environments were estimated by hemispheric photograph using a Nikon Coolpix 4300 digital camera equipped with a hemispherical fish-eye lens (180°) set on a tripod 1.5 m above the ground and positioned at the center of the plot towards the magnetic north (Reis et al., 2021). All photos were taken at the beginning of the morning or in cloudy days to avoid overexposure. The total transmitted gap light (referred here as light transmission) at the center of each plot was calculated using Gap Light Analyzer (GLA) software (Reis et al., 2021). Transmitted light radiation corresponds the sum of direct and diffuse solar radiation transmitted by the canopy and a better indicator of light condition at the understory (Cerqueira et al., 2021). Photographs were taken in the first four month of planting and at the end of data collection and mean values of the months were used to describe plants growth conditions. Soil nutrient analysis was performed using a pool of samples collected from each plot in the two areas.

After one year of growth in field, leaves of all available seedlings were collected to conduct the leaf thermotolerance test. One leaf per seedling was collected and maintained in separated plastic bags sprayed with water and maintained in dark and cool temperature to avoid tissue damage. In the next morning the leaves were transported to the UESC's Plant Physiology laboratory for the measurements of leaf traits and thermotolerance.

Leaf traits

For each tree seedling, we evaluated the leaf mass per area (*LMA*), leaf thickness (*LT*), and chlorophyll index of one selected leaf per individual. The leaf or leaflet areas (*LA*) were estimated using the ImageJ software (Abràmoff et al., 2005; Brito-Rocha et al., 2017) from photos taken by a smartphone. For this, the leaves were placed on a standard white background sheet, which contained a scale used to calculate the *LA*. After the end of leaf thermotolerance essays, the leaves and leaflets were dried in a forced air circulation oven at

60°C until constant mass to obtain the dry mass (*DM*). The *LMA* was estimated through the formula $LMA = DM/LA$. The leaf thickness (*LT*) was measured using an analogic micrometer (Zaas Precision, Brazil). The chlorophyll index was measured with a SPAD-502 portable chlorophyll meter (Minolta, Japan) for nursery experiment and with the ClorofiLog portable chlorophyll meter (Falker, Brazil) for field experiment.

Leaves of the seedlings used in the nursery experiment were collected for leaf anatomy analysis. One healthy, fully developed and mature leaf per individual of *A. pyricollum*, *G. marginata* and *H. chrysotrichus* was collected and fixed in a 2.5% Formal-Acetic-Alcohol (FAA) solution in 0.1 M phosphate buffer for anatomical analysis. Anatomical analysis was conducted at Jardim Botânico do Rio de Janeiro Research Institute at Rio de Janeiro (Brazil). Specimens were dehydrated in an increasing ethanolic series and embedded in hydroxymethylacrylate resin (Leica). They were then sectioned and stained with 0.5% toluidine blue (O'Brien & McCully 1981). Anatomical transversal sections of 200 µm were photographed using a BX-50 microscope (Olympus, USA) and measured using Image Pro-Plus (Media Cybernetics, USA). The total mesophyll area, palisade and spongy parenchyma thickness were measured.

Leaf thermotolerance

Immediately after evaluation the leaf attributes, the chlorophyll fluorescence emission of each leaf was measured using a Pocket PEA portable fluorometer (Hansatech Instruments, UK), to calculate the maximum quantum efficiency of photosystem II (F_v/F_m). This measurement corresponds to the initial F_v/F_m value at standard laboratory temperature (23°C). To avoid mechanical stress of cutting leaf discs, that resulted in water loss problems in previous tests (unpublished data), we used entire leaves or leaflets for all the thermotolerance tests. After measuring the chlorophyll fluorescence, the leaves or leaflets were placed individually in sealed plastic bags to avoid water loss and direct contact with hot water. Then, the bags were immersed in a preheated water bath using a Sous-Vide system for slow cooking (Huispark). Leaves were exposed to seven categories of temperature (*i.e.*, 30, 35, 40, 45, 50, 55 e 60°C), we chose to use 60°C as maximum temperature due to irreversible damage is already visually observed. The water temperature was measured by the multi-purpose thermometer TM879 (Equitherm) and no differences were found between the preprogrammed values on the Sous-Vide water heater and the reference thermometer. After 15 minutes at each temperature, the leaves or leaflets were placed between two layers of wet paper towels for another 15 minutes

in the dark at room temperature for F_v/F_m measurements. Then, the leaves or leaflets were heated again at a temperature 5°C hotter and the process was repeated until 60°C. The use of the same leaves occurred due to a limitation in the number of leaves available in seedlings and to simulate the gradual increase in temperature (Geange et al., 2021). The water temperature and F_v/F_m data were used to determine the temperature that promotes the loss of 50% of the initial F_v/F_m (referred as T_{50}) and the temperature at which F_v/F_m decreases dramatically (referred as T_{Crit}) (Kumarathunge et al., 2019; Marias et al., 2017; Perez and Feeley, 2020). We used the equations described in Slot, Nardwattanawong, et al. (2021) to estimate the T_{50} and T_{Crit} . During the thermotolerance tests, the temperature of the laboratory's air conditioning was set at 23°C.

Relative distance plasticity index (RDPI)

We measured the species plasticity through the relative distance phenotypic plasticity index (RDPI) according to Valladares et al., (2006) of all species in the nursery experiment. The RDPI ranges from 0 to 1 and is calculated based on the distances among mean trait values for each environment combination, divided by the number of distances. In our study, the RDPI value was calculated for each variable, and each species, considering the six light environments.

Carbon isotope analysis

The dried leaves of thermotolerance teste were transported to the Stable Isotopes Center of the Universidade Estadual Paulista—UNESP, Brazil in order to determine the leaves carbon isotope percentages of nursery and field plants. Leaves samples were homogenized in a cryogenic mill (Geno/Grinder 2010—SPEX SamplePrep, USA) using liquid nitrogen at –196 °C and assembled in a homogenized sample of each light treatment for nursery plants, and for each field plot for field plants. The samples were analyzed in a CF-IRMS continuous-flow isotope ratio spectrometry system using an IRMS (Delta V Advantage, Thermo Scientific, Germany) coupled to an Elemental Analyzer (Flash 2000, Thermo Scientific, Germany) using a gas interface (ConFlo IV, Thermo Scientific, Germany) to determine the isotopic ratio of Carbon R ($^{13}\text{C}/^{12}\text{C}$).

Statistical analysis

The leaf thermotolerance curves were fitted with the “*nlsLM*” function from the *minpack.lm* package in R version 4.2.1 (Elzhov et al., 2022). From the curves, we estimated individual values of the of T_{50} and $F_v/F_{m,Max}$ for each plant individual and calculate the mean species' values with their standard errors. T_{Crit} was estimated as the temperature at which a line describing the slope of the F_v/F_m decline at T_{50} intersected with a horizontal line indicating $F_v/F_{m,Max}$ (Slot et al., 2021). Individuals' outliers were excluded to achieve a better adjustment of the curve models. Six individuals of *G. marginata* were excluded due to low initial F_v/F_m (below 0.69). All thermotolerance curves of were compared using maximum likelihood for best fit and were selected according to the lowest Akaike information criterion corrected (AIC_c) for small samples (Berk, 2018).

The leaf traits (T_{50} , T_{Crit} , LMA , LT , SPAD and anatomical variables) were compared according to the light availability. For experimental data of nursery, we performed an analysis of covariance (ANCOVA) with the leaf traits and DLI for each treatment and an analysis of variance (ANOVA) to describe the difference for each species. A Shapiro test was used to test normality of the residuals and in case of need, we transformed in log to acquire normality. Anatomical variables were legitimized to normalize data. Difference between each species response for light was tested using a Tukey test. For field data, we used a linear regression to test the relationship between the leaf traits and the light transmission in the two areas. All analysis were made with R (R Core Team 2022).

Results

Nursery experiment

The growth and leaf traits of the four analyzed species were observed to be influenced by the range of DLI under nursery conditions. Notably, all seedlings presented survival and health at the end of the experiment across the six DLI environments. All leaf traits presented a significative influence of DLI on the response except for parenchyma spongy thickness (Table 2). There was also a difference in the response to light at species level for all traits tested. The interaction of light and the species response was only present in the LMA , LT and mesophyll area, meaning that this response was species dependent to all species tested.

Table 2. Analysis of covariance (ANCOVA) summary results for variables from four species in different light intensity at nursery conditions. Anatomical variables were legitimized to normalize data. p values obtained for covariate DLI, p-level for the seedlings of four species

e p-interaction between DLI and seedlings. Species correspond to: *Aspidosperma pyricollum* (Asp), *Citharexylum myrianthum* (Cit), *Goniorrhachis marginata* (Gon) and *Handroanthus chrysotrichus* (Han).

Variable	DLI	p-level (Sp)	p-interaction (DLI*Sp)	Species
Leaf mass per area	< 0.001 ***	< 0.001 ***	< 0.001 ***	Asp, Cit, Gon, Han
Leaf thickness	< 0.001 ***	< 0.001 ***	0.001 **	Asp, Cit, Gon, Han
SPAD index	< 0.001 ***	< 0.001 ***	0.735	Asp, Cit, Gon, Han
<i>T</i>₅₀	< 0.001 ***	< 0.001 ***	0.085	Asp, Cit, Gon, Han
<i>T</i>_{crit}	< 0.001 ***	< 0.001 ***	0.372	Asp, Cit, Gon, Han
Mesophyll area	< 0.001 ***	< 0.001 ***	0.046 *	Asp, Gon, Han
Palisade thickness	< 0.001 ***	< 0.001 ***	0.056	Asp, Gon, Han
Spongy thickness	0.059	< 0.001 ***	0.219	Asp, Gon, Han

p > 0.05 (not significant); p < 0.05(*); p < 0.01(**); p < 0.001(***)

The *LMA* increased linearly with DLI all species apart from *A. pyricollum* (Tab. 2, Fig. 2A). The difference in *LMA* for *C. myrianthum* was of 5x in the light environment to the shade. For *G. marginata* the difference was of 1.4x, while for *H. chrysotrichus* the difference was of 2.5x. The *LT* also had a positive and significant increment with DLI, except for *A. pyricollum* (Fig. 2B). For the other three species the change in the *LT* at the light environment to the shade 1.3x to 2x. The SPAD response showed an increased value of 1.3x to 1.6x along the decrease in light availability (Fig. 1C).

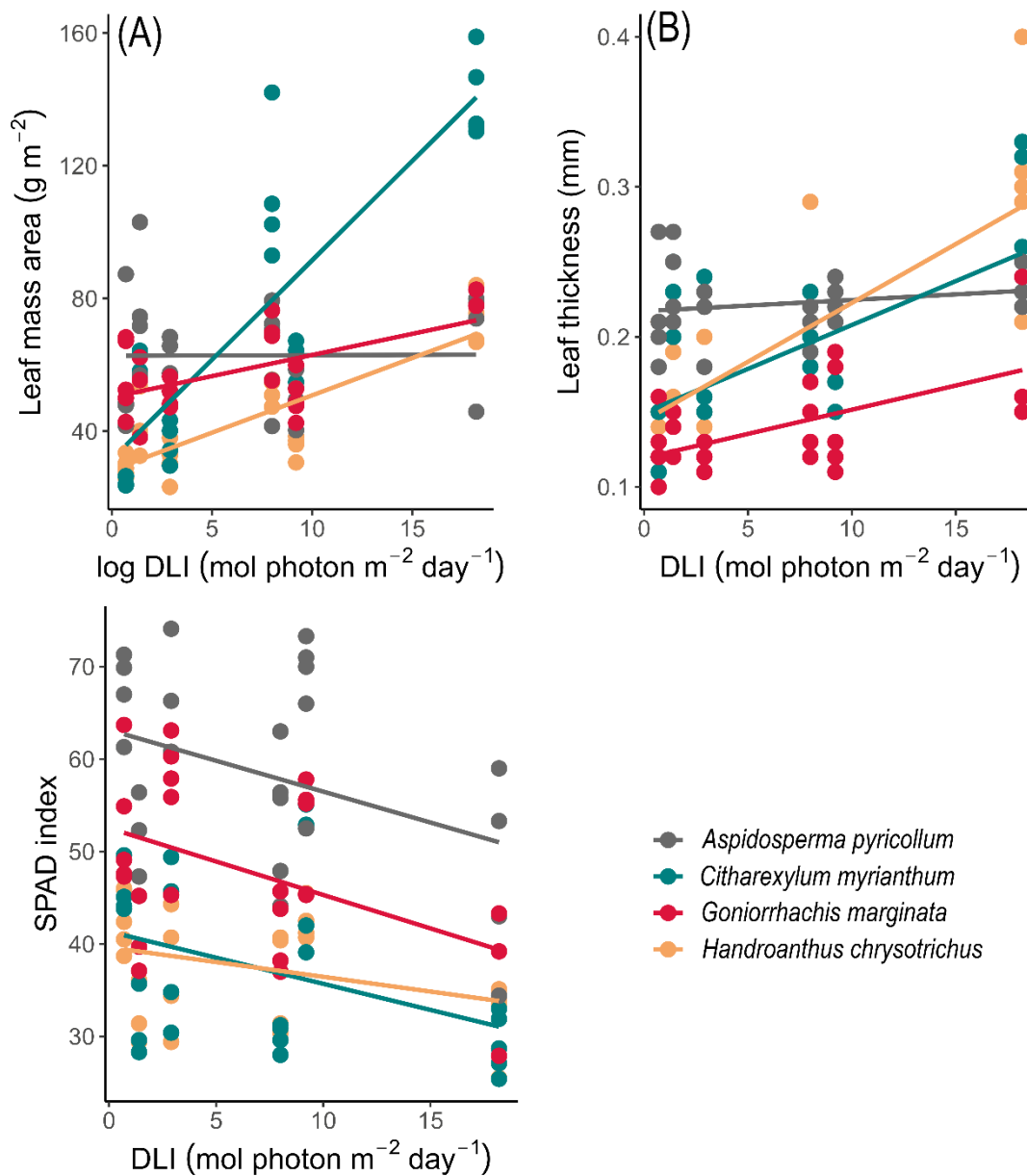


Figure 1. Relationship between leaf traits and day light integral (DLI) in four different species. Gray - *Aspidosperma pyricollum*, green - *Citharexylum myrianthum*, red - *Goniorrhachis marginata* and yellow - *Handroanthus chrysotrichus*. (A) leaf mass area; (B) leaf thickness, and (C) SPAD index. Lines indicate the relationship according to analysis of covariance (ANCOVA, $p < 0.05$).

The three species used for the leaf anatomy analysis presented a mesomorph mesophyll (Fig. S5). The mesophyll area and palisade thickness showed linear and positive relationships with DLI, although almost did not change in *A. pyricollum* (Fig. 2). There was no increase in spongy parenchyma thickness to DLI for all tree species analyzed.

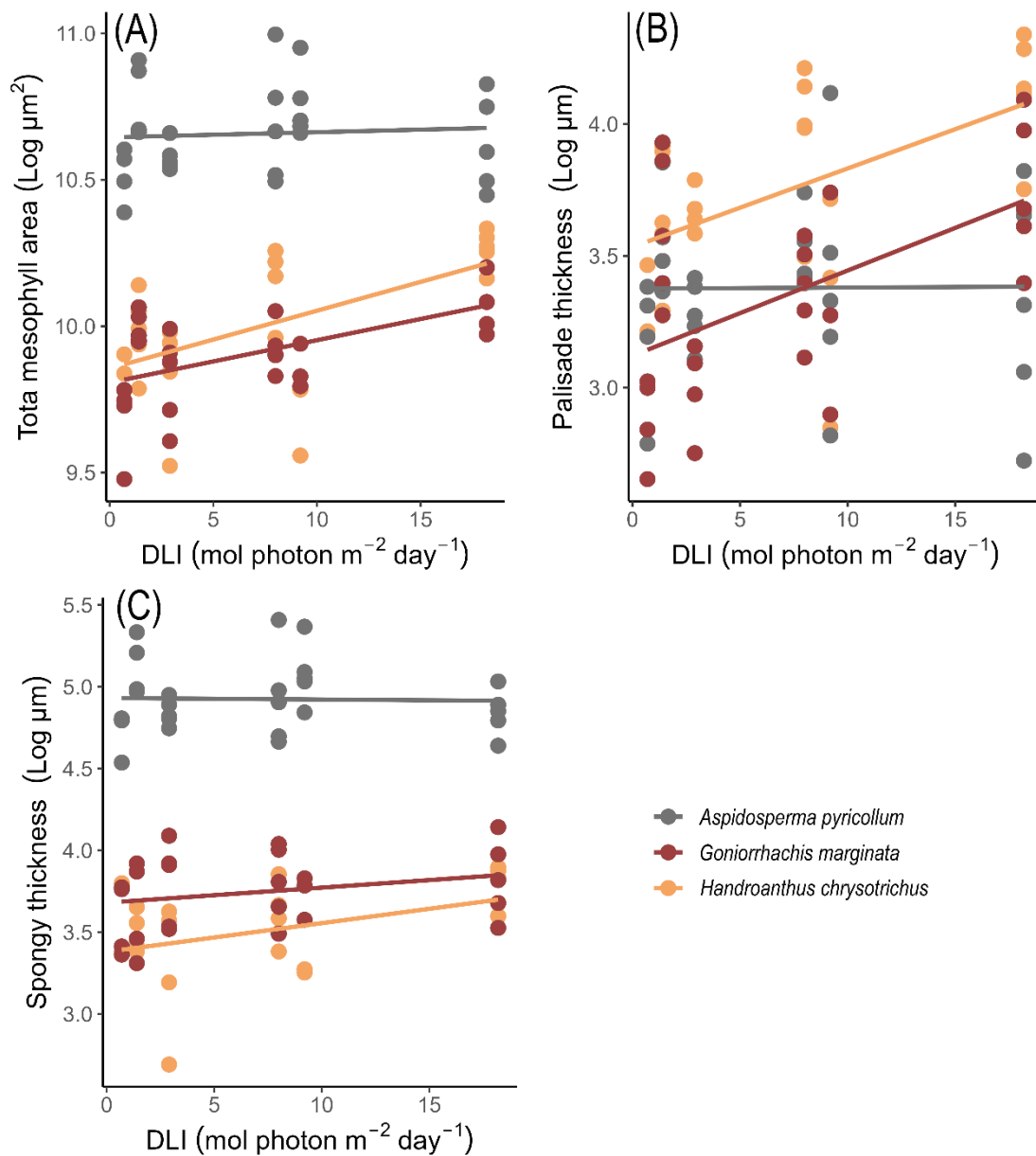


Figure 2. Relationship between leaf traits and day light integral (DLI) in four different species. Gray - *Aspidosperma pyricollum*, red - *Goniorrhachis marginata* and yellow - *Handroanthus chrysotrichus*. (A) mesophyll area, (B) palisade thickness and (C) spongy thickness. Lines indicate the relationship according to analysis of covariance (ANCOVA, $p < 0.05$).

The F_v/F_m ratio decreased with increasing temperature, reaching zero at 60°C in most individuals, regardless of species or light environment (Fig. S1-S4). There was a significant linear and negative relationship between thermotolerance and DLI. This slight decrease with DLI was observed for both thermotolerance traits, T_{crit} and T_{50} (Fig. 3A-B). This difference

under different DLI environments was approximately 1.1- to 1.3x for the four species. The most striking response was the T_{crit} of *C. myrianthum*, which showed a difference of 1.3x under the DLI range.

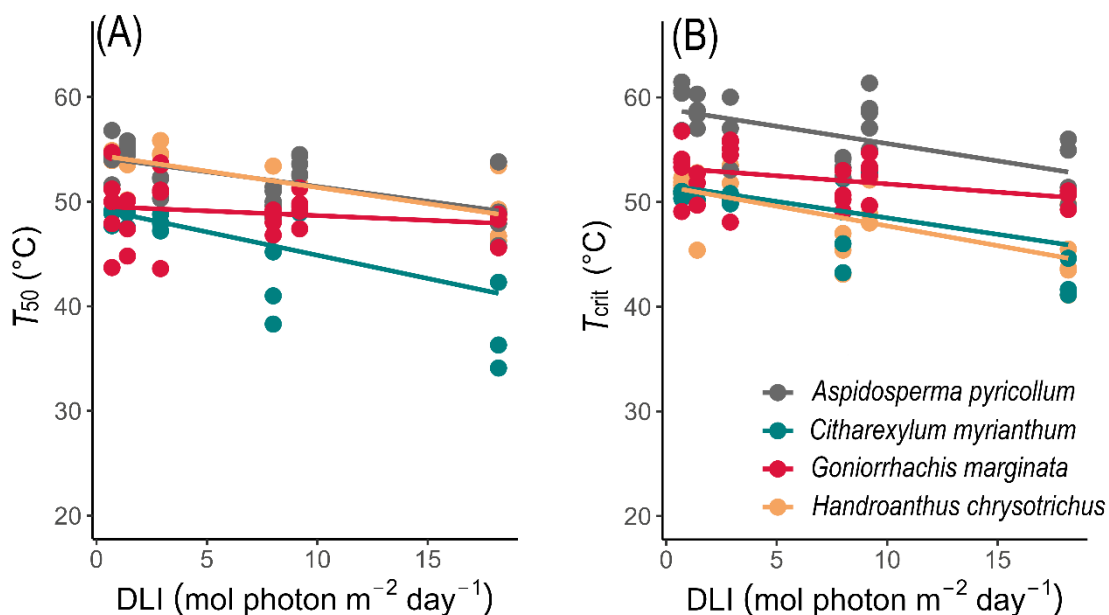


Figure 3. Relationship between leaf thermotolerance traits and day light integral (DLI) in four different species. Gray - *Aspidosperma pyricollum*, green - *Citharexylum myrianthum*, red - *Goniorrhachis marginata* and yellow - *Handroanthus chrysotrichus*. (A) T_{crit} ; (B) T_{50} . Lines indicate the relationship according to analysis of covariance (ANCOVA, $p < 0.05$).

RDPI varied for the leaf traits and species. *LMA* presented the lowest values for *A. pyricollum*, while the highest values were observed in *C. myrianthum*. The same species had the highest RDPI values also for *LT*, *SPAD*, T_{crit} and T_{50} (Table 3). *A. pyricollum* also presented the lowest values for RDPI for *LT*, mesophyll area and palisade parenchyma thickness.

Table 3. Relative distance plasticity index (RDPI) by species light environments of *Aspidosperma pyricollum* (Asp), *Citharexylum myrianthum* (Cit), *Goniorrhachis marginata* (Gon) and *Handroanthus chrysotrichus* (Han).

	Asp	Cit	Gon	Han
Leaf mass per area	0.091	0.365	0.205	0.205
Leaf thickness	0.036	0.186	0.089	0.156
SPAD index	0.094	0.127	0.104	0.075
T_{50}	0.030	0.043	0.017	0.026

T_{crit}	0.029	0.057	0.015	0.035
Mesophyll area	0.059	--	0.086	0.124
Palisade thickness	0.090	--	0.205	0.189
Spongy thickness	0.083	--	0.056	0.115

Field experiment

The two areas differed in light availability in the understory, with the AGF having more diverse light availability in its plots (Table S1). After one year of growth in the field, most *A. pyricollum* seedlings survived and showed modest growth in height. However, after the introduction of *C. myrianthum* into plots with varying degrees of canopy cover, all seedlings began to show signs of herbivory. At the end of the one-year experiment, over 21% of the seedlings did not survive, while the remaining plants showed significant herbivore damage. Although, *H. chrysotrichus* presented no apparent signs of herbivory, 30% of all seedlings were lost and approximately 50% were in poor condition to conduct an experiment. Due to the lack of healthy seedlings, both species were excluded from the final analysis.

There were few leaf traits that presented significant relationship with light transmission (Table 4). At AGF area, the T_{crit} presented small significant linear negative relationship with light transmission. Similar, at the RA, the LT presented linear negative relationship with light transmission (Fig.4)

Table 4: Relationship between leaf traits and light transmission in *A. pyricollum* at two areas: agroforestry (AGF) and restoration area (RA). R^2 indicate the coefficient of determination for linear regression tendency, p -value the significance of the equation for the linear model.

	AGF			RA		
	p-value	R ²	equation	p-value	R ²	equation
<i>LMA</i>	0.164	--	--	0.573	--	--
<i>LT</i>	0.35	--	--	0.013	0.260	$y = -0.006x + 0.272$
Chl index	0.396	--	--	0.429	--	--
<i>T</i> ₅₀	0.369	--	--	0.021	0.064	$y = 0.32x + 51.971$
<i>T</i> _{crit}	0.033	0.217	$y = -0.19x + 54.104$	0.147	--	--

$p > 0.05$ (not significant); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

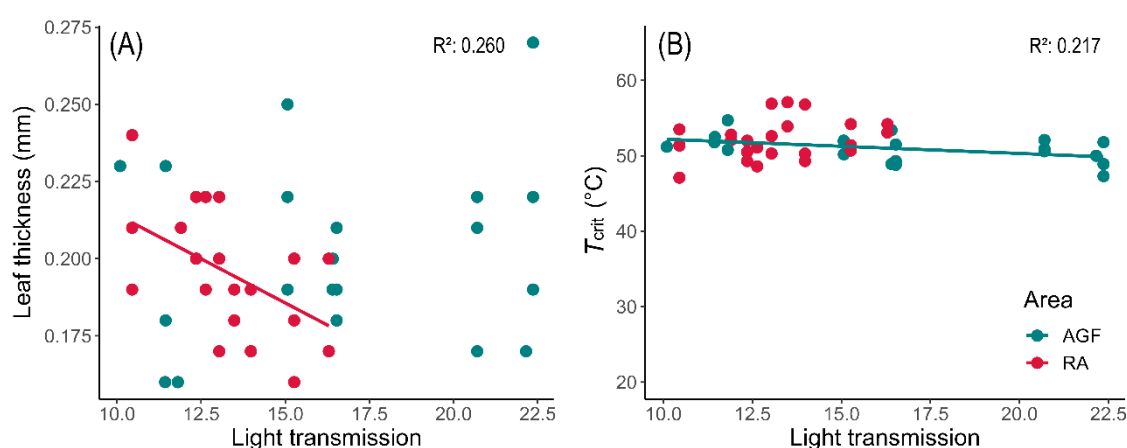


Figure 4. Relationship between leaf traits and light transmission in *A. pyricollum* at two areas. Green: agroforestry (AGF) and red: restoration area (RA). (A) Leaf thickness and (B) T_{crit} . Lines indicate the relationship with p -value < 0.05 .

Carbon content

There was no significant difference in carbon total percentage between the tree areas, ranging around 49.97% in all areas. Although, there was difference in all areas comparing the carbon isotopic ratio ($^{13}\text{C} + ^{12}\text{C}$) (Fig S2). The area with higher $\delta^{13}\text{C}$ was the regeneration area, followed by the AGF, whereas the nursery presented the lowest values for $\delta^{13}\text{C}$.

Discussion

We formulated this study to explore the response of leaf thermotolerance in seedling of species to be used in forest enrichment plantations to varying light gradients under both nursery and field conditions. Contrary to our initial hypothesis, our results revealed higher

thermotolerance levels under deep shade conditions, rather than the anticipated lower thermotolerance. These results contrast with the results of Slot et al., (2019) and Krause et al. (2015), who found a positive effect of light availability on the heat tolerance of sun leaves of tropical seedlings, but without assessing thermotolerance across a wide gradient of light availability. Some previous studies suggested that excess light intensifies high-temperature stress in plants under high light conditions and induces lower thermotolerance (Dongsansuk et al., 2013; Krause et al., 2015). However, our experiment in the nursery received approximately 60% of the light exposure of full sun, supporting that there was no stress due to high light conditions in the seedlings at the nursery experiment. This is also confirmed by the initial F_v/F_m values at the species level that were around 0.83. Consequently, the observed decrease in thermotolerance cannot be attributed to photostress or excess light. The simultaneous increase in leaf thermotolerance and chlorophyll index under deep shade observed in our study, provides a possible link to the response of thermotolerance to shade. However, further studies are essential to fully understand the mechanisms driving the physiological basis of the responses of thermotolerance to light.

Light is indeed the most important physical environmental factor for plants (Valladares et al., 2016; Van Ieperen, 2012). This resource is particularly important for tropical tree seedlings, as light availability is the most limited resource in the forest understory (Valladares et al., 2012, 2002). In order to deal with this limited resource, many species resort to leaf plasticity, which is a solution to heterogeneous environments (Xu et al., 2008). Examples of leaf plasticity in response to light environment are seen in *LMA*, *LT*, chlorophyll index and anatomy and could be considered as very important plastic responses to light availability (Rozendaal et al., 2006). The decrease in *LMA* under shade conditions usually reflects an attempt to enhance the light interception under low light environment, consistent with a shade avoidance strategy (Kalve et al., 2014; Poorter and Rozendaal, 2008; Valladares et al., 2012; Xu et al., 2008). Larger leaves may intercept more light, but this is often at the expense of thinner and less dense leaves, resulting in lower *LMA* and *LT* values (Poorter et al., 2009). This adaptive strategy was observed in all species, especially in *C. myrianthum*, which showed a remarkable fivefold decrease in *LMA* under shade and a doubling of *LT*, reflected in RDPI values of 0.37 and 0.19, respectively. In deep shade, *G. marginata* and *H. chrysotrichus* also showed significant reduction in *LMA* and *LT*, although less pronounced than *C. myrianthum*. The decrease in leaf thickness was reflected in the mesophyll area, as indicated by our anatomical results for *G. marginata* and *H. chrysotrichus*. The observed

increase in palisade parenchyma under high light conditions in these two species is consistent with the typical response to increased light, which is often associated with enhanced photosynthetic performance (Ashton and Berlyn, 1994). In contrast to the pronounced increase observed in *C. myrianthum* under shaded conditions, *A. pyricollum* showed small changes in *LMA* and *LT*, reflecting in a low RDPI, suggesting that it is a very conservative species. The only leaf trait that had substantial change for *A. pyricollum* was the chlorophyll index under nursery conditions. There was an increase in chlorophyll index at deep shade environment for *A. pyricollum* and the other tree species. Chlorophyll content usually increases under low light to increase the photosynthetic capacity (Souza and Válio, 2003). Overall, these results underline the high leaf trait plasticity with which all those tree species respond to different light conditions.

Compared to the other leaf traits, the plasticity of the leaf thermotolerance was low for all the species, which is reflected in the changes of 5-19% for T_{50} and 4-30% for T_{crit} . The only exception was the T_{crit} of *C. myrianthum* with a decrease of 30% with increasing light and an RDPI of 0.043 for T_{50} and 0.057 for T_{crit} . In the other three species, the RDPI for the two thermotolerance traits remained at 0.017 and 0.035. This result contradicts our second hypothesis of thermotolerance plasticity, as the response of thermotolerance to light appears to be low compared to the other leaf traits. Plasticity of thermotolerance of T_{50} and T_{crit} was already seen in previous studies with many environmental conditions. Konôpková et al. (2018) demonstrate the plasticity of T_{crit} and T_{15} (temperature where there is 15% of F_v/F_m loss) in silver fir (*Abies alba*), a temperate climate tree species, for different provenances origins with trees from warmer locations presenting an overall higher photochemical performance and better heat-stress response. In another study, Sastry & Barua (2017) also reported leaf thermotolerance plasticity and acclimation to temperature as of 33 tropical tree species, half had higher thermotolerance of T_{50} in the hot-dry season compared to cooler wet season. Local and seasonal acclimation of leaf thermotolerance was also reported for Zhu et al. (2018) where they found not only a higher T_{crit} warmer sites but also a seasonal acclimation of T_{crit} for different plant species. For bromeliads, Chaves et al. (2018) found significantly higher thermotolerance in shaded *A. nudicaulis* individuals compared to sun-exposed plants. Although bromeliads differ from tropical trees in terms of morphological and physiological traits, all these studies indicate that thermotolerance also changes in relation to environmental conditions. Although, the observed changes in thermotolerance were often only a few degrees, emphasizing the limited extent to which these traits adjust in the face of

varying environmental conditions. This limited plasticity, was demonstrated also by Konôpková et al. (2018), Sastry & Barua (2017), Slot et al. (2019), and implies a limited ability of the tree species to acclimate to future extreme heat waves events. As a result, irreversible heat-induced damage could prove to be a significant stress factor, especially for the sun-exposed leaves of tropical forest trees where the leaf temperatures usually exceed photosynthetic optima at midday (Miller et al., 2021; Slot et al., 2019). This limited plasticity raises concerns about the ability of species to endure fast temperature changes, potentially impacting ecosystem dynamics and warranting proactive strategies to mitigate heat-induced damage (Zhu et al., 2018). Also, it confirms thermotolerance as a useful method for determining the limits of a species' heat stress and, as a conservative trait, a helpful physiological approach to improve criteria for classifying species vulnerability risk for its conservation in regard to climate change (Geange et al., 2021; Wikelski and Cooke, 2006).

Although limited plasticity may be a concern for many species under the climate change context, high plasticity does not necessarily mean better fitness (Nicotra et al., 2010). The response of *A. pyricollum* emphasizes this point, as the leaf traits of *A. pyricollum* changed hardly in response to light availability but survived in all environments under field conditions. Due to the failure of the other two species, *A. pyricollum* was the only species evaluated under field conditions that showed almost no relationship with leaf traits and light transmission, apart from *LT* and thermotolerance. Therefore, the third hypothesis of similar results under experimental forest enrichment plantations was partially confirmed and further experiments with other species are highly recommended. Still, the increase of thermotolerance under shade conditions for *A. pyricollum* at the nursery experiment and in the forest enrichment plantation pose as a possible similar response for thermotolerance under the two experiments. These results are promising for the advocacy of forest enrichment strategies, especially with regard to their association with the temperature moderated understory microclimate (De Frenne et al., 2021). Seedlings grown in deep shade exhibited increased resistance to heat waves, suggesting a viable strategy for mitigate the impacts of extreme heat waves. Applying such approaches to forest management could prove helpful in promoting resilience against the effects of rising temperatures and contribute to overall climate change mitigation efforts.

There is little information on *A. pyricollum*, still we know that this species is widely distributed in different biomes, as there are registers in AF and reports on savannas and

caatinga (Do Carmo et al., 2015; Vital, 2018). Carvalho (2006) used it as a synonym of *A. olivaceum*. Despite the status of *A. pyricollum* as a species is still valid (Flora do Brasil 2020), the author discusses its use in restoration and describes it as a heliophilic species with broad thermotolerance (15.5°C to 27.6°C) (Carvalho, 2006). This is in accordance with the description of the multiple-biome species and is consistent with the response of the species to light and temperature in our experiment. Therefore, this species seems to be able of acclimating to different environmental conditions, but with very low plasticity in relation to the morphological, anatomical and thermotolerance leaf traits. Regardless of the low plasticity by light, *A. pyricollum* had higher mean values for T_{50} and T_{crit} than the other three species. The success at field conditions also confirm that it is an excellent species for reforestation (Carvalho, 2006). In addition, the high ability to cope with extreme heat events despite the light environment, suggests that this species is excellent for reforestation at different stages as well as for enrichment plantations.

Compared to all species, *C. myrianthum* exhibited the lowest thermotolerance. This species is reported as a pioneer with excellent natural regeneration in different successional stages and is also used to attract animals with its fruits and flowers (Amaral et al., 2013; Rocca and Sazima, 2006; Silveira et al., 2013). However, under field conditions, most *C. myrianthum* seedlings suffered from severe herbivory and died before the conclusion of the experiment. At the end of this experiment, only about one third of the seedlings survived healthy in open gap plots, with of these individuals exhibiting considerable growth, reaching 1-3 meters in height (Fig. S6). While the strategy of increasing leaf area to enhance light capture may improve light interception, it also increases palatability and thus the risk of herbivory damage (Poorter et al., 2009). Consequently, increasing leaf area and reducing *LMA* under shaded conditions proved detrimental to *C. myrianthum*, representing a maladaptive response (Nicotra et al., 2010). In addition, light under field conditions tend to be more limited than under nursery experiment. The period of direct exposure to light and filtering by surrounding trees diminish the light exposure throughout the day at field conditions, making the open canopy plots in the field more similar to a mid-shaded environment of the nursery experiment. In this sense, despite the species' reduced leaf thermotolerance in high light environments, its plantation within intermediate canopy gaps remains essential for its survival as canopy plays a critical role in seedling establishment in enrichment plantations (Forbes et al., 2020; Laughlin and Clarkson, 2018). This nuanced ecophysiological behavior underscores the complexity of plant responses to the light environment.

Conclusion

Leaf thermotolerance has been shown to have a positive relationship with increased shade, which was observed in the nursery and field experiments, and suggests the effectiveness of forest enrichment in mitigating the impacts of extreme heat waves. Although light significantly influenced most leaf traits studied, its impact on leaf thermotolerance was notably lower compared to other leaf traits and appears to be species specific. Despite the low plasticity to light availability of leaf thermotolerance compared to other traits it remains a valuable tool for assessing species' heat stress limits and could help in species-specific conservation efforts.

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Appendix

Table S1. Environmental information from nursery and field areas (mean \pm s.d.).

	Nursery	Agroforestry	Regeneration area
Light			
Day light integral (mol photons m ⁻² day ⁻¹)	0.7 to 18.2	--	--
Mean canopy openness	--	10.46 \pm 3.03	7.96 \pm 1.41
Light transmission	--	16.31 \pm 4.23	13.34 \pm 1.77
Temperature (°C)			
Mean	23.54 \pm 1.85	21.72 \pm 2.05	20.20 \pm 0.43
Minimum	19.59 \pm 2.51	13.15 \pm 0.68	14.04 \pm 0.76
Maximum	28.52 \pm 2.09	32.78 \pm 2.98	28.59 \pm 1.82
Soil (kg/kg)			
Coarse sand	0.372	0.223	0.451
Fine sand	0.303	0.113	0.122
Silt	0.101	0.146	0.048
Clay	0.224	0.518	0.379
Textural classification	Clay-Sandy Loam	Clay	Clay-sandy
Chemistry			
pH	5.15	5.05	3.74
P (mg/dm ³)	58.0	2.9	7.5
K (mg/dm ³)	49	63	61
Ca ²⁺ (cmol/dm ³)	4.74	9.06	1.22
Mg ²⁺ (cmol/dm ³)	2.09	3.45	0.66
Al ³⁺ (cmol/dm ³)	00.00	0.00	2.02
N (dag/kg)	0.178	0.416	0.271

Table S2. Leaf carbon content (¹³C + ¹²C) and carbon isotopic ratio (¹³C + ¹²C) in the areas studied. AGF: agroforestry; RA: regeneration area; and nursery (mean \pm s.d.).

Area	C (%)	$\delta^{13}C$
AGF	50.70 \pm 1.92	-33.39 \pm 0.86
RA	49.36 \pm 1.67	-35.27 \pm 0.62
Nursery	49.67 \pm 1.04	-30.62 \pm 0.97

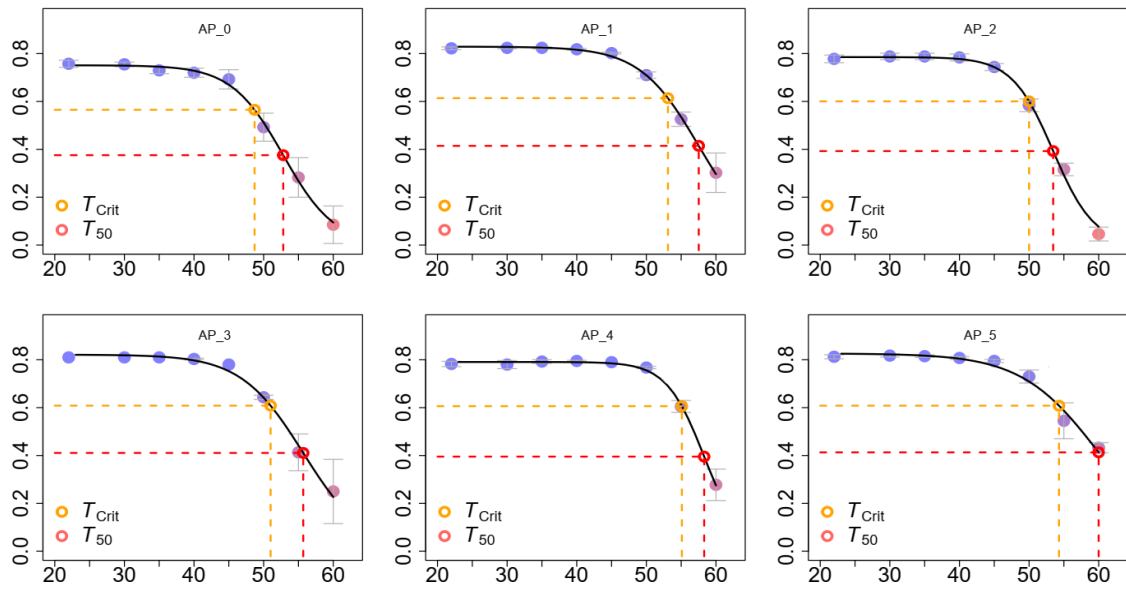


Figure S1. F_v/F_m curve of *Aspidosperma pyricollum* (AP) in distinct light environments, plotting F_v/F_m along the y-axis against temperature ($^{\circ}\text{C}$) on the x-axis. The AP values, ranging from 0 to 5, denote varying degrees of shade intensity. Red line represents the T_{50} value, while the orange line, the T_{crit} within the graph.

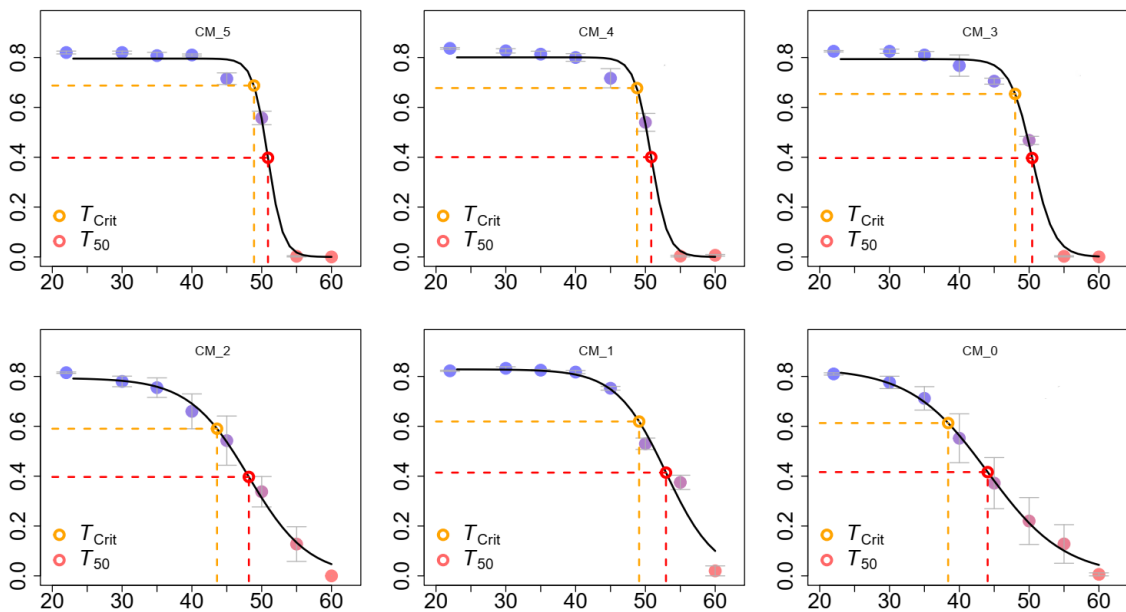


Figure S2. F_v/F_m curve of *Citharexylum myrianthum* (CM) in distinct light environments, plotting F_v/F_m along the y-axis against temperature ($^{\circ}\text{C}$) on the x-axis. The CM values, ranging from 0 to 5, denote varying degrees of shade intensity. Red line represents the T_{50} value, while the orange line, the T_{crit} within the graph.

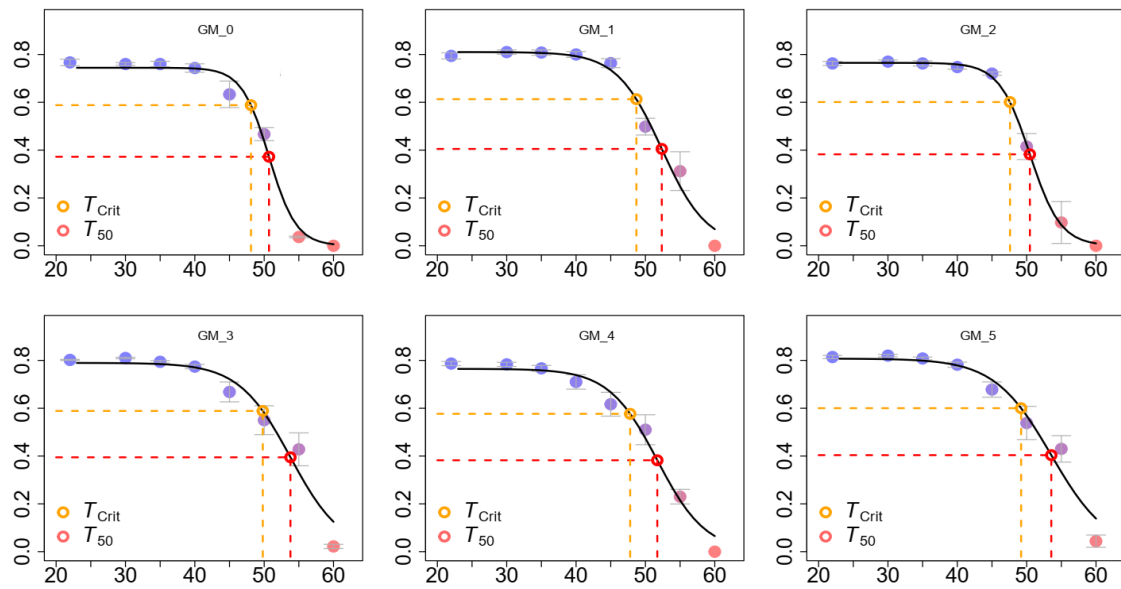


Figure S3. F_v/F_m curve of *Goniorrhachis marginat* (GM) in distinct light environments, plotting F_v/F_m along the y-axis against temperature ($^{\circ}\text{C}$) on the x-axis. The GM values, ranging from 0 to 5, denote varying degrees of shade intensity. Red line represents the T_{50} value, while the orange line, the T_{crit} within the graph.

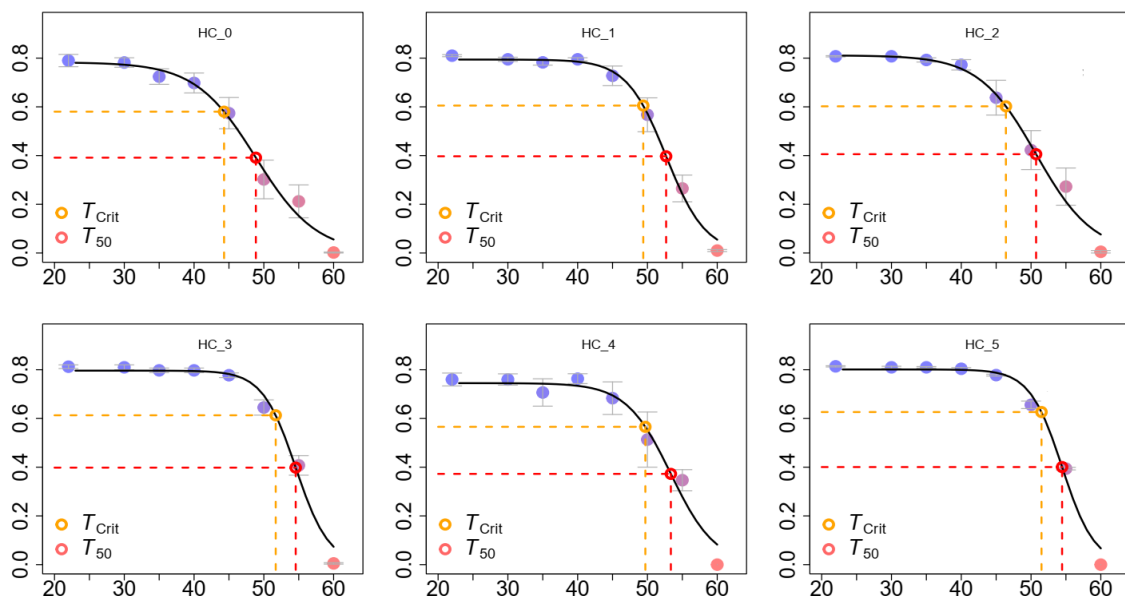


Figure S4. F_v/F_m curve of *Handroanthus chrysotrichus* (HC) in distinct light environments, plotting F_v/F_m along the y-axis against temperature ($^{\circ}\text{C}$) on the x-axis. The GM values, ranging from 0 to 5, denote varying degrees of shade intensity. Red line represents the T_{50} value, while the orange line, the T_{crit} within the graph.

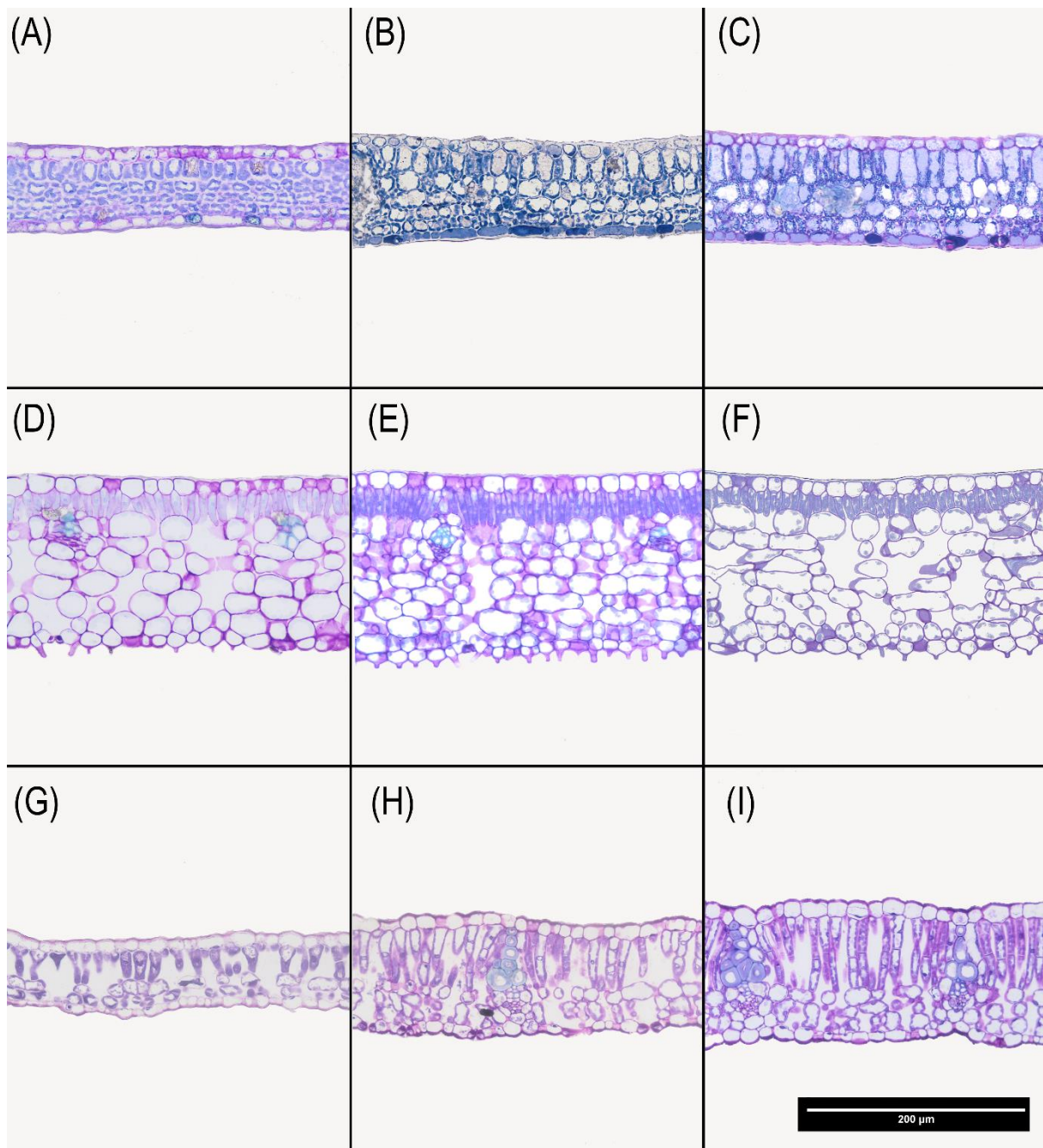


Figure S5 Anatomy of *A. pyricollum* (A-C), *G. marginata* (D-F) and *H. chrysotrichus* (G-I) in three light environments. 18.2 mol photons m⁻²day⁻¹ in A, D and G; low shade (8.0 mol photons m⁻²day⁻¹) in B, E and H; and deep shade (0.7 mol photons m⁻²day⁻¹) in C, F and G.



Figure S6. *Citharexylum myrianthum* under different light environments and developmental stages in field settings. (A) A mature individual grown reaching a height exceeding 3.00 meters by the experiment's conclusion. (B) Seedling in the field experiment early stages; this seedling did not survive through later stages. Asterisks highlight points of herbivory.

CONSIDERAÇÕES FINAIS

A atividade humana tem modificado rapidamente a superfície terrestre, promovendo uma vantagem competitiva para algumas espécies em detrimento de outras, diminuindo a presença de espécies nativas. Em especial essa atividade tem provocado as mudanças climáticas, que têm acarretado o aumento de eventos climáticos extremos, aumentando a ocorrência, intensidade e duração de ondas de calor. Como a temperatura impacta o funcionamento das plantas, desde a fotossíntese até seu crescimento, esta tese teve como objetivo investigar como espécies lenhosas respondem ao aumento da temperatura no contexto de conservação.

No primeiro capítulo através de uma meta-análise foi possível confirmar a fotossíntese como um processo bioquímico muito suscetível ao aumento da temperatura. Observou-se que plantas lenhosas jovens nos trópicos não são especialmente suscetíveis a aumentos de temperatura em comparação com outras regiões. Contudo, apesar de um padrão de aclimação na fotossíntese líquida (A_n) em resposta ao aquecimento, o aumento da temperatura tende a elevar a taxa de respiração (R_d), contribuindo para um desequilíbrio no balanço de carbono, o que pode levar à redução na produção de biomassa vegetal, especialmente em plantas jovens. Nesse sentido, existe uma preocupação maior em densas florestas tropicais, pois apesar de não serem mais suscetíveis a aumentos na temperatura, florestas tropicais tem uma porcentagem muito reduzida de luz fotossinteticamente ativa no sub-bosque. Dessa forma, qualquer desbalanço na aquisição de carbono, pode ser custoso para a sobrevivência desses juvenis que são dependentes da produção de biomassa ou investimento em estruturas defensivas.

A fotorrespiração provou ser o principal mecanismo de resposta ao aumento da temperatura. Considerando que a quase totalidade das espécies de árvores conhecidas apresentam metabolismo do tipo C3, ela tende a aumentar fortemente com o aumento da temperatura, evidenciando uma perda energética. A relevância da fotorrespiração na resiliência das plantas a temperaturas elevadas ressalta sua importância adaptativa, porém, também suscita preocupações para a regeneração florestal em face de futuros aumentos de temperatura e eventos extremos de calor.

Dentro do processo fotossintético, a etapa fotoquímica, em especial o fotossistema 2 (*PSII*) é altamente suscetível à variações da temperatura. Quando a temperatura ultrapassa um limite

crítico, há um comprometimento do funcionamento do *PSII*, resultando em uma limitação severa na capacidade fotossintética, que pode ser medida pela redução na razão F_v/F_m . Essa medida permite o cálculo dos parâmetros clássicos de termotolerância, T_{crit} e T_{50} , que foram testados dos capítulos 2, 3 e 4. Com base nessa metodologia avaliou-se, no capítulo 2, se a termotolerância varia de acordo com a distribuição geográfica original das espécies. Não foi possível relacionar a termotolerância com a distribuição geográfica das espécies, o que pode ter sido ocasionado tanto pelo número de espécies como pelos parâmetros de termotolerância utilizados. Além disso, não foram observadas relações significativas entre os atributos foliares mensurados e a termotolerância. Por outro lado, o uso dessa metodologia permitiu verificar que a termotolerância está mais relacionada com as especificidades de cada espécie do que com a sua amplitude de distribuição geográfica, sendo possível identificar espécies mais vulneráveis aos eventos extremos, como o caso de *Arapatiella psilophylla*.

Quando testada se existe diferença entre a termotolerância de espécies invasoras e nativas em áreas de Restinga, um ecossistema associado a Mata Atlântica, observou-se que a termotolerância foliar é similar entre espécies invasoras e nativas, apesar de suas distribuições globais e capacidades competitivas distintas. No entanto, a alocação contrastante de biomassa, evidenciada pela massa específica e espessura foliar, apresentou diferenças entre essas espécies, sendo as espécies invasoras mais adaptadas às estratégias de crescimento rápido. Apesar da termotolerância semelhante, as espécies invasoras detêm vantagens competitivas sobre as espécies nativas, caracterizadas por taxas de crescimento rápido, alta taxa de reprodução e dispersão. Dessa forma, as espécies nativas estão em desvantagem em ondas de calor. Em especial a espécie nativa *Allagoptera arenaria* demonstrou um rendimento fotoquímico inferior em condições de campo, sugerindo a presença de estresse em seu habitat natural que será maior em episódios de ondas de calor

O quarto capítulo foi desenvolvido visando a aplicação prática da metodologia empregada nos estudos de termotolerância foliar na conservação, levando em consideração a necessidade da restauração ecológica frente as mudanças climáticas. Neste, a termotolerância foliar provou ter uma relação positiva com a sombra, o que foi observado tanto num experimento realizado no viveiro como no campo, e sugere a eficácia do enriquecimento florestal na mitigação dos efeitos de ondas de calor extremo. Embora a luz tenha influenciado significativamente a maioria das características foliares estudadas, o seu impacto na termotolerância foliar foi menor em comparação com outras características foliares, além de

ser espécie-específica. Portanto, a seleção de um ambiente de luz para o plantio de mudas em ações de enriquecimento florestal deve ser específica para cada espécie. A termotolerância foliar apresentou pequena plasticidade entre as espécies, com poucas alterações em T_{50} e T_{crit} . Esta plasticidade restrita implica a capacidade limitada das árvores para se aclimatarem a futuros eventos de aquecimento rápido e intenso. Apesar da baixa plasticidade na termotolerância em comparação com outros atributos foliares, estudos da termotolerância foliar com base na emissão de fluorescência da clorofila *a* continuam a ser uma ferramenta valiosa para avaliar os limites de estresse térmico e pode ajudar nos esforços de conservação das árvores tropicais. Por se tratar de um atributo conservativo e capaz de determinar a vulnerabilidade de espécies, os atributos de termotolerância foliar possuem potencial significativo para aprimorar a classificação de espécies conforme seu risco de extinção em cenários climáticos de médio e longo prazos.

Esta tese contribui para a compreensão da resposta ao aumento da temperatura em espécies tropicais nativas e não nativas, e contribui em questões de conservação relacionadas a mudanças climáticas, particularmente em locais de alta diversidade de árvores, dentro de regiões consideradas mais vulneráveis às mudanças climáticas.