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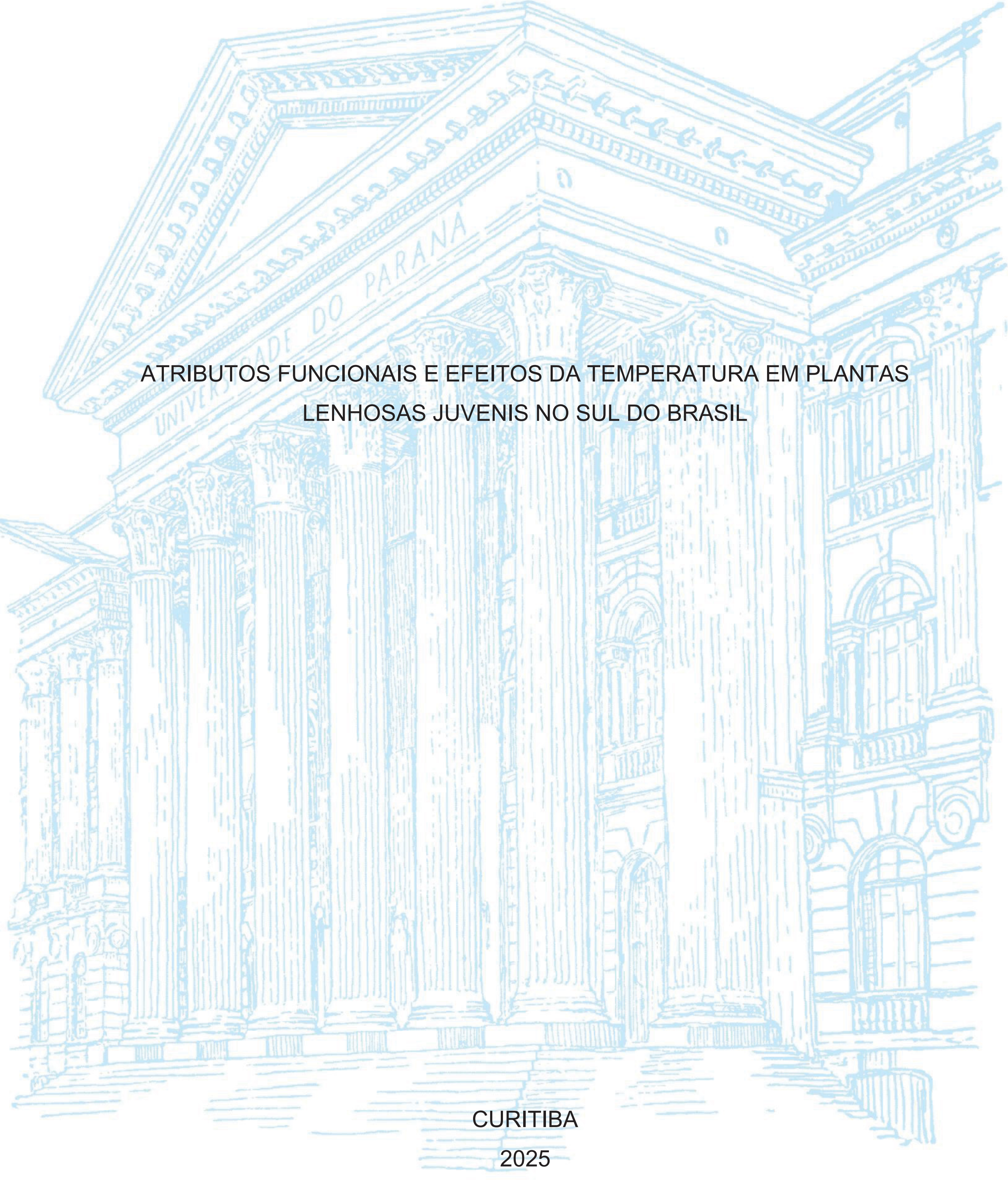

ATRIBUTOS FUNCIONAIS E EFEITOS DA TEMPERATURA EM PLANTAS LENHOSAS JUVENIS NO SUL DO BRASIL

CURITIBA
2025

ADLER DVORAK BARBOZA

ATRIBUTOS FUNCIONAIS E EFEITOS DA TEMPERATURA EM PLANTAS LENHOSAS JUVENIS NO SUL DO BRASIL

Dissertação apresentada ao curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ecologia e Conservação.

Orientador: Dr. Marcos Bergmann Carlucci

Coorientadora: Dra. Marina Correa Scalon

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ADLER DVORAK BARBOZA**, intitulada: **Atributos funcionais e efeitos da temperatura em plantas lenhosas juvenis no sul do Brasil**, sob orientação do Prof. Dr. MARCOS BERGMANN CARLUCCI, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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DEDICATÓRIA

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“from the roots to the foliage, you notice that plants are constructed on a diffuse model, the opposite of the centralised animal model. It is an organisation that allows for freedom and strength at the same time.” (MANCUSO, S., 2019)

RESUMO

A temperatura é uma das condições essenciais do nicho ecológico das espécies. Dentro do espaço de nicho, os organismos enfrentam extremos térmicos por meio de distintos mecanismos: fisiológicos, morfológicos, comportamentais, fenológicos e espaciais. As plantas, por exemplo, são organismos sésseis que precisam tolerar variações de temperatura, expressando essa capacidade por meio de seus atributos funcionais – características que refletem seu desempenho individual no ambiente onde vivem. Diante do avanço das mudanças climáticas globais, torna-se essencial direcionar os estudos para ecossistemas de elevada biodiversidade e sujeitos a essas mudanças. No Brasil, a Mata Atlântica constitui um ambiente ideal para investigar como sua diversificada flora, marcada por um complexo histórico biogeográfico, responde às variações térmicas iminentes. Neste estudo, avaliamos a interação entre plantas e temperatura sob múltiplas abordagens e em diferentes níveis de organização ecológica. No primeiro capítulo, investigamos a tolerância de comunidades vegetais a eventos frequentes de geada ao longo de um gradiente campo-floresta. Focamos em arvoretas e arbustos, analisando a correlação entre atributos funcionais das folhas e os impactos da geada na estrutura foliar. Os resultados demonstraram diferenças significativas na composição funcional entre comunidades de campo e de floresta, com plantas de campo apresentando folhas mais espessas e resistentes que as de floresta. As condições ambientais, como a cobertura do dossel e o adensamento lenhoso, explicam parte da variação nos danos foliares, sendo que plantas de sub-bosque mostraram maior retenção foliar pós-geada, possivelmente devido à proteção microclimática. Dada a crescente ameaça climática sobre mudanças na frequência e intensidade das geadas, esse fenômeno pode não ser mais eficaz no controle de plantas lenhosas invadindo os mosaicos campo-floresta. No segundo capítulo, investigamos como atributos de resistência térmica se correlacionam com a história biogeográfica das linhagens ancestrais das espécies. Utilizando parâmetros de tolerância ao calor e ao frio em mudas de espécies nativas, analisamos se esses limites diferem entre linhagens de dois contingentes biogeográficos distintos da Mata Atlântica: linhagens austrais que migraram para o norte e linhagens tropicais que migraram para o sul. Também correlacionamos esses limites com atributos morfológicos das folhas e variáveis climatológicas associadas à distribuição das espécies. Os resultados indicam que a história biogeográfica influencia a tolerância térmica das espécies a temperaturas elevadas, sendo os parâmetros fisiológicos fortemente correlacionados à espessura foliar e à altitude em que as espécies ocorrem. Esses padrões evidenciam o impacto potencial das mudanças climáticas na sobrevivência das mudas, estádio sensível do ciclo de vida das plantas. Houve ausência de diferença na tolerância ao frio entre as espécies, o que pode estar relacionada à falta de aclimatação, um processo fundamental para a manutenção da folhagem durante o inverno. Em síntese, esta dissertação demonstra como a interação entre plantas e temperatura pode ser analisada sob diferentes perspectivas, contribuindo para uma compreensão mais aprofundada da sensibilidade das espécies vegetais da Mata Atlântica diante da emergência climática corrente.

Palavras-chave: Conservadorismo de nicho; ecofisiologia; ecologia funcional; mosaicos campo-floresta; termotolerância.

ABSTRACT

Temperature is a fundamental factor shaping the ecological niche of species. Within the niche space, organisms endure extreme temperature conditions through diverse mechanisms: physiological, morphological, behavioural, phenological, and spatial adaptations. As sessile organisms, plants must tolerate temperature fluctuations, expressing this adaptation through their functional traits — characteristics linked to individual performance within their environmental context. Given the accelerating pace of global climate change, it is critical to investigate ecosystems that harbour high biodiversity yet face significant threats from anthropogenic and climatic pressures. The Atlantic Forest in Brazil represents an excellent study model to assess how its rich plant diversity, shaped by a complex biogeographical history, responds to imminent temperature variations. In this study, we examine the interaction between plants and temperature using multiple approaches and across distinct levels of ecological organisation. In the first chapter, we investigate how plant communities along a forest-grassland gradient respond to frequent frost events. We focused on treelets and shrubs, examining the correlation between leaf functional traits and the frost-induced foliar damage. Our findings reveal that the functional structure of grassland and forest communities differs significantly. Mean values of functional traits and environmental conditions (such as canopy cover and woody density) vary between ecosystems, influencing how plants are affected by frost. While leaf damage levels are primarily explained by foliar traits, post-frost reductions in foliage quantity suggest competitive interactions similar to those observed among understory plants. As climate change threatens the frequency and intensity of frost events, we recognise frost as a crucial factor in maintaining the forest-grassland ecosystems, with its absence likely facilitating increased woody encroachment into grasslands. In the second chapter, we explore how the thermal physiology of saplings correlates with the biogeographical history of ancestral species lineages. Using heat and cold tolerance parameters, we assess whether these limits differ between two distinct Atlantic Forest floristic contingents: austral lineages that migrated northwards and tropical lineages that migrated southwards. We also correlate these tolerance limits with leaf morphological traits and bioclimatic variables related to species distribution. Our findings indicate that biogeographical history influences species' heat tolerance, with physiological parameters highly correlated with leaf thickness and elevation where the species can occur. These patterns highlight how climate change may affect sapling survival, one of the most vulnerable stages in plant development. There was a lack of difference among the species’ cold tolerance, which may come from the absence of hardening, a crucial process enabling species to retain foliage during winter. In summary, this dissertation demonstrates how the plant-temperature interaction can be examined through different perspectives, offering a deeper understanding of the sensitivity of Atlantic Forest plant species in the face of the upcoming climatic emergency.

Keywords: Ecophysiology; forest-grassland mosaics; functional ecology; niche conservatism; thermotolerance.

SUMÁRIO

1 INTRODUÇÃO GERAL

Como organismos sésseis, plantas estão sujeitas a uma seleção para tolerar ou resistir a diferentes estresses ambientais. Dentro de um cenário de mudanças globais, as ondas de calor, os períodos de seca, as geadas esporádicas e a combinação desses eventos proporcionam um nível superior de estresse a elas (JENTSCH; BEIERKUHNLEIN, 2008; REYER et al., 2013). No entanto, a temperatura é um fator crucial para o desenvolvimento das plantas (WAHID et al., 2007) e elas, ao longo do tempo evolutivo, encontraram diferentes maneiras de lidar com condições extremas (ARAÚJO et al., 2013).

A temperatura é um dos muitos aspectos do nicho ecológico dos organismos. Para entendermos a interação entre temperatura e desempenho biológico, podemos lançar mão de múltiplas perspectivas: fisiológica, morfológica, fenológica e espacial (VISAKORPI et al., 2024; WAHID et al., 2007; ZANNE et al., 2014). Cada uma dessas perspectivas revela nuances essenciais para a compreensão da interação dos organismos com a temperatura. Essa relação torna-se ainda mais complexa quando se considera como os limites de tolerância diferem ao longo da ontogenia (NIINEMETS, 2010), com mudas sendo mais sensíveis do que plantas adultas a extremos climáticos (POORTER; MARKESTEIJN, 2008; TESKEY et al., 2015; VITASSE et al., 2014). Assim, combinar a utilização de ensaios de termotolerância em estádios ontogenéticos juvenis permite uma compreensão mais detalhada da tolerância térmica nas plantas além do que é comumente estudado.

A fisiologia vegetal da termotolerância pode investigar desde fluidez de membrana, espécies reativas de oxigênio, desnaturação de proteínas e sinalização celular (BOKHORST et al., 2018; DEMIDCHIK et al., 2014; GEANGE et al., 2021; WAHID et al., 2007). Em estudos ecológicos, é comum a investigação do desempenho do aparato fotossintético frente a gradientes de temperatura, tanto positivas quanto negativas, através da quantificação da fluorescência da clorofila (GAST; RÖMERMANN; BUCHER, 2020; GEANGE et al., 2021; TIWARI et al., 2021). Nessa abordagem, o estresse térmico induz o interrompimento do fluxo de elétrons e diminuição da concentração de clorofila (BUKHOV et al., 1999; GOVINDACHARY et al., 2004), afetando a capacidade de fluorescência total (KNIGHT; ACKERLY, 2002). Todavia, é possível visualizar um efeito tecidual de impacto através da mensuração de vazamento de eletrólitos. O estresse promove a formação de espécies reativas de oxigênio, efluxo de potássio e, consequentemente, a morte celular programada (DEMIDCHIK et al., 2014). Essa técnica é comumente utilizada na ecofisiologia para avaliar o impacto de temperaturas congelantes sobre os tecidos vegetais (GEANGE et al., 2021; GURVICH et al., 2002; KOVALESKI; GROSSMAN, 2021).

Embora os estudos ecofisiológicos ofereçam medidas refinadas, suas limitações metodológicas dificultam uma compreensão ampla e acessível da tolerância térmica (BELLUAU; SHIPLEY, 2018). Diante disso, características morfológicas têm sido adotadas como proxies eficientes para avaliar essa tolerância, uma vez que permitem mensurações rápidas e aplicáveis em escalas ecológicas mais amplas (DA SILVA; ROSSATTO, 2022; KNIGHT; ACKERLY, 2003). Essas características são referenciadas como atributos funcionais, ou seja, são conectadas a um desempenho de performance individual e resposta ao ambiente (VIOLLE et al., 2007). De mensuração mais rápida e prática, os atributos funcionais podem ser utilizados para entender padrões em nível de individuo, população e comunidade, quanto ao efeito do ambiente (no caso, tanto biótico quanto abiótico) no seu nicho e desempenho (BERG; ELLERS, 2010; KLEYER et al., 2012; MENEZES et al., 2020). Sendo assim, torna-se mais prático e acessível avaliar atributos morfológicos do que fisiológicos para encontrar associações de desempenho individual com condições extremas do ambiente ao redor. Essa compensação de acessibilidade pode ser usada para classificar atributos funcionais em: *soft traits*, os de fácil mensuração e associação mais indireta ao ambiente; e *hard traits*, com associação mais direta ao ambiente e de difícil mensuração (BELLUAU; SHIPLEY, 2018; HODGSON et al., 1999). Por exemplo, em ecossistemas abertos, atributos funcionais como folhas mais espessas estão associados à diminuição de perda de água por transpiração (FLETCHER; WOOD; HABERLE, 2014; LEIGH et al., 2012) e à resistência quando expostas a eventos de fogo e geadas (DE ANTONIO; SCALON; ROSSATTO, 2023; JARDIM et al., 2025; MARACAHIPES et al., 2018; MÜLLER et al., 2007).

No contexto espacial, é preciso ressaltar como a interpretação de tolerância varia quanto à escala de observação. Em micro e mesoescala, estudos de relação entre ambiente e comunidades vegetais fornecem um limiar mais preciso dos limites de ocorrência (GÓMEZ-APARICIO et al., 2008; SOHLBERG; BLISS, 1984). Como ilustração, estudos indicam que a estrutura de uma floresta é influenciada por seus microclimas, tal como o gerado pelo efeito de borda (RIES et al., 2004; VAN MELIS et al., 2021). O adensamento de espécies lenhosas sobre ecossistemas abertos também é influenciado pelo microclima gerado por elementos nucleadores, como as plantas-berçário (*nurse plants*), que podem ser árvores, arbustos, ou touceiras de gramíneas, ou mesmo rochas-berçário *(nurse rocks).* Esses elementos nucleadores favorecem a germinação e o desenvolvimento inicial de espécies vegetais pouco tolerantes às condições de ecossistemas abertos (ABREU et al., 2021; CARLUCCI; DUARTE; PILLAR, 2011; DUARTE et al., 2006). Por outro lado, quando a escala passa a ser mais ampla, ocorre uma generalização do que se entende como tolerância ambiental (GRACE, 1987). Assim, o uso dos dados de ocorrência das espécies é uma aproximação, embora com muitas cautelas, de condições favoráveis para o desenvolvimento da espécie (BOCSI et al., 2016; COLWELL; RANGEL, 2009). A correlação entre a tolerância mecanística, entendida como a resposta fisiológica das espécies às variações ambientais, e a tolerância espacialmente informada, baseada no clima presente na distribuição geográfica observada, pode ou não ser significativa (RUEDA; GODOY; HAWKINS, 2017; VISAKORPI et al., 2024).

Avaliar ecossistemas utilizando essas múltiplas abordagens permite construir um panorama geral no que diz respeito às respostas aos extremos de temperatura (CUSTER et al., 2024). Considerando as lacunas de conhecimento atuais sobre tolerância térmica, os *hotspots* de biodiversidade merecem uma atenção especial dado ao avanço das mudanças climáticas (TREW; MACLEAN, 2021). Projeções estimam que os efeitos das mudanças climáticas acoplados ao avanço da pressão econômica da agropecuária ameaçam principalmente os *hotspots* tropicais, como os da Ásia, África e América do Sul (HABEL et al., 2019). No Brasil, a Mata Atlântica é um desses locais com alta biodiversidade e muito fragilizado pela ação antrópica (HABEL et al., 2019; MARQUES; GRELLE, 2021). Sua distribuição original se estende desde o litoral do Ceará até o litoral do Rio Grande do Sul, compreendendo diferentes tipos vegetacionais (florestas tropicais, campos, restingas e manguezais; COLOMBO; JOLY, 2010). Dentre eles é possível destacar a Floresta com Araucária, uma vegetação associada a altas altitudes e caracterizada pela presença marcante da araucária [*Araucaria angustifolia* (Bertol.) Kuntze], conífera emergente no dossel florestal (CARLUCCI; MARCILIO-SILVA; TOREZAN, 2021; HOEHNE, 1930; MAACK, 1949). Além da araucária, ocorrem espécies de Lauraceae, Myrtaceae e Fabaceae, e de muitas outras famílias, algumas com certa resistência ao eventos de geada frequentes na região (ARAUJO FRANGIPANI; CRISTINA MÜLLER; ANAND, 2021; CARMO; MORO; NOGUEIRA, 2007; DORNELLES et al., 2022; OLIVEIRA-FILHO et al., 2015).

Evolutivamente, cabe destacar a peculiaridade da biogeografia histórica da Floresta com Araucária. Ela é formada por uma mistura de linhagens com ancestralidades distintas (CARLUCCI; MARCILIO-SILVA; TOREZAN, 2021). Linhagens de espécies como *A. angustifolia, Drimys brasiliensis* (Winteraceae)*, Podocarpus lambertii* (Podocarpaceae)*, Hennecartia omphalandra* (Monimiaceae), *Cinnamodendron dinisii* (Canellaceae) são traçadas a origens no antigo supercontinente de Gondwana (PENNINGTON; DICK, 2004; SEGOVIA; ARMESTO, 2015), tendo se diversificado no cone sul da América do Sul e migrado ao norte devido à orogênese dos Andes, que criou condições mésicas favoráveis a essas linhagens (MURILLO-A; STUESSY; RUIZ, 2016). Essas linhagens de legado austral convivem com espécies de linhagens originárias de biomas tropicais, como Amazônia e Cerrado, além de regiões da África e sudeste Asiático, que migraram ao sul devido ao fim do Último Máximo Glacial (ANTONELLI et al., 2009, 2018; OLIVEIRA-FILHO et al., 2015). As migrações em plantas estão associadas a um rastreamento de habitats propícios (ACKERLY D., 2003; DONOGHUE, 2008). A preferência de habitat pode representar uma similaridade de nicho em linhagens descendentes de uma mesma região biogeográfica (ACKERLY, 2009; CAVENDER-BARES et al., 2016), o que pode proporcionar tolerâncias ambientais similares entre espécies filogeneticamente distantes. Considerando a diversidade biogeográfica histórica, haveria a possibilidade de conservadorismo de nichos tropicais (WIENS; DONOGHUE, 2004) ou nichos austrais (SEGOVIA; ARMESTO, 2015) entre as linhagens de plantas atuais, influenciando suas tolerâncias a extremos de temperatura.

Dada a crescente ameaça das mudanças climáticas à biodiversidade global, em especial aos ecossistemas tropicais, é crucial entender como as plantas toleram diferentes temperaturas para prever padrões futuros de sobrevivência. No entanto, são poucos os estudos que combinam abordagens fisiológicas, morfológicas e biogeográficas (ver CAVENDER-BARES et al., 2016; SKLENÁŘ et al., 2012). Esta pesquisa pretende preencher essa lacuna, oferecendo uma análise detalhada de como diversas plantas da Mata Atlântica respondem a variações de temperatura. O primeiro capítulo aborda o impacto das geadas em plantas jovens ao longo de um gradiente campo-floresta, com objetivo de avaliar a correlação de estrutura das folhas com os efeitos de eventos de geadas no inverno. O segundo capítulo analisa como a resposta fisiológica de juvenis de algumas espécies arbóreas é influenciada pela história biogeográfica de suas linhagens ancestrais, avaliando também a relação entre respostas fisiológicas, atributos morfológicos e variáveis climatológicas na distribuição dessas espécies. Desta forma, este trabalho visa ampliar o conhecimento sobre a interação entre temperatura e plantas, conectando diferentes aspectos dessa relação em uma das regiões mais ricas em biodiversidade do mundo.

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2 CHAPTER I: Functional composition and frost effects on woody plant juveniles in a forest-grassland ecotone.

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FUNCTIONAL COMPOSITION AND FROST EFFECTS ON WOODY PLANT JUVENILES IN A FOREST-GRASSLAND ECOTONE

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ABSTRACT

Aims: The impact of climatic events on plants may vary depending on their functional traits and the surrounding environment. We aimed to assess whether and how leaf functional traits in woody plant juvenile communities occurring in the contrasting environments of an encroached grassland and a forest understory interact with frost events.

Location: Paraná State, Brazil.

Methods: We mapped leaf functional traits from woody plant juvenile occurring in an Araucaria mixed forest patch and an adjacent grassland under woody plant encroachment. We investigated the relationship of leaf functional traits with environmental conditions using the fourth-corner approach. We evaluated foliage volume and health before and after the 2024 winter frosts. The observed leaf damage was modelled as a function of the individuals' traits and the environment to identify patterns of the distinct frost impacts on woody plant communities.

Results: Leaves of plant individuals in forest and grassland communities were functionally distinct, differing significantly in leaf width, dry mass content, and thickness. Following frost events, leaf damage occurred in both communities: forest individuals exhibited less proportional damage than grassland ones, however, overall damage proportions for each vegetation did not differ significantly (p-value > 0.05). The observed damage patterns were linked to leaf structure, shape, plant height, and canopy cover.

Conclusions: While frosts are known to disturb plant communities in open ecosystems, their effects are generally not severe in well-established woody plants. Plants' functional traits integrated with their microenvironmental conditions protect them from harsh frost damage. The tendency of more oscillating temperatures and the unpredictable frosts may have no effect on forest-grassland mosaics maintenance, not negatively interfering in the woody plant encroachment as predicted.

KEYWORDS: Climatic events; Environmental pressure; Forest-grassland mosaics; Frosts; Functional traits; Saplings, Shrubs, Woody plant encroachment.

2.1 INTRODUCTION

Woody plant species evolved certain traits that allow them to survive and grow under the numerous adversities of open environments, such as high sun exposure, propensity to fires, and large herbivore activities. On the other hand, in forest understories, woody plant juveniles must grow in shady, humid, light-deprived conditions. In the forest-grassland ecotones, the coexistence of forests and grasslands in the same region depends on specific regimes that support each ecosystem as a stable state (Bond, 2008; Hirota et al., 2011; Hoffmann et al., 2012; Oliveras & Malhi, 2016). The stability of forest-grassland ecotones is affected if regime thresholds are surpassed (Hirota et al., 2011; de L. Dantas et al., 2013; Beckett & Bond, 2019; Beckett et al., 2022). For instance, woody plant encroachment occurs in open ecosystems due to the decrease in disturbances such as fire or herbivory by large animals (Carlucci, Teixeira, et al., 2011; Abreu et al., 2021; Müller et al., 2024), while the transition of forest to open ecosystems may be caused by intense and frequent fires (Beckett et al., 2022). However, extreme climatic events also affect this maintenance (Fahey et al., 2018; Hoffmann et al., 2019; Joshi et al., 2020). Climatic extreme events may affect plant physiology, compromise organ health, and lead to plant mortality (De Boeck et al., 2011; Niu et al., 2014). Frosts are one of these events, potentially leading to plant tissue damage or even individual death (Botha et al., 2020; Pilon et al., 2022), especially for the ground-layer vegetation. These impacts produce dead biomass from forbs, grasses, and woody plants that may fuel subsequent fires (Holdo, 2005; Ripley et al., 2015; Hoffmann et al., 2019).

Frosts are generally expected during the cold season in subtropical zones, whereas at lower latitudes they may occur at high elevations (Marengo et al., 2023).

Frost impacts on plants can be visually attested through monitoring of organ shedding (Wagner et al., 2021; de Antonio et al., 2023), slower regrowth (Araujo Frangipani et al., 2021) and the mortality of sensitive species (Ripley et al., 2015; Botha et al., 2020). However, the escalating global climatic change is altering the settings of climates with regional impacts. For instance, in South America, frost events are decreasing in frequency, precipitation volume is increasing in the southeast, while warm and dry days are becoming more frequent in the north (Cai et al., 2020; Gulizia et al., 2022; Feron et al., 2024). The decreasing frequency of frosts reinforces the urgency of studying their effects while they remain common, to understand their impact on vegetation under future climatic conditions. The novel climates – defined as climatic conditions with no close analogue conditions regionally or globally – are beginning to emerge in some regions and are expected to spread by the end of the 21st Century (Williams & Jackson, 2007; Trew et al., 2024). The transition toward novel climate regimes is characterised by more frequent hot days and irregular precipitation patterns (Feron et al., 2019; Gulizia et al., 2022), with some abnormal temperature drops during cold waves (Marengo et al., 2023), and greater temperature variations overall (Karl, 1988; Vasseur et al., 2014). These climate alterations are further intensified by other meteorological phenomena, such as El Niño and extratropical cyclones, both of which are changing their frequency and impact (Reboita et al., 2021; Tedeschi & Sampaio, 2022; Arizmendi et al., 2022). Although significant temperature fluctuations are becoming more frequent (Vincze et al., 2017; Feron et al., 2019; Wu et al., 2025), the studies on the impacts of interspersed heat and cold waves on terrestrial ecosystems remain scarce (but see Vasseur et al., 2014), with most research focusing only on heatwave effects (Seidl et al., 2017; Feng et al., 2021; Breshears et al., 2021).

Using plant functional traits is an informative way of investigating ecosystem responses to climate change (Huxley et al., 2023), because they reveal whether plants are filtered or not by environmental conditions (Violle et al., 2007; Kraft et al., 2015). Functional traits that respond to the environment can be understood both at the species level, as species adaptations, and at the community level, as scaled-up patterns that may also affect ecosystem processes (Laughlin, 2014; Funk et al., 2017; Lepš & de Bello, 2023). Frosts may generate functional responses that depend on different dimensions of the plant economic spectra, e.g., shifts in leaf buds, flower buds, and leaf phenology (Wisniewski et al., 2014; Vitasse, Lenz, Hoch, et al., 2014; de Antonio et al., 2021). While there is no single trait-environment relationship (Kleyer et al., 2012), certain leaf traits exhibit consistent associations with stress tolerance strategies, reflecting evolutionary trade-offs between resource acquisition and conservation (Weemstra et al., 2016; Maracahipes et al., 2018). Leaf traits, for instance, may express variables (e.g., wider blades, higher thickness, small areas) linked to conservative resource-use strategies that also enhance persistence after frost events (de Antonio et al., 2023). At the community scale, such adaptations can drive functional filters in frost-prone habitats, where favouring species with specific trait values (Reich, 2014; Laughlin et al., 2020).

The values of functional traits can be understood as plant ecological strategies and can be interpreted along a continuum of resource acquisition strategies (Reich, 2014). Regarding leaves, the continuum of resource acquisition expresses “fast” strategies at one end (hereafter, acquisitive), related to higher productivity and “slow” strategies at the other end (hereafter, conservative), related to lower productivity (Shipley et al., 2006; Reich, 2014). The “fast-slow” continuum is demonstrated in ecological gradients, such as forest interiors to open ecosystems (Maracahipes et al., 2018). Forest plants from the understorey stratum would present more acquisitive trait strategies related to leaf construction, such as larger lamina areas, lower mass of structural carbon, and wider blades compared to those from open ecosystems (Carlucci et al., 2012; Klipel et al., 2021, 2023). The acquisitive strategy is hypothesised to be related to a goal of maximum carbon intake to compensate for the lower light availability and asymmetric competition while downgrading mechanical resistance (Reich, 2014; Müller et al., 2021). In contrast, more conservative strategies of resource investment are selected for plants in open ecosystems (i.e., grasslands and savannas). The limitations of the open ecosystem environment are high irradiance, herbivory, and fires (Bond, 2008; de L. Dantas et al., 2013; Maracahipes et al., 2018), which may favour thicker leaves with narrower blades and higher mass of structural carbon (Maracahipes et al., 2018). Notably, such trait values confer cross-resistance to temperature extremes and mechanical damage: tough leaves in savanna plants are linked to both fire survival (Flake et al., 2021) and frost tolerance by reducing freeze-induced mesophyll ice formation (da Silva & Rossatto, 2022; Pilon et al., 2022; de Antonio et al., 2023). These strategies suggest a convergent evolutionary solution where conservative traits serve as multi-stress adaptations across open environments.

During the process of woody plant encroachment in open ecosystems, there is a selection for multi-stress tolerant species, with both grassland specialists and generalist species colonizing this ecosystem (Jardim et al., 2025). In this study, we investigated the functional composition and the effects of frost on woody plant juveniles (i.e., shrubs and trees) located along a forest-grassland ecotone in subtropical Brazil. We aimed to test the following hypotheses: (H1) in the forest would exhibit acquisitive leaf traits, such as higher specific leaf area (SLA) and lower leaf thickness, to optimize light capture under the shaded understory (Maracahipes et al., 2018), whereas those colonizing the grassland would display conservative traits, including lower SLA and higher leaf thickness, to resist frost and drought (Pilon et al., 2022) and reduce fire risk (Flake et al., 2021). (H2) Community composition would be more strongly influenced by vegetation structure, particularly canopy cover and stem density, than by functional trait patterns alone (Botha et al., 2020; Erdős et al., 2024). (H3) Regarding frost damage, we propose two alternative hypotheses: (H3a) It would be primarily driven by vegetation structure, with lower damage under dense canopy due to buffering effects (Hoffmann et al., 2011; De Frenne et al., 2019). (H3b) Alternatively, functional traits of leaf structure are better to predict frost damage, with acquisitive-leaved species suffering greater damage regardless of canopy cover (de Antonio et al., 2023; Jardim et al., 2025).

2.2 METHODS

2.2.1 Study area

The study took place in Vila Velha State Park (VVSP) (25°15’02” S, 49°59’59” W, at a mean altitude of 900 m), which covers 3,083 hectares and is in Ponta Grossa municipality, Paraná state, Brazil (Figure 1). The park is situated in the Campos Gerais region, which encompasses the Araucaria Forest range within the Atlantic Forest domain (Ab’Sáber, 2003) and relicts of the Cerrado domain (Silva et al., 2016). The landscape in protected areas showcases forest patches interspersed with grasslands, creating a vegetational mosaic (Ritter et al., 2010). The protected areas in the region have fire management actions to preserve open ecosystems and retard woody plant encroachment.

The composition of the forest patches has *Araucaria angustifolia* (Araucariaceae) emerging above the canopy. Species of the Lauraceae and Aquifoliaceae family are the most frequent in the upper strata, while the understorey has an abundance of Rubiaceae and Myrtaceae species (Maack, 1949; Carmo et al., 2007). In the areas of forest encroachment over grasslands, common woody plants include *Clethra scabra* Pers. (Clethraceae), *Myrsine umbellata* (Less.) H. Robinson (Primulaceae), *Frangula polymorpha* Reissek (Rhamnaceae), and some woody Asteraceae representatives (Carmo et al., 2007; Appendix S1).

The climate on VVSP can be described as Cfb (temperate oceanic) on Koppen’s classification (Cruz, 2007). This is a temperate climate category, with average temperatures below 18ºC in the coldest month and 22ºC in the warmest month. Mean annual precipitation is 1,554 mm, which is well distributed throughout the year. There is no distinct dry season, with precipitation levels only lowering in August and September. Frosts occur primarily during winter (June to September), with varying frequency each year.

2.2.2 Sampling design

Given that some other grassland areas in VVSP were highly encroached by shrubs or were recently burned, our sampling area was located on top of a hill at approximately 1000 m a.s.l. The area was burned in 2017, setting a sharp forest edge, but nowadays, a tenuous edge is expanding over grasslands. We established two adjacent 70 × 70 m plots, one in the forest interior and the other in the grassland under encroachment (Figure 1C). Each local plot area was divided into 49 100-m² subplots. In these subplots, we recorded adult trees (diameter at breast height - DBH > 5 cm). For grasslands, due to the low ramification height, the diameter for adults was measured at the ground level. In these same subplots, a 16-m² square was established for recording juvenile trees and shrubs (height > 130 cm and diameter at ground level - DGL < 5 cm). Of the 49 subplots, 16 were systematically sampled in each block (Figure 1D). The recorded plants were identified at the species level through the updated database of Brazilian flora (‘Flora e Funga do Brasil’, 2023). For the following methods, we primarily considered the plants in the 16-m² subplots.

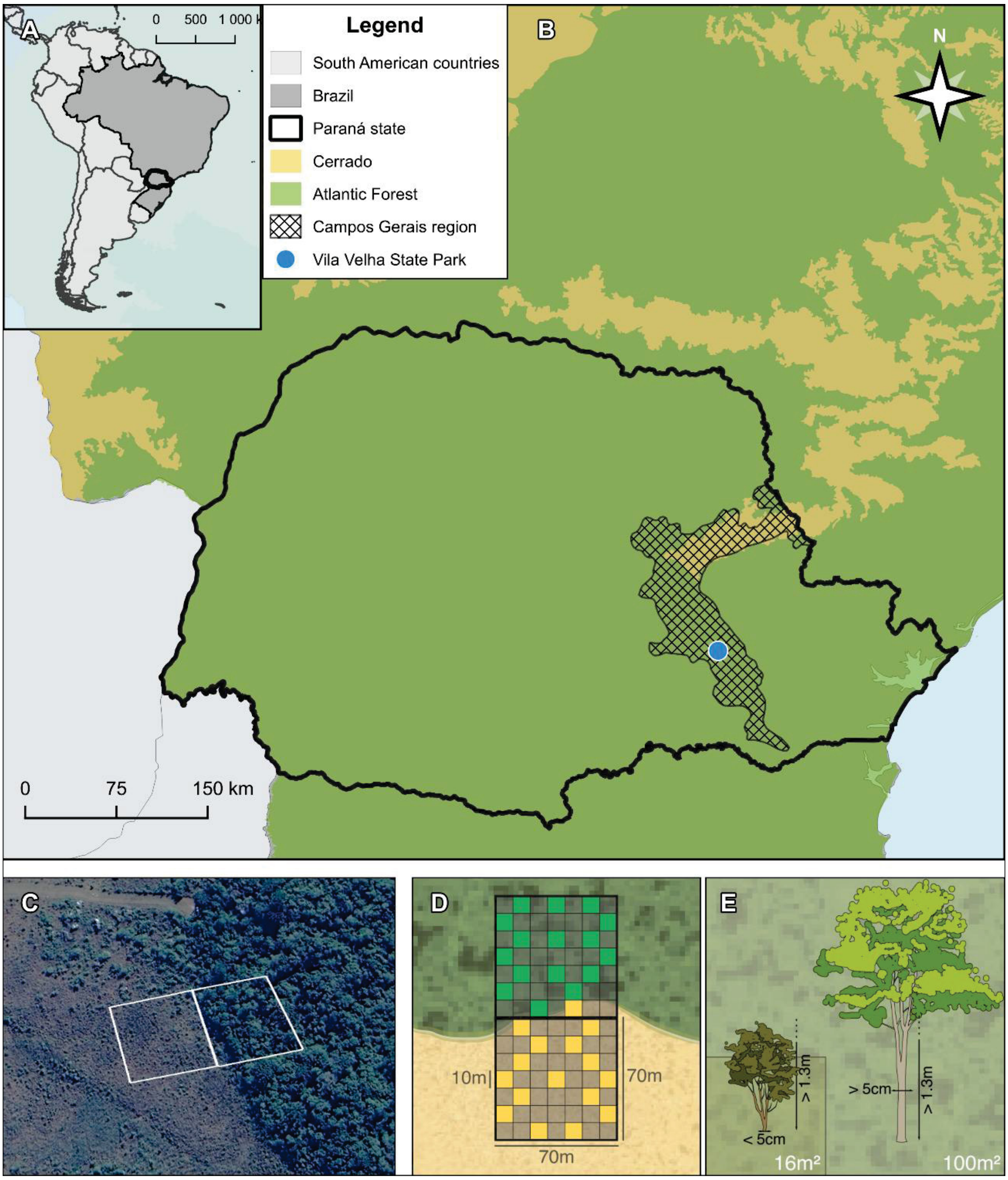

Figure 1. Study site and sample design. Legend: A - South American countries; B - Paraná state highlighted onto biomes; C - Satellite image of the study area; D – Illustrative scheme of plant sampling design; E – Illustrative scheme of plant size criteria for sampling in each plot size. Satellite images from 2023 (Airbus, Google Earth Pro). Biomes are delimited according to IBGE's (2012) classification.

2.2.3 Community structure covariates

To evaluate the covariates influencing the effects of frost, the following structural parameters of the community were calculated for each subplot: leaf area index (LAI) and community closure index (CCI). The LAI measures the total amount of leaves per unit ground, and it is directly related to the light coming to the storeys

(Charles-Dominique et al., 2018). To obtain it, we took canopy photos with an EOS REBEL T100 camera with a ROKINON 8MM F35 HD fisheye lens 130 cm above the ground at the centroid of each 16 m² plot. For LAI analysis, we standardised image exposure using Adobe Lightroom and made them binary using the Hemispherical_2.0 plugin for ImageJ (Beckschäfer, 2015). The analysis of LAI was conducted utilising the *hemispheR* package (Chianucci & Macek, 2023). The CCI approximates light availability and climatic buffering by considering the sum of the woody volume (i.e., assuming trees have a cone shape = basal area × height/3) in the plot (de L. Dantas et al., 2013). We calculated CCI for both 100-m² (considering adult trees) and 16-m² plots (considering juveniles trees and shrubs).

2.2.4 Assessment of frost effects

Frost events during 2024 were confirmed through public frost alerts (https://www.idrparana.pr.gov.br/Pagina/Alerta-Geada) and communication with the VVSP staff, leading to field campaigns for evaluation of the vegetation. Few cold fronts with different intensities were recorded throughout winter (Figure 2). Low precipitation and heat waves also occurred during the season, although ENSO (El Niño-Southern Oscillation) ended in the first semester of 2024.

Two foliage evaluations, one in autumn (April) and the other at the end of winter (September), were conducted on juvenile individuals (i.e., 16-m² plots), thus accomplishing the combined effect of all frost events. To parameterise both the quantity (i.e., amount of leaves held by the plant) and health of the foliage (i.e., appearance of the leaf blades), we adapted the Fournier index (Fournier, 1974): 1 – 100-76% of foliage quantity or integrity (unaffected); 2 – 75-51% of quantity or integrity (slightly affected); 3 – 50-26% of quantity or integrity (substantially affected); and 4 – 25-1% of quantity or integrity (strongly affected). The evaluations considered pathogens and other processes that also damage leaf tissue appearance, since they are similar and can be confused with the harmful effects of frost (Appendix S2).

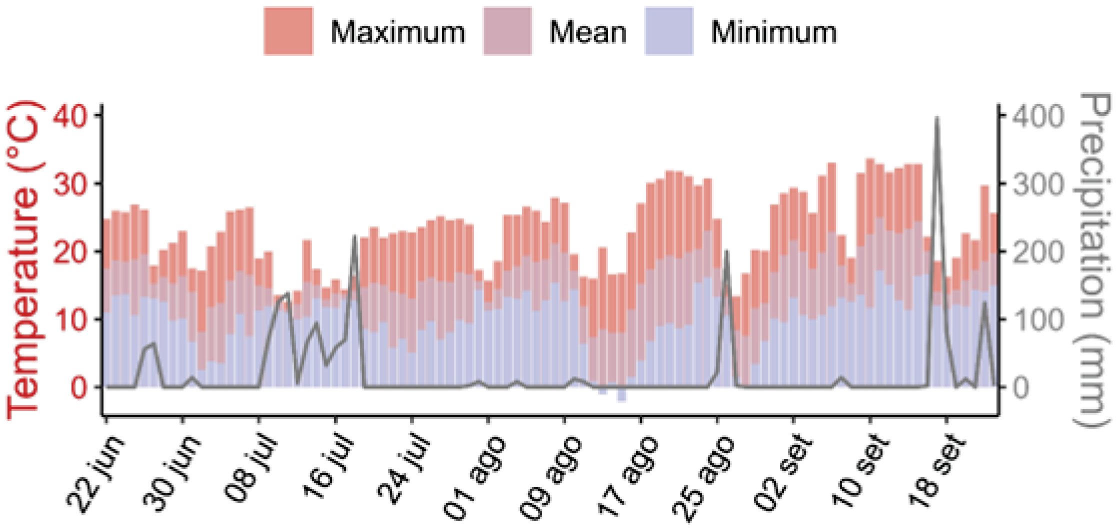

Figure 2. Daily temperature (coloured bars) and precipitation (gray line) plot of Ponta Grossa municipality throughout the 2024 winter. Source: SIMEPAR (Sistema Meteorológico do Paraná).

2.2.5 Functional traits measurement

Foliage material was collected from all juvenile individuals sampled within the 16m² plots to analyse the functional structure comprehensively. In strongly damaged or scarce foliage cases, healthier individuals of the same species within a 2-meter buffer were utilised for material collection. This alternative was only needed for 27 individuals. The fresh leaves were collected in the field and transported to the Functional Community Ecology Lab (LABEF-UFPR) for processing. The standard protocol was followed for collecting, storing, and measuring the selected functional traits (hereafter, FT; Pérez-Harguindeguy et al., 2013).

The selected FT encompasses the leaf economic spectrum and relates leaf sensitivity to external factors (Table 1), comprising trade-offs between resource acquisition and leaf resistance (Wright et al., 2004). Six morphological traits were selected: leaf area (LA), specific leaf area (SLA), leaf dry mass content (LDMC), leaf blade width (LWidth), leaf margin complexity (NDMC), and leaf thickness (LThick). Trait measurement included ten healthy, fully expanded leaves without evidence of pathogens or herbivory per individual. Exceptional cases include compound leaves, in which we considered the leaflets as lamina units, and naturally leafless plants (e.g., *Baccharis crispa* Spreng.), in which stem appendages were considered as photosynthetic structures (Pérez-Harguindeguy et al., 2013).

The processing of the traits began with weighing the fresh leaves using a precision scale and scanning with a portable scanner (CANON Lide 300 ®). The leaves were oven dried at 70ºC for 72 hours before being weighed again. This dual mass measurement allows for calculating the LDMC, the ratio between dry mass and fresh mass of the leaf, related to the structural content of leaf tissues (Wright et al., 2004). The scans were used to measure LA, LWidth, and NDMC using ImageJ software version 1.54 (Schneider et al., 2012). For LA, the area of the leaf blade along with the petiole was considered, whereas only the leaf blade was considered for LWidth and the margin complexity index. The margin complexity index is calculated by the normalised difference of marginal complexity (NDMC), using the perimeters of the leaf margin and its convex envelope: (margin – convex hull) / (margin + convex hull) (Leigh et al., 2017). This index ranges from 0 to 1, with leaves having entire margins close to zero and those with more complex margins and blades close to one. The division of leaf area by dry mass refers to the SLA, a trait indicative of growth rate and resistance (Wright et al., 2004). LThick was estimated by calculating the fresh mass divided by the leaf area (Vile et al., 2005).

Table 1. Description and expectation for the selected functional traits measured.

Trait	Acronym	Unit	Functional significance	References	Prediction	
					Forest	Grassland
Leaf area	LA	cm²	Respond to nutrient limitations, adverse environmental conditions, and stresses.	Díaz et al., 2016; Donovan et al., 2011; Reich, 2014; I. J. Wright et al., 2004	High	Low
Specific leaf area	SLA	cm². g^{-1}	Proxy for growth rate and resistance. It is related to a trade-off between carbon gain and longevity.	Same as LA	High	Low
Leaf dry mass content	LDMC	g. g^{-1}	Related to investment in structure, longevity and defence.	Same as LA	Low	High
Leaf blade width	LWidth	mm	Related to the thermic	Fauset et al., 2018; Leigh et	Low	High

			balance of the mesophyll.	al., 2017; Pérez-Harguindeguy et al., 2013		
Normalised difference of the marginal complexity	NDMC	Unitless	Index related to the leaf marginal aspect. The margin complexity involves mechanisms of hydraulic balance. It has been hypothesised as a proxy of cold tolerance.	Leigh et al., 2017; Royer & Wilf, 2006; Wang et al., 2022	Low	High
Leaf thickness	LThick	mm	It relates to resource acquisition and allocation and correlates with leaf toughness.	Gast et al., 2020; Gurvich et al., 2002; Vile et al., 2005	Low	High

2.2.6 Data analyses

2.2.6.1 Functional composition

The trait matrix was grouped by vegetation (i.e., grassland and forest) for analysis. This matrix was standardised to a mean of zero and a standard deviation of one to equalise variation between variables; we used the *'decostand'* function from the *'vegan'* package (Oksanen et al., 2022). The dimensionality of the FT was reduced through a principal components analysis (PCA). The functional investigation and differentiation of grassland and forest individuals were analysed through an analysis of variance (ANOVA). The models were performed using the '*adonis2*' function from the vegan package (method = 'Euclidean, permutation = 999'), followed by post hoc analysis using a pairwise two-sample permutation test. A multivariate kernel density estimation was calculated to represent the probability of occurrence of specific functional trait combinations in a two-dimensional space defined by the PCA axes (Carmona et al., 2016). This technique reveals areas of high occupancy density in the functional space. We used the '*stat_density_2d*' function from the '*ggplot2*' package to generate the plots based on the '*kde2d*' function from the '*MASS*' package for density estimation.

All analyses were conducted in R software version 4.3.2 (R Core Team, 2023).

2.2.6.2 Trait-community structure relationships

We applied a double-constrained correspondence analysis to relate community structure variables to trait values and community composition (dc-CA; ter Braak et al., 2018). The dc-CA method is a regression-based technique to assess the "fourth-corner problem" (Dray & Legendre, 2008). This method utilises correlation to link species traits with environmental variables by analysing three datasets: species' trait values, site environmental conditions, and species abundances per site. The approach optimises the correlation between traits and environmental conditions through weighted least squares, considering species and site abundances. Focusing on the fourth-corner correlation, dc-CA investigates how environmental conditions influence community functional composition and the relationships between species' niche centroids (SNC) and their traits (ter Braak et al., 2018). SNCs represent the average environmental conditions in which species thrive, weighted by their abundances, and help uncover whether traits influence species presence. The considered community structure parameters were the CCI for 16-m² plots and 100-m² plots, and the LAI for 16-m² plots. For traits, we summarised the values for each species in each vegetation type. We separated generalist species that occur in the two different environments.

2.2.6.3 Frost interactions with the environment and traits

Firstly, we assessed the variation in leaf damage between individuals from both vegetation types. Final damage levels were calculated by treating foliage classifications as integer values and subtracting posterior evaluation scores from preview scores. Positive values indicated damage, while negative values indicated recovery. Subsequently, we considered the proportion of damage levels for each vegetation type for a chi-square test. We analysed foliage quantity and leaf integrity separately.

To understand how community structure and functional diversity were related to frost effects on plants, we performed two classification models (i.e., one for foliage quantity and the other for leaf integrity) under the random forest algorithm using the *tidymodels* framework (Kuhn & Wickham, 2020) and the “ranger” engine (Wright & Ziegler, 2017). We considered only the posterior evaluation for frost response at the individual level as the dependent variable, individuals' trait values, their individual heights, and community parameters (i.e., LAI, CCI for 100-m² subplots, and CCI for 16-m² subplots) repeated for each individual as independent variables.

The data were split into training and testing sets using an 80/20 ratio. The training set was used for model development, while the test set was reserved for model evaluation. For model parameterisation (i.e., the number of predictors randomly selected at each split and the minimum number of data points in a terminal node), we tuned the parameters with random values using 3-fold cross-validation generated from the training set. The best parameter values were chosen based on higher values of the Kappa index (Cohen, 1960). We considered the Kappa index and the balanced accuracy on the final model fitting for performance evaluation. Afterwards, we obtained the variable importance as the average change in performance after a random permutation (see Table 5 for variable importance in the prediction of each class using Shapley Additive Explanation).

2.3 RESULTS

2.3.1 Functional composition

The first two PC axes of species traits accounted for 67.6% of the variation in the functional space composed of six traits (Figure 3). The first axis revealed a spectrum of leaf structural investment versus resource acquisition, but poorly reflected differences between forest and grassland. Individuals with high SLA presented lower values of LA, LThick, and LWidth (Table 2), showing that both forest and grassland harbour species located along the major dimension of the leaf economic spectrum (Figure 4A; $F(1, 375) = 3.73$, $\eta^2 = 0.01$, p-value = 0.054). The second axis better differentiated grassland and forest, with individuals in grasslands with higher LDMC and NMDC but lower LA, SLA, and LWidth (Figure 4B, Table 2; $F(1, 373) = 261.8$, $\eta^2 = 0.41$, p-value < 0.001). The higher kernel density for the two vegetation individuals is strongly correlated with PC2 (Figure 3), highlighting common trade-offs of LA and LWidth versus LDMC and NDMC among the individuals.

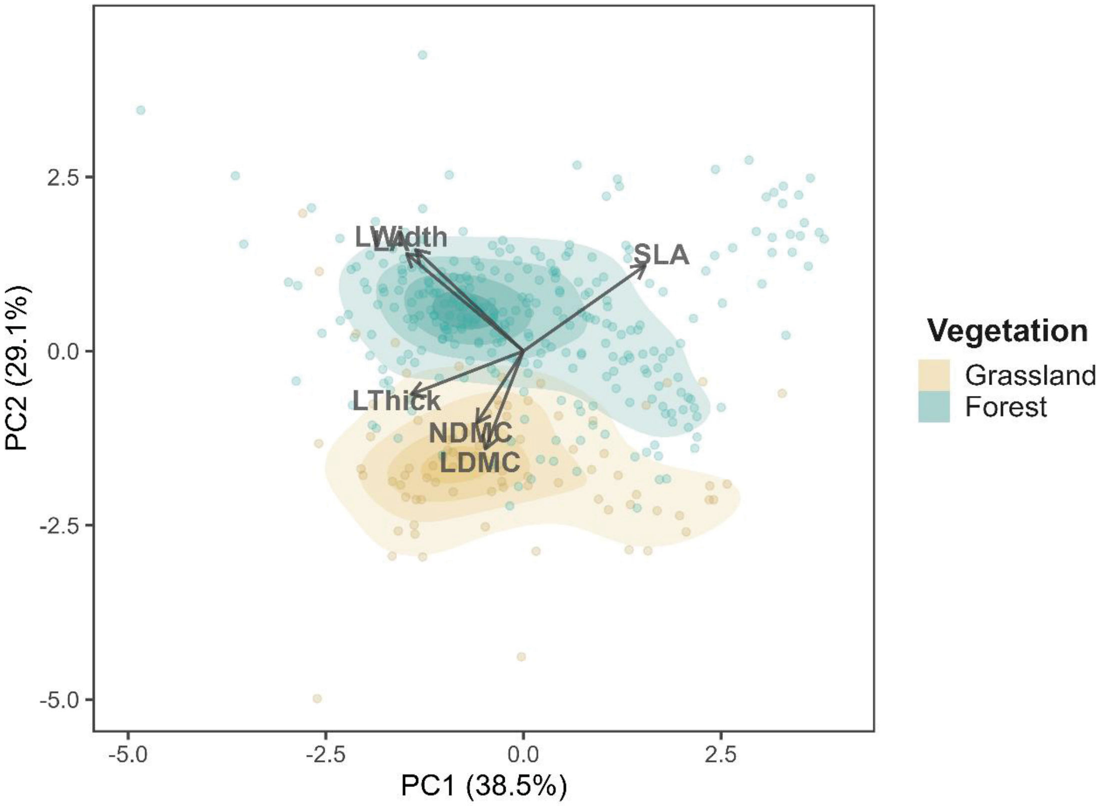

Figure 3. Ordination scatterplot of Principal Component Analysis (PCA) on leaf functional traits of juvenile woody plants individuals from Vila Velha State Park. Deep hues signify areas of functional hotspots within the multivariate functional space, highlighting regions with a greater concentration of individuals. Legend: LA – leaf area; SLA – specific leaf area; LDMC – leaf dry mass content; LWidth – leaf width; NDMC – normalised difference of margin complexity; LThick – leaf thickness.

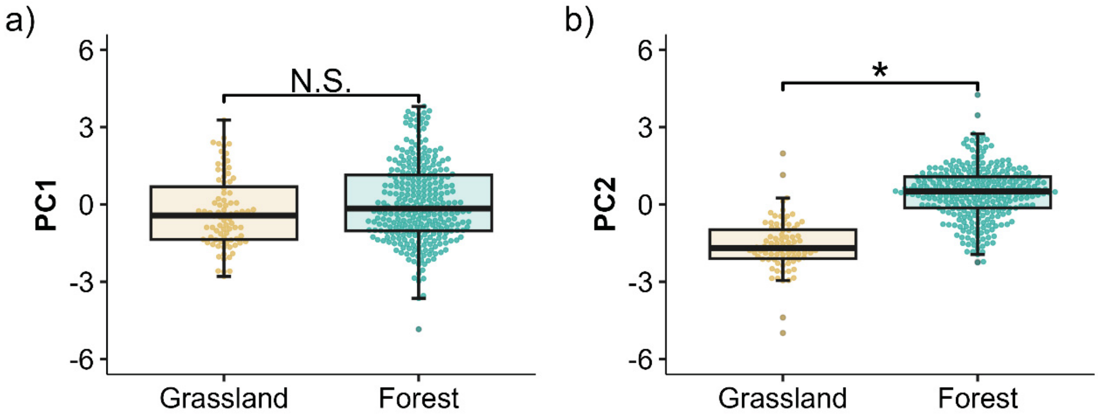

Figure 4. Boxplots of individuals' habits on Principal Component Analysis (PCA) first (A) and second (B) axes. Points behind the boxplots represent individuals. Annotation above boxplots represent the results of an Analysis of Variance: asterisks are for p-value < 0.05, NS. stands for "non-significant" (p-value > 0.05).

Table 2. Trait loadings of the principal component analysis (PCA) on treelets individuals' traits. Trait values were standardised to mean 0 and standard deviation 1. Legend: LDMC – leaf dry mass content,

LA – leaf area, SLA – specific leaf area, LThick – leaf thickness, LWidth – leaf width, NDMC – normalised difference of marginal complexity.

Functional traits	PC1 (38.5%)	PC2 (29.1%)	PC3 (16.6%)
LDMC	-0.161	-0.468	0.714
LA	-0.492	0.466	0.083
SLA	0.514	0.41	-0.125
LThick	-0.472	-0.206	-0.429
LWidth	-0.454	0.485	0.177
NDMC	-0.195	-0.342	-0.503

2.3.2 Trait-environment relationships

The first two dc-CA axes revealed significant relationships between traits and environmental conditions (max-test, p-value = 0.001; Figure 5, Table 3). The first axis describes a gradient from the grassland to the forest regarding light availability, with forests being shadier than any grassland community, but also tending to have species with entire leaf margins and thinner leaf blades (Figure 5). Variations in woody plant density mostly explained the second dc-CA axis, showing a negative correlation in communities regarding the closure of understory (i.e., plots of 16m²) and in communities to the closure of mature tree plots (i.e., plots of 100m²). The trait gradient associated with the second axis segregates communities with species with leaves with lower dry mass content and complex margins from those with species of lighter leaves and entire margins (Figure 5).

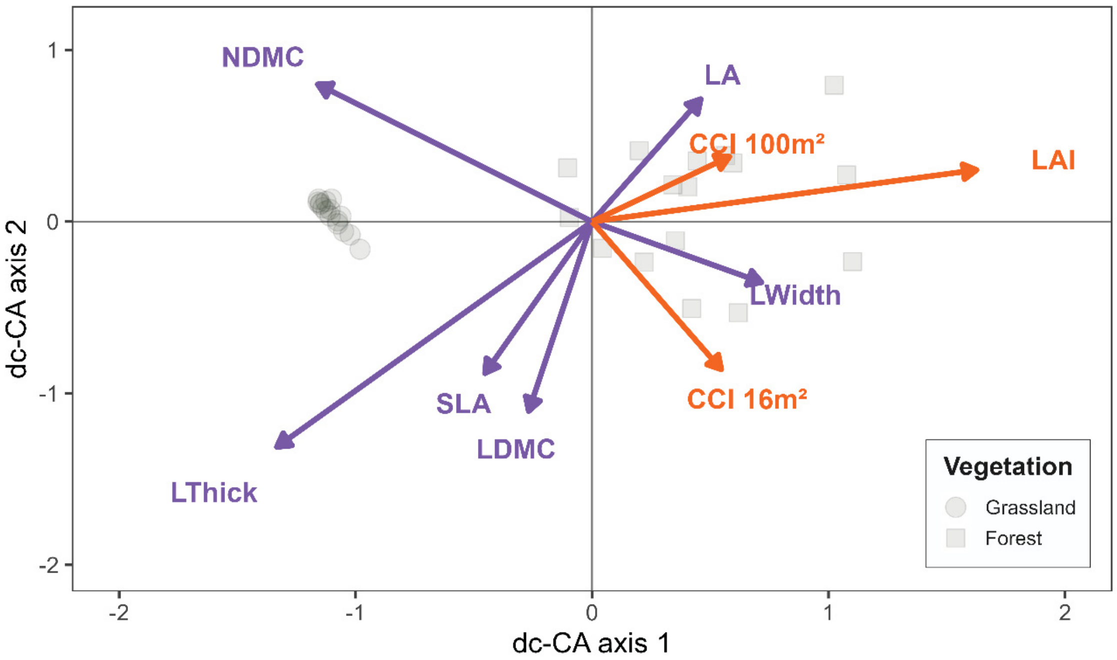

Figure 5. Multivariate space of vegetation plots (16 m²) in the forest-grassland ecotone of VVSP. This diagram highlights the canonical weights of community variables and the scaled correlations of traits, consolidating the coefficients derived from multiple regressions of community abundance-weighted means (CWMs) of traits against environmental predictors. It also displays the constrained scores that indicate the positions of the samples (plots) within the dc-CA biplot. Legend: LDMC – leaf dry mass content, LA – leaf area, SLA – specific leaf area, LThick – leaf thickness, LWidth – leaf width, NDMC – normalised difference of marginal complexity, CCI 16m² - community closure index for 16m² plots, CCI 100m² - community closure index for 100m² pots, LAI – leaf area index.

Traits and community variables were good predictors of species composition (fourth-corner correlation of 71%, Table 3). Leaf traits were strong predictors of the species niche centroids, accounting for 40% of the variance explained (Table 3). In contrast, environmental variables were less effective in capturing the variation in community abundance-weighted trait means (27.13% of variance explained, Table 3).

Table 3. Statistics from double-constrained correspondence analysis (dc-CA). E = environmental (climate) variables; T = functional traits; CWM = community weighted mean traits; SNC = species niche centroids.

Statistic	Axis 1	Axis 2	Axis 3
dc-CA eigenvalues	0.50	0.10	0.00
fourth-corner correlations (rFC)	0.71	0.32	0.07
% Explained fitted variation (cum.)	82.14	99.3	100.00
% CWM variation expl. by E (adj R2)	18.3	27.13	27.49
% SNC variation expl. by T (adj R2)	32.85	40.03	40.36

2.3.3 Frost interactions with the environment and traits

Foliage parameters showed a high number of individuals affected before frost events (Figure 6). At the end of the winter, foliage quantity and leaf integrity changed in the evaluated individuals (Figure 6, Appendix S3). Many individuals that initially exhibited perfect foliage conditions decreased leaf quantity and integrity after the frost events (Figure 6; Table 4). The number of individuals classified in the worst conditions doubled by the end of the season (Figure 6; Table 4). It is worth noting that some plants improved the classification in the posterior evaluation (i.e., an increase in leaf amount and/or health). When comparing the damage proportions between forest and grassland individuals, we found no significant difference for foliage quantity ($X^2 = 0.318$, $p = 0.99$) and leaf integrity ($X^2 = 0.147$, $p = 0.99$).

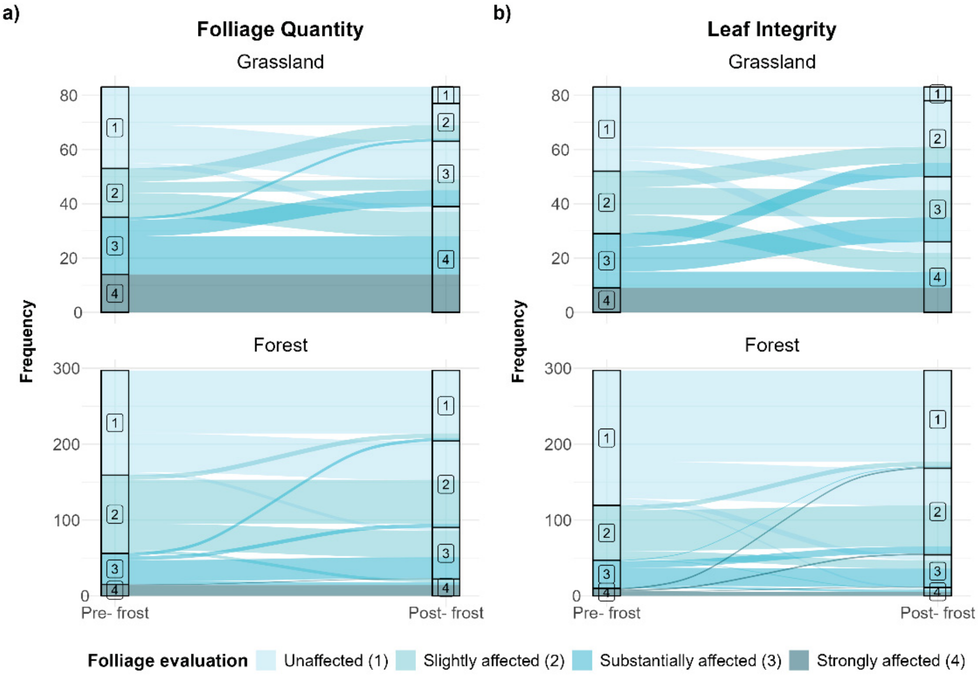

Figure 6. Alluvial plots of state change in a) foliage quantity and b) leaf integrity of juvenile woody plant individuals before and after frost events in both areas (i.e., grassland and forest). Foliage evaluation was performed using an adaptation of the Fournier index. We classified individuals from 1 (unaffected foliage) to 4 (strongly affected foliage).

Table 4. Table of the number and proportion of individual classifications that changed after frosts compared to before for each vegetation type.

Parameters	Class	Grassland	Forest

		Pre-frosts	Post-frosts	Proportion of change (%)	Pre-frosts	Post-frosts	Proportion of change (%)
Foliage quantity							
	Unaffected	30	6	-28.9	138	93	-15.2
	Slightly affected	18	14	-4.82	103	114	3.7
	Substantially affected	21	24	3.61	41	68	9.09
	Strongly affected	14	39	30.1	15	22	2.36
Leaf integrity							
	Unaffected	31	5	-31.3	178	129	-16.5
	Slightly affected	23	28	6.02	72	114	14.1
	Substantially affected	20	24	4.82	37	43	2.02
	Strongly affected	9	26	20.5	10	11	0.337

The random forest models performed moderately when testing the influence of functional traits and environmental variables on predicting frost effects, considering the indices chosen. For foliage quantity, the best model had a Kappa index of 0.229 and a balanced accuracy of 0.622. Among the predictor variables, individual height was the most important to distinguish each class (Figure 7A, Table 5). For leaf integrity, the Kappa index was 0.367, and the balanced accuracy was 0.681. The NDMC trait was the most influential variable in the model, with the others presenting similar values of importance (Figure 7B, Table 5).

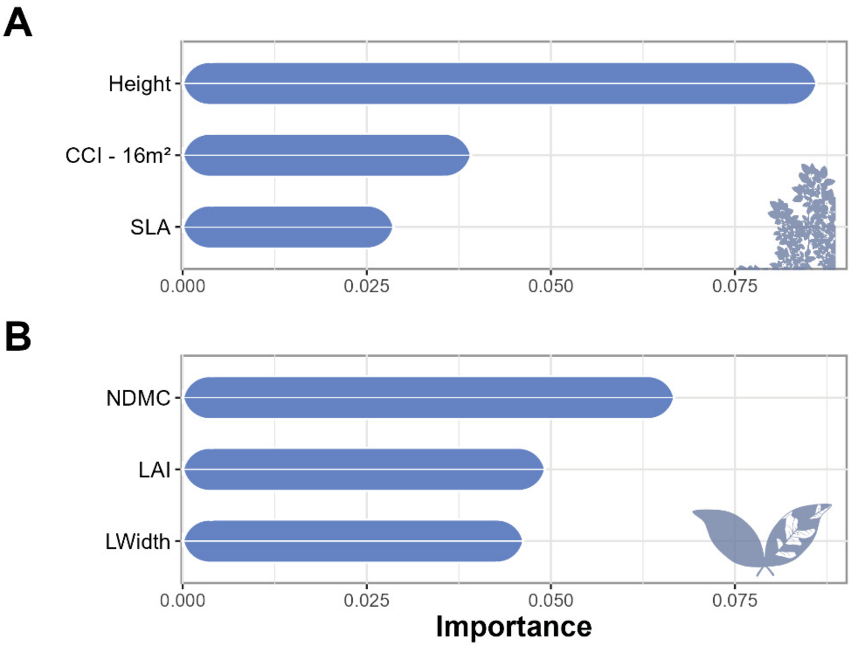

Figure 7. Barplots represent the three most important variables for classifying observations in the random forest models for foliage quantity (a) and leaf integrity (b). Variable importance is calculated using the permutation method that shuffles each predictor variable and observes the impact on the model's performance. Legend: CCI 16m² – community closure index for 16m² plots, SLA – specific leaf area, NDMC – normalised difference of the marginal complexity, LAI – leaf area index, LWidth – leaf width.

Table 5. Table of Variable Importance assessed using the Shapley Additive Explanation (SHAP) method. The SHAP method returns the importance of each environmental/trait variable for classifying observations into one of the four categories within the two foliage parameters (i.e., foliage quantity and leaf integrity). Legend: LDMC – leaf dry mass content, LA – leaf area, SLA – specific leaf area, LThick – leaf thickness, LWidth – leaf width, NDMC – normalised difference of marginal complexity, CCI 16m² - community closure index for 16m² plots, CCI 100m² - community closure index for 100m² pots, LAI – leaf area index.

	Foliage quantity				**Leaf integrity**			
	Unharmed	Slightly harmed	Substantially harmed	Strongly harmed	Unharmed	Slightly harmed	Substantially harmed	Strongly harmed
LDMC	0.02	0.022	0.023	0.017	0.038	0.043	0.023	0.011
LA	0.021	0.037	0.026	0.03	0.032	0.035	0.023	0.022
SLA	0.026	0.028	0.028	0.015	0.032	0.042	0.041	0.012
LThick	0.033	0.026	0.026	0.025	0.041	0.039	0.033	0.012
LWidth	0.023	0.04	0.024	0.031	0.024	0.028	0.021	0.019
NDMC	0.031	0.021	0.021	0.022	0.081	0.061	0.034	0.023
Height	0.076	0.044	0.039	0.019	0.033	0.043	0.024	0.013
CCI 16m²	0.03	0.034	0.032	0.025	0.05	0.032	0.033	0.028
CCI 100m²	0.024	0.051	0.032	0.032	0.066	0.04	0.038	0.028
LAI	0.03	0.039	0.025	0.034	0.067	0.05	0.025	0.03

2.4 DISCUSSION

Frost's impact on woody plants in a forest-grassland ecotone depends on the individual functional traits and the environmental structure. The quantity of foliage after several frost effects could express the direct impact of frosts (Brando & Durigan, 2005), nonetheless, the most important variables from the random forest models showed competition-related factors (e.g., height and CCI; Figure 7A). There is a well-known relationship between plants' maximum height and light acquisition (Falster et al., 2017), which also influences leaf production in certain understorey species (Kursar & Coley, 1999). The SLA values, the third most explainable variable to classify plant foliage quantity after frosts, are related to leaf lifespan (Wright et al., 2004). Plants that invest in high values of SLA (i.e., larger area per low dry mass) tend to have more short-lived leaves (Reich et al., 1991; Kunstler et al., 2016).

Conversely, leaf integrity classification after frosts is explained mainly by some leaf traits (i.e., NDMC and LWidth) and the LAI (Figure 7B). NDMC, although the most important variable, is hard to link with the plant fitness regarding temperature tolerance. Leaf marginal complexity is a weak proxy of leaf physiology (Leigh et al., 2017), and does not have a single correlation pattern with a specific environment, as higher values are a possible result to different environmental conditions, such as low temperatures, high precipitation and high air relative humidity (Royer & Wilf, 2006; Feild & Arens, 2007; Royer et al., 2012; Carlucci et al., 2012; Li et al., 2016; Shi et al., 2019; Frye et al., 2020; Iszkuło et al., 2024). In contrast, the leaf blade width is related to thermal tolerance, with narrower leaves better regulating thermal stresses (de Antonio et al., 2023; Posch et al., 2024). Moreover, the classification of plants into different categories of leaf damage was associated with the LAI of communities, here also expressing the amount of buffering the canopy provides to the overstorey layer in harsh conditions (i.e., frosts, heatwaves, and hailstorms; De Frenne et al., 2019).

There were explicit correlations of functional traits and environmental variables for individuals and communities. While SLA effectively differentiates forest individuals from those in grasslands, this distinction does not hold when examining communities' weighted means. This outcome may come from the species niche centroids controlling community patterns in forest plots (Peng et al., 2021). Additionally, species' spatial preferences are not well distinguished in small spatial scales (Viana & Chase, 2019), potentially due to stochastic processes shaping local occurrences (Ren et al., 2022). Despite these complexities, individuals and communities clearly show a pattern regarding LWidth associated with forest conditions. Given that LWidth is also associated with LAI and CCI at the community level, the positive correlation among them results in wider leaves in crowded and light-scarce plots. These values likely reflect the scarce light availability in the forest understorey and a strong competition for resource acquisition (King & Maindonald, 1999). In grasslands, individuals and communities present higher values of NDMC. The construction of more dissected leaves is hypothesised to be a consequence of both climate and genetics (Nicotra et al., 2011). Studies conducted on physiological processes have failed to establish a straight correlation between marginal complexity and thermal tolerance (Leigh et al., 2017). There is no consensus on the correlation between this phenotype and its development mechanisms on a global scale, with variations observed between deciduous and evergreen species (Iszkuło et al., 2024). Our results showed multiple ways for plants to cope with freezing temperatures. It is known that plants can present different strategies to deal with stress (Levitt, 1980; Sklenář et al., 2012; Oliveira et al., 2021; Tarvainen et al., 2022). When facing temperature stresses, for instance, plants may shed their leaves to escape any damage (Williams et al., 2008; Hasselquist et al., 2010), but some species possess other adaptations to avoid or tolerate these conditions (Wisniewski et al., 2014; Posch et al., 2024). Avoidance can be described as the minimisation of damage through structural characteristics, such as cell format and disposition leading to water supercooling (Wisniewski et al., 2014), control of stomata (Sato et al., 2024), and soil-deepened organs (Wisniewski et al., 2014; Lubbe et al., 2021). Both strategies would be preferable for plants that face freezing conditions for short periods (di Francescantonio et al., 2020). Nevertheless, damage minimisation mechanisms depend on gradual acclimation (Yamori et al., 2014). Climate change has the potential to induce significant alterations in temperature patterns, resulting in an increased probability of anomalous warm spells occurring during the winter months (Vincze et al., 2017; Feron et al., 2019), leading to deacclimation and loss of frost resistance in plants (Loveys et al., 2006; Vyse et al., 2019). Deacclimation is known to affect ecosystems dependent on cold (Pagter & Arora, 2013; Bokhorst et al., 2018), however, it has not yet been fully comprehended in other systems that experience short moments of freezing temperatures. Our results suggest that current occasional frosts, even when interspersed with heatwaves, do not hinder the establishment or development of woody plants in subtropical open ecosystems. This pattern may be associated with woody plant encroachment under future scenarios of increasingly rare frosts; however, further studies are required to test this hypothesis (Joshi et al., 2020).

In subtropical Brazil forest-grassland ecotones, cold deacclimation may favour the establishment and growth of frost-resistant woody species through nurse elements (e.g., grasses, shrubs, rocks), accelerating plant encroachment in open ecosystems.

Our evaluations showed a high number of individuals with affected foliage parameters before frosts events. This pattern could arise from pathogens, as pointed in the methods, but also because of the leaf senescence processes started in the autumn (season of the pre-frost evaluation). Despite the high proportion of severely damaged plants in the grassland following the frosts, the overall pattern of change did not differ significantly between the two vegetation types. It is known that the effects of frost vary along a vertical gradient (Stutsel et al., 2020). Studies focusing only on ground-level plants in open ecosystems report greater impacts depending on soil cover and herbaceous canopy structure (Pilon et al., 2022; de Antonio et al., 2023), which in turn delays the establishment of tree seedlings (Botha et al., 2020; Joshi et al., 2020). One could assume that tree survivors in grasslands exhibit greater resistance to frost (de Antonio et al., 2021) and/or were facilitated by other elements (i.e., trees, shrubs, rocks; Duarte et al., 2006; Carlucci, Duarte, et al., 2011; Abreu et al., 2021; Müller et al., 2024). This study found that more severe effects (e.g., complete foliage death) are not uniformly observable in already established woody plants. Their occurrence as tall individuals likely results from the absence of major disturbances such as fire (Müller et al., 2012). Such an undisturbed environment promotes the clear development of woody species, enhancing their ability to survive fire and frost as they age and grow taller (Botha et al., 2020).

The occasional frosts in subtropical Brazil are a class of disturbance in open ecosystems, however, it is more selective in their impact than fire, for instance. By evaluating the impact of frosts on woody plants across a forest-grassland ecotone, this study showed that plants' environmental context and functional traits play a crucial role in moderating the frost effects. We showed that woody plant juveniles from forests present leaves with distinct resource use strategies from grassland individuals. Such variation in functional traits is mostly explained by the buffering of the canopy and neighbouring woody plants. Although isolating the frost effects from other causes was not feasible, their occurrence changed at certain degree the plants' foliage characteristics. Leaf traits, height, and microenvironment were key influences on frost effects on the plants evaluated. The mild response of woody plant juveniles suggests that, while disruptive for the ground-layer vegetation of open ecosystems, frosts do not significantly threaten established woody plants, as they have already withstood other environmental pressures over time. Looking forward, advancing our understanding of how climate disturbances influence species-rich ecotones remains a critical frontier in ecological research. Future studies should examine the interplay between temperature fluctuations and community dynamics, using in-field experiments and laboratory approaches to refine our predictions about plant resilience and ecosystem stability in frost-prone ecosystems.

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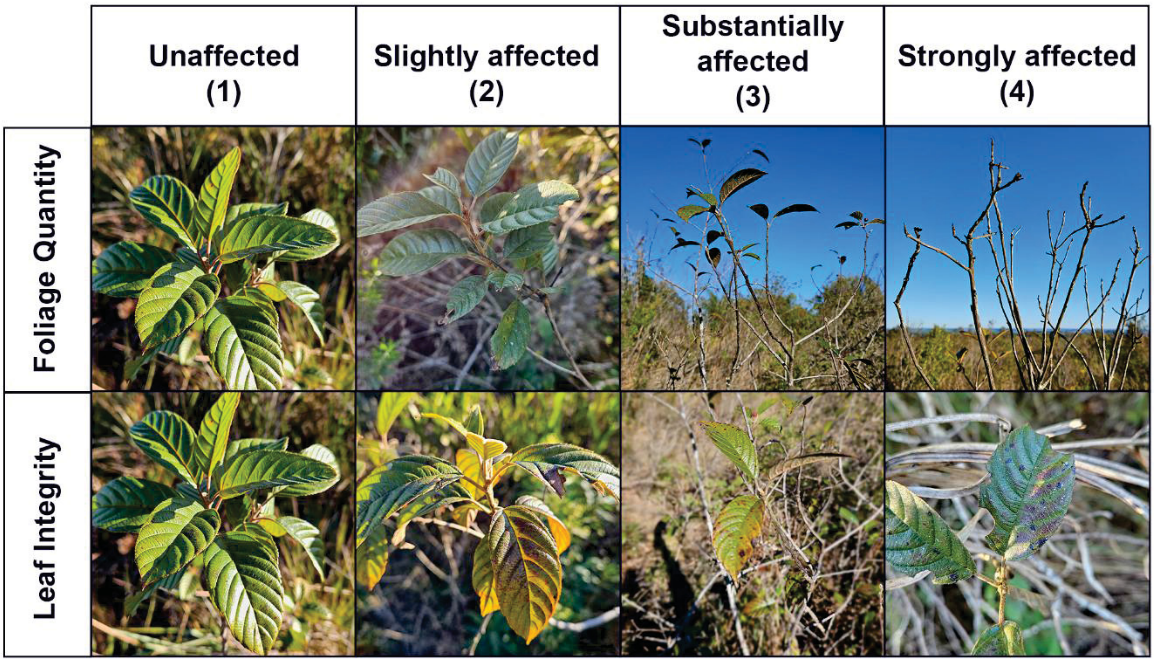
2.5 SUPPORTING INFORMATION

Appendix S1. List of juvenile tree and shrub species with their abundance in the 16-m² subplots.

Species	Family	Abundance	
		Grassland	Forest
Ilex paraguariensis	Aquifoliaceae	0	1
Ilex theezans	Aquifoliaceae	0	6
Asteraceae sp1.	Asteraceae	1	0
Baccharis cf. oblongifolia	Asteraceae	1	0
Baccharis crispa	Asteraceae	18	0
Baccharis dracunculifolia	Asteraceae	4	0
Baccharis uncinella	Asteraceae	6	0
Chromolaena laevigata	Asteraceae	1	0
Grazielia intermedia	Asteraceae	6	0
Moquiniastrum polymorphum	Asteraceae	0	2
Monteverdia gonoclada	Celastraceae	0	6
Clethra scabra	Clethraceae	30	3
Alchornea triplinervea	Euphorbiaceae	0	1
Dalbergia brasiliensis	Fabaceae	0	1
Vitex megapotamica	Lamiaceae	0	4
Nectandra grandiflora	Lauraceae	0	1
Strychnos brasiliensis	Loganiaceae	0	1
Leandra aurea	Melastomataceae	0	1
Miconia sellowiana	Melastomataceae	0	4
Mollinedia clavigera	Monimiaceae	0	7
Sorocea bonplandii	Moraceae	0	7
Campomanesia adamantium	Myrtaceae	0	1
Eugenia longipedunculata	Myrtaceae	0	2
Eugenia neoverrucosa	Myrtaceae	0	1
Myrcia glomerata	Myrtaceae	0	9
Myrcia selloi	Myrtaceae	0	1
Myrcia splendens	Myrtaceae	1	6
Myrciaria floribunda	Myrtaceae	0	5
Myrciaria cuspidata	Myrtaceae	0	1
Myrtaceae sp1.	Myrtaceae	0	1
Plinia peruviana	Myrtaceae	0	1
Myrsine gardneriana	Primulaceae	0	2
Myrsine umbellata	Primulaceae	7	15
Roupala montana	Proteaceae	0	1
Frangula polymorpha	Rhamnaceae	10	5
Prunus myrtifolia	Rosaceae	0	2
Coussarea contracta	Rubiaceae	0	36
Palicourea sessilis	Rubiaceae	0	19
Psychotria suterella	Rubiaceae	0	20
Rudgea sessilis	Rubiaceae	0	10

Species	Family	Abundance	
		Grassland	Forest
Esenbeckia grandiflora	Rutaceae	0	97
Casearia decandra	Salicaceae	0	6
Casearia sylvestris	Salicaceae	0	3
Allophylus edulis	Sapindaceae	0	1
Matayba elaeagnoides	Sapindaceae	0	4
Solanum argenteum	Solanaceae	0	1
Solanum pseudoquina	Solanaceae	0	1

Appendix S2. Example of classification of foliage characteristics on *Frangula polymorpha* Reissek (Rhamnaceae).



Appendix S3. List of mean classification of species regarding their foliage quantity and integrity before and after frosts. Values represent the average classification and standard deviation. Difference between after and before evaluations are interpreted as condition downgrading (positive mean values) or upgrading (negative mean values).

Species	**Pre-frosts**		**Post-frosts**		**Difference**	
	Quantity	Integrity	Quantity	Integrity	Quantity	Integrity
GRASSLAND						
Asteraceae sp1.	1±0	2±0	4±0	4±0	3±0	2±0
Baccharis crispa	2.41±1	2.06±0.9	3.29±0.85	2.71±0.85	0.88±0.93	0.65±0.93
Baccharis dracunculifolia	3.25±0.96	2.25±1.5	3.75±0.5	3.25±1.5	0.5±1	1±1.41
Baccharis sp.	3±0	2±0	4±0	2±0	1±0	0±0
Baccharis uncinella	2.5±1.05	1.67±1.21	3.17±1.33	3.17±1.33	0.67±0.82	1.5±1.38
Chromolaena laevigata	1±0	1±0	2±0	2±0	1±0	1±0
Clethra scabra	1.9±1.12	2.1±1.03	3.13±0.73	3.03±0.72	1.23±0.9	0.93±0.78
Frangula polymorpha	2.4±0.97	1.6±0.84	3.3±1.06	2.2±0.79	0.9±0.88	0.6±1.26
Grazielia intermedia	3.5±0.84	3±1.26	4±0	3.33±1.03	0.5±0.84	0.33±0.52
Myrcia splendens	2±0	3±0	3±0	3±0	1±0	0±0
Myrsine umbellata	1.14±0.38	2.14±0.9	1.57±0.53	2.57±1.13	0.43±0.53	0.43±0.53
FOREST						
Alchornea triplinervea	4±0	2±0	4±0	2±0	0±0	0±0
Allophylus edulis	3±0	1±0	3±0	1±0	0±0	0±0
Campomanesia adamantium	3±0	1±0	4±0	2±0	1±0	1±0
Casearia decandra	2.5±1.38	2.33±1.21	2.17±1.47	1.67±0.82	-0.33±0.82	-0.67±1.21
Casearia sylvestris	1.33±0.58	1±0	1.67±1.15	1±0	0.33±0.58	0±0
Clethra scabra	3.67±0.58	1.67±0.58	3.33±0.58	1.67±0.58	-0.33±0.58	0±0
Coussarea contracta	1.89±0.89	1.94±0.92	2±0.83	2.11±0.85	0.11±0.67	0.17±0.74
Dalbergia brasiliensis	3±0	3±0	3±0	3±0	0±0	0±0
Esenbeckia grandiflora	1.55±0.61	1.36±0.62	1.99±0.68	1.59±0.66	0.44±0.52	0.23±0.51
Eugenia longipedunculata	1±0	1±0	1±0	1.5±0.71	0±0	0.5±0.71
Eugenia neoverrucosa	1±0	1±0	1±0	1±0	0±0	0±0
Frangula polymorpha	2.6±1.14	1.2±0.45	3.2±1.1	2.2±1.1	0.6±1.14	1±1.22
Ilex paraguariensis	2±0	1±0	2±0	2±0	0±0	1±0
Ilex theezans	1.17±0.41	1±0	1.17±0.41	1±0	0±0	0±0
Leandra aurea	2±0	1±0	3±0	3±0	1±0	2±0
Matayba elaeagnoides	1±0	1.75±0.96	1±0	2.5±1	0±0	0.75±0.96
Miconia sellowiana	2.5±1.29	1.25±0.5	3±0.82	2.5±1.29	0.5±0.58	1.25±1.26
Mollinedia clavigera	1.57±0.98	1±0	1.71±0.76	1.14±0.38	0.14±0.69	0.14±0.38
Monteverdia gonoclada	2±0.63	1.67±1.21	2.17±1.17	1.83±1.33	0.17±0.75	0.17±0.41
Moquiniastrum polymorphum	3.5±0.71	2.5±2.12	3.5±0.71	1.5±0.71	0±0	-1±2.83
Myrcia glomerata	2.44±0.73	1.67±0.71	2.89±0.78	1.89±0.6	0.44±0.53	0.22±0.44
Myrcia selloi	3±0	3±0	3±0	2±0	0±0	-1±0
Myrcia splendens	2±0.89	1.33±0.52	2±0.89	1.33±0.52	0±0	0±0.63

Species	**Pre-frosts**		**Post-frosts**		**Difference**	
	Quantity	Integrity	Quantity	Integrity	Quantity	Integrity
Myrciaria floribunda	2.2±1.3	1.2±0.45	2.2±1.3	1.2±0.45	0±0	0±0
Myrciaria cuspidata	4±0	1±0	4±0	1±0	0±0	0±0
Myrsine gardneriana	2.5±0.71	1.5±0.71	2.5±0.71	1.5±0.71	0±0	0±0
Myrsine umbellata	1.93±0.88	3±0.76	2±0.76	2.47±0.83	0.07±0.88	-0.53±0.92
Myrtaceae sp1.	2±0	1±0	1±0	1±0	-1±0	0±0
Nectandra grandiflora	2±0	2±0	2±0	2±0	0±0	0±0
Palicourea sessilis	1.53±0.7	1.47±0.61	1.84±0.83	2.16±0.6	0.32±0.95	0.68±0.58
Plinia peruviana	1±0	1±0	1±0	1±0	0±0	0±0
Prunus myrtifolia	1.5±0.71	2±0	2.5±0.71	2.5±0.71	1±0	0.5±0.71
Psychotria suterella	1.4±0.5	1.6±0.94	1.65±0.81	1.7±0.92	0.25±0.55	0.1±0.45
Roupala montana	4±0	1±0	4±0	1±0	0±0	0±0
Rudgea sessilis	1.8±0.92	1.7±0.82	2.2±0.92	1.7±0.82	0.4±0.52	0±0
Solanum argenteum	2±0	1±0	2±0	1±0	0±0	0±0
Solanum pseudoquina	2±0	1±0	4±0	1±0	2±0	0±0
Sorocea bonplandii	1±0	1.43±0.79	1.29±0.49	1.43±0.53	0.29±0.49	0±0.58
Strychnos brasiliensis	1±0	2±0	2±0	2±0	1±0	0±0
Vitex megapotamica	2.25±1.26	2.25±0.96	3.25±1.5	3.25±1.5	1±1.15	1±0.82

3 CHAPTER II: Thermotolerance of subtropical Atlantic Forest trees: a clash of floristic contingents

Manuscript format commands the requirements of the New Phytologist *journal*.

THERMOTOLERANCE OF SUBTROPICAL ATLANTIC FOREST TREES: A CLASH OF FLORISTIC CONTINGENTS

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SUMMARY

- Thermal limits tolerated by plants may reflect ancestral legacies. Such tolerance can be assessed through multifaceted approaches, ranging from physiology to species distribution. Here, we investigate whether the biogeographic history of Atlantic Forest tree lineages influences their resistance to temperature extremes, using a multi-approach framework.
- We selected 14 subtropical Atlantic Forest tree species based on their historical biogeographic origins: either northern (i.e. Cenozoic, Equatorial rainforests) or southern (i.e. ancient Gondwanan mesic forests). We exposed sapling leaves to freezing and heating to obtain physiological measurements. Thermotolerance traits were then correlated with leaf morphology and species-specific bioclimatic variables.
- Physiological traits varied across floristic contingents, with Equatorial-origin species exhibiting greater heat tolerance. Leaf thickness was a strong predictor of both heat and cold thermotolerance. While bioclimatic variables explained heat resistance, cold tolerance remained unrelated to them.
- Atlantic Forest trees keep niche legacies from their historical contingents, suggesting niche conservatism within the lineages within the floristic contingent. Their physiological tolerance is influenced by leaf functional traits while also intersecting with environmental parameters. Our multifaceted

approach framework contributes to a more comprehensive understanding of plant thermal tolerance in a changing world.

Keywords: biogeographic history, climate change, climatic niche, ecophysiology, niche conservatism, stress tolerance.

3.1 INTRODUCTION

Although plants primarily respond to environmental pressures through shifts in distribution rather than through rapid genetic adaptations (Donoghue, 2008), significant gaps remain in our understanding of their resistance to abiotic stresses in the context of the current climate emergency (Lancaster & Humphreys, 2020; Geange *et al.*, 2021). While functional traits (i.e., morphological, phenological, and physiological characteristics) are critical for assessing current thermal tolerance (Violle *et al.*, 2007; Wahid *et al.*, 2007), they have been shaped by eco-evolutionary processes, thereby potentially holding evolutionary and biogeographic history legacies (Cavender-Bares *et al.*, 2016). For instance, leaf traits (e.g., photosynthetic sensitivity, cellular integrity, and leaf morphology and structure) are widely used as proxies for thermotolerance (Gurvich *et al.*, 2002; Knight & Ackerly, 2003, Geange *et al.*, 2021), reflecting trade-offs in carbon investment and stress resistance (Wright *et al.*, 2004; Sastry & Barua, 2017). Nevertheless, relying solely on trait-based assessments may neglect the lasting impact of lineage history, because ancient adaptations continue to influence modern ecosystems as evolutionary legacy effects (Cavender-Bares *et al.*, 2004, 2016).

The thermotolerance spectrum, i.e., the range of tolerances an organism exhibits to contrasting temperatures, is deeply tied to evolutionary processes and patterns (Becklin *et al.*, 2016). Phylogenetic niche conservatism (Wiens & Donoghue, 2004) suggests that closely related lineages retain similar climatic preferences, including thermal tolerances shaped by ancestral environments. This concept is further elaborated in the tropical niche conservatism hypothesis, which seeks to explain the high biodiversity observed near the Equator (Wiens & Donoghue, 2004). In this explanation, lineage diversification is greater in the Equator because of higher speciation and lower extinction rates than in the subtropics; there are also some adaptive radiations polewards, but glacial cyclical events generate widespread extinctions at higher latitudes (Gerhold *et al.*, 2018). However, this framework does not account for other origins of diversification that still leave major imprints on biogeographical patterns. For instance, the Neotropics present lineages' distributions that do not match only a Cenozoic tropical origin (Segovia & Armesto, 2015). The southern cone of South America has a significant influence of several Gondwanan survivor lineages (*sensu* Kooyman *et al.*, 2014) that find their mesic habitat preferences in high elevations in the region (Carlucci *et al.*, 2017; Gerhold *et al.*, 2018). This representation has led many clades to prefer temperate-like climates, highlighting a possible phenomenon postulated as the austral niche conservatism (Segovia & Armesto, 2015; Jin & Qian, 2025). Legacy effects frequently appear as conserved trait syndromes (Ackerly D., 2003), for instance, in traits such as freezing tolerance or deciduousness. These legacy effects may influence present-day distribution patterns, as evidenced in the genus *Myrceugenia* (Myrtaceae), which possesses high leaf freezing tolerance and exhibits a distribution tightly linked to high elevations in the Andes and the southern Brazilian highlands (Pérez *et al.*, 2014; Murillo-A *et al.*, 2016). The syndromes of deep-past relative lineages may continue to influence community assembly and ecosystem functioning, even if they no longer align with current climatic conditions (Pérez *et al.*, 2014; Cavender-Bares *et al.*, 2016).

The sources of many plant lineages in southern South America, along with climate conditions, have strongly influenced the region's distribution and composition of biomes (Oliveira-Filho *et al.*, 2015; Cantidio & Souza, 2019). In the Atlantic Forest of Brazil, a vital biodiversity hotspot characterised by a high species diversity and climatic variation (Marques & Grelle, 2021), different lineages and floristic contingents cooccur across several biomes. These include the Araucaria mixed forests, tropical dense rainforests, and Campos grasslands. The Atlantic Forest's flora can be described in two major biogeographic units with distinct evolutionary histories, North and South (Costa *et al.*, 2000). The northern sector shows stronger Amazonian floristic influences (Antonelli *et al.*, 2018; Lins-e-Silva *et al.*, 2021), while the southern part exchanged more biodiversity with the Andes and has a strong presence of Gondwanan lineages (Sanmartín & Ronquist, 2004; Carlucci *et al.*, 2021). The primary biogeographic break between these blocks occurs around 19°S, in the *Rio Doce* valley, a region that experienced a strong floristic turnover, particularly within the dense rainforest formations, since the Pleistocene (Carnaval & Moritz, 2008; Fiaschi & Pirani, 2009). Furthermore, the recent diversification of the Cerrado flora allowed many exchanges of plant lineages with the southern block (Simon *et al.*, 2009; Hughes *et al.*, 2013; Antonelli *et al.*, 2018), characterising a migration of more Equatorial lineages.

Despite the known contrasting biogeographic influences on species composition in the southern block, the consequences of these different historical legacies for species ecological niches remain largely unexplored. The varying biogeographic influences on both sections of the Atlantic Forest overlapped with the varying environmental conditions, likely contributing to the evolution of distinct temperature tolerances within its flora.

Given the ongoing climate change, plant resistance to extreme temperatures should receive greater attention (Chaudhry & Sidhu, 2022). It is well-documented that Atlantic Forest plants are changing their distribution range (Colombo & Joly, 2010; Teixeira *et al.*, 2016; Dos Santos *et al.*, 2020; Wilson *et al.*, 2021; Bernardinis *et al.*, 2023; dos Santos Alves *et al.*, 2024; Bergamin *et al.*, 2024), potentially contributing to distributional shifts of species in tropical and subtropical regions. The shifts are often associated with temperature rising (Sommer *et al.*, 2010; Zwiener *et al.*, 2017), a factor that interacts heavily with land use changes (e.g., Calambás-Trochez *et al.*, 2021), as habitat fragmentation can impede species migration and adaptation. To contribute to increasing and connecting natural areas on a landscape scale, restoration efforts are needed to buffer the future impacts on biodiversity (von Holle *et al.*, 2020). While ecological restoration increasingly considers climatic tolerance (Timpane-Padgham *et al.*, 2017; Bradford *et al.*, 2021; de Lima *et al.*, 2021; Fremout *et al.*, 2022) and conservation attempts are growing, there remains a notable lack of data on species-specific thermotolerance, especially in saplings – a critical life stage for forest regeneration. Therefore, these knowledge gaps hinder abilities to help support the floristic biodiversity in the face of environmental change within the Atlantic Forest.

The threats posed by climate change to the subtropical Atlantic Forest (Gulizia et al., 2022), along with the lack of dedicated research on thermal tolerances in the region (Lancaster & Humphreys, 2020), highlight the need for a multi-faceted research approach. Integrating evolutionary history with trait-based ecology can bridge this gap (Cavender-Bares *et al.*, 2016), especially for critical life stages such as saplings. Given the evolutionary, biogeographical, and climatic factors influencing thermal tolerance of trees, here we address the following key questions: (1) Do plant lineages associated with two distinct floristic contingents in the southern block of the Atlantic forest (i.e., Equatorial vs. Austral) display differences in their physiological tolerances to extreme temperatures (hereafter referred to as thermotolerance)? (2) Are morphological traits correlated with thermotolerance traits in saplings? (3) How spatial climatic niches of trees in the subtropical Atlantic Forest influence their thermotolerance? To explore these questions, we classified tree species as lineage representatives into two floristic contingents based on the literature. Using thermotolerance assays, we assessed the physiological tolerance of saplings from each species to freezing and elevated temperatures. After that, we compared tolerance parameters with leaf morphological traits and the spatial occurrence climate of species. By investing in such an approach, we aimed to enhance our understanding of current ecophysiological responses to climate change through the lens of the history of lineages and floristic contingents.

3.2 MATERIAL AND METHODS

3.2.1 Study system

Our study system consisted of species representing lineages of different floristic contingents in the subtropical Atlantic Forest. We selected a workable number of species that co-occur in the Atlantic Forest of the east of Paraná state in Brazil (Supplementary Information Fig. S1). The area encompasses the Araucaria Mixed Forest range within the Atlantic Forest domain (Ab’Sáber, 2003), with the influence of the Cerrado flora at its northern boundaries (Ritter *et al.*, 2010). It is a transitional zone between recently diversified plant species (i.e., Cerrado flora; Simon *et al.*, 2009) with more ancient lineages (i.e, Araucaria Mixed Forest; Rezende *et al.*, 2017).

From a list of regional tree species (Dvorak *et al.*, 2025), we conducted a literature search for studies that reconstructed the ancestral geographical ranges of internal nodes in their phylogenies. Selection criteria included the use of ancestral character reconstruction methods on dated phylogenies and the inclusion of one or more species of interest (Supplementary Information Methods S1).

We categorised the species with the ancestral occupation area into floristic contingents. Due to variation in the classification of regions in the studies, species were classified into two large groups: Austral-Antarctic, when the clades had an evolutionary origin in landmasses that are now the current continents of Oceania, Antarctica, or in the extreme southern region of South America; and Equatorial-Brazilian, when the origin of the clades traced back to the central and northern areas of South America, as well as non-South American tropical origins (e.g., Africa, Central America/Caribbean, and Southeast Asia).

After the research, we compared these classified species with those available in local nurseries. Considering the availability of the species and seedlings taller than

30 cm, seven species per contingent were selected (Table 1; Supplementary Information Table S1).

Table 1 Chosen species of each floristic biogeographic contingent.

Austral-Antarctic	Equatorial-Brazilian
Myrtaceae - *Campomanesia guazumifolia* (Cambess.) O.Berg	Meliaceae - *Cabralea canjerana* (Vell.) Mart.
Canellaceae - *Cinnamodendron dinisii* Schwacke	Cannabaceae - *Celtis iguanaea* (Jacq.) Sarg.
Myrtaceae - *Eugenia involucrata* DC.	Erytroxylaceae - *Erythroxylum cuneifolium* (Mart.) O.E.Schulz
Anacardiaceae - *Lithraea brasiliensis* Marchand	Lythraceae - *Lafoensia pacari* A.St.Hil.
Sapindaceae - *Matayba elaeagnoides* Radlk.	Rosaceae - *Prunus myrtifolia* (L.) Urb.
Monimiaceae - *Mollinedia clavigera* Tul.	Rubiaceae - *Randia armata* (Sw.) DC.
Myrtaceae - *Psidium cattleyanum* Sabine	Rutaceae - *Zanthoxylum rhoifolium* Lam.

The parent trees were in or near the nursery (Supporting Information Fig. S1), avoiding location biases. The saplings were kept in a greenhouse and regularly watered until thermotolerance assays.

3.2.2 Thermal stress assays

We conducted the assays near the end of the growing season (December to March) to guarantee leaves of similar maturing ages. From the assays, we obtained five different physiological traits of thermotolerance (Table 2).

3.2.2.1 Heating tolerance

The heat tolerance was estimated from photosynthetic parameters. To access it, we chose the leaves of three individuals from each species. Using two leaf discs for each individual for each treatment, we acclimated them to darkness at 25ºC for 25 minutes. To estimate heat tolerance, the leaf discs were exposed for 40 minutes in an incubator at 30, 35, 40, 45, 50, 55, 60, and 65ºC. We assessed chlorophyll fluorescence before and after treatments using a mini-PAM fluorometer (Walz GmbH, Effeltrich, Germany). The fluorometer provides three key measurements: F_0, the initial fluorescence before excitation of photosystem II (PSII); F_m, the maximum fluorescence when PSII is fully excited; and F_v, the variable fluorescence, calculated as the difference between F_m and F_0 (Knight & Ackerly, 2003). The final measure is the maximum potential quantum yield of photosystem II, calculated as the ratio of variable to maximum fluorescence in dark-adapted chlorophyll (F_v/F_m; Björkman & Demmig 1987).

We fitted a four-parameter logistic curve for the F_v/F_m measurements across the temperature spectrum of the experiments using the *'drc'* package (Ritz *et al.*, 2015). We extracted three parameters from the fitted curves: a) T_5, the temperature at which F_v/F_m declines to 95% of its maximum; b) T_{50}, the temperature at which F_v/F_m reaches 50% of its maximum; and c) T_{95}, the temperature at which F_v/F_m declines to 5% of its maximum (Bannister *et al.*, 2005). We calculated the decline width (DW) as the difference between T_5 and T_{95}, that is, the range where F_v/F_m declines from 95% to 5% of its maximum (Tiwari *et al.*, 2021).

3.2.2.2 Freezing tolerance

We estimated the cold tolerance using the electrolyte leakage method. We cut 5 mm leaf discs of three individuals from each species using a cork cutter. The discs were washed for two hours in ultrapure water to remove leaking cell contents resulting from the mechanical damage. For each treatment, we placed two discs per individual in a tube with 2 ml of ultrapure water and exposed the tubes to controlled temperatures for 14 hours in the dark (i.e., 25 and -15°C). At the end of exposure, the tubes were allowed to reach room temperature in the dark. We measure the solution conductivity with an AKSO AK-83 conductivity meter (Rio Grande do Sul, Brazil). After that, we boiled the tubes for 15 minutes to allow full cell lysis before measuring conductivity again (Pérez-Harguindeguy *et al.*, 2013). These measurements are taken to calculate the percentage of electrolyte leakage (PEL) as follows:

$$PEL = (e_C/e_T) \times 100,$$

where e_C is the solution conductivity right after the treatment, and e_T is the conductivity after boiling. High values of PEL imply full cell damage due to the freezing temperatures and, therefore, frost sensitivity.

To correct any species-specific anatomy biases, we calculate the effective PEL (PEL_e) by subtracting the PEL of the freezing treatments from the PEL of the control treatment at 25°C.

Table 2 Physiological traits of thermotolerance and their meanings.

Trait	Acronym	Evaluated temperature	Meaning	Reference
Temperature of 95% decline in PS II functioning	T_5	Heating	Higher values imply a delay of the initial damage to the photosynthetic apparatus as the temperature alters.	Bannister *et al.*, 2005
Temperature of 50% decline in PS II functioning	T_{50}	Heating	Higher values imply a delay of the possible reversible damage to the photosynthetic apparatus as the temperature alters.	Bannister *et al.*, 2005
Temperature of 5% decline in PS II functioning	T_{95}	Heating	Higher values imply a delay of the total damage to the photosynthetic apparatus as the temperature alters.	Bannister *et al.*, 2005
Width of temperature tolerance	DW	Heating	Related to the biochemical protection mechanisms of photosynthesis. Higher values imply thermal sensitivity, as any temperature change affects the functioning and other processes that facilitate the photosynthetic pathway. The opposite, lower values, imply a sustained resistance and better functioning without facilitation mechanisms.	Tiwari *et al.*, 2021
Percentage of effective electrolyte leakage	PEL_e	Freezing	It is the effective amount of dissolved intracellular content after programmed cell death triggered by an external factor. It discards the total electrolyte dissolved after mechanical damage caused by the method. Higher values imply a severe damage in the leaf tissue caused by induced cell death (e.g.,	Demidchik *et al.*, 2014; Pérez-Harguindeguy *et al.*, 2013

			ice nucleation, membrane damage, and/or apoptosis).	

3.2.3 Leaf morphological traits

To correlate thermotolerance values with leaf morphology, we considered leaf thickness (L_{th}) and specific leaf area (SLA), which are key traits representing the leaf economic spectrum related to resource acquisition, investment, and leaf longevity (Wright et al., 2004). We selected three leaves from three individuals per species to assess their traits following the global protocol (Pérez-Harguindeguy et al., 2013). L_{th} was measured using a digital calliper, with the tip positioned at the fresh leaf blade, avoiding prominent veins. L_{th} was calculated as the average of three measurements: at the bottom, middle, and top of the leaf blade. We scanned the fresh leaves in a flatbed scanner and measured their areas using *ImageJ* software (Schneider et al., 2012). Leaves were weighed at full turgor and dried after 72 hours in an oven at 70ºC for new weighing. SLA was obtained by dividing the fresh leaf area by its dry mass.

3.2.4 Bioclimatic tolerance

To retrieve the environmental conditions at which the species can exist in their distribution range, we used recorded geographic points of species occurrence (Supplementary Information Table S2), which have been compiled for a previous study (Trindade & Marques, 2024). The occurrence data were obtained from five biodiversity data sources: GBIF (GBIF, 2023), Jabot (Silva *et al.*, 2017), NeoTropTree (Oliveira-Filho, 2017), and SpeciesLink (Canhos *et al.*, 2022). Occurrence points were cleaned by excluding misrecorded occurrences (e.g., open ocean, territory centroids, locations of biodiversity institutions), using packages CoordinateCleaner (Zizka *et al.*, 2019) and florabr (Trindade, 2024).

We used the occurrence data for each species to extract the values of the environmental variables associated with each point in a 30 arc-second resolution raster. As environmental variables, we chose bioclimatic and elevation data. The climate data were the potential evapotranspiration (PET, kg m^{-2} $month^{-1}$), and the minimum temperature in the coldest month (BIO06, hereafter MTCM, °C) gathered from CHELSA V 2.1 (https://chelsa-climate.org/; Brun *et al.*, 2022a,b) from 1981 to 2010. The elevation information was obtained from the WorldClim V 2.1 raster (https://www.worldclim.org/; Fick & Hijmans, 2017). These variables are influential on plant development, and they are less correlated with others available (Kibler *et al.*, 2023; Bergamin *et al.*, 2024). We treated all variables as percentiles: PET at the 50th (refers to the median PET at which all specimens occur), MTCM at the 5th (refers to the minimum temperature among the MTCM of all occurrences, discarding extreme values), and elevation at the 95th percentile (refers to the maximum elevation of the occurrences, discarding extreme values; Supplementary Information Table S2).

3.2.5 Statistical analyses

3.2.5.1 Comparison between floristic contingents

We omitted phylogenetic relatedness from all analyses - more information in the Supplementary Information Methods S2.

To evaluate whether the species' thermotolerances differed between the floristic contingents, we used the physiological measurements (i.e., heating and freezing related traits from Table 2) to perform an Analysis of Variance (ANOVA).

To investigate trade-offs among extreme tolerances, we conducted a Principal Component Analysis (PCA) with physiological parameters as loadings to investigate how species occupy the thermotolerance multivariate space. For this, we previously standardised traits to a zero mean and unit variance to equalise the discrepancy of values in different traits. We compared PC axes between floristic contingents using an ANOVA.

3.2.5.2 Morphological traits and physiological tolerance

We examined the covariation between species' morphological leaf traits and thermotolerance traits using plant trait network analysis (He et al., 2020). Trait networks depict how different plant traits are interconnected, with each trait represented as a node and the relationships between them as edges. These connections can reflect trade-offs, co-optimisation, or allometric relationships, often grounded in biomechanical or physiological constraints (He et al., 2020). Trait networks were constructed using all species' mean trait values through the *igraph* package (Csárdi *et al.*, 2023). To retain only significant relationships among traits ($p < 0.05$), we applied a permutation test with 9,999 iterations to Spearman's rank correlation coefficients using the *jmuOutlier* package (Garren, 2019). For each trait, we calculated two centrality metrics (He *et al.*, 2020): the degree, defined as the number of edges connected to a node, and the weighted degree, defined as the sum of all significant coefficients of correlation of a node. Traits with higher degree and weighted degree regulate the values of the others, something described as phenotypic integration (Murren, 2002). We also generated trait networks considering all species at the contingent level.

3.2.5.3 Relationships among species' physiological and bioclimatic tolerance

We analysed the variation in species' heat and cold physiological tolerances across bioclimatic and altitudinal factors. To evaluate the most important variables that influence thermotolerance, we applied regression models using the random forest algorithm within the *tidymodels* framework (Kuhn & Wickham, 2020) and the "ranger" engine (Wright & Ziegler, 2017). We considered DW for the heat parameter, while PEL_e was considered for cold tolerance. The independent variables included PET, MTCM, and Elevation. To ascertain the importance of variables on model performance, we set a model with 10,000 trees and executed it 1,000 times, calculating variable importance through permutation for each run.

All analyses were conducted in R version 4.3.2 (R Core Team, 2023).

3.3 RESULTS

3.3.1 Physiological tolerances between floristic contingents

The lineages representatives of the two floristic contingents exhibited marked differences in their physiological thermotolerances. In the heat tolerance assay (Fig. 1, Supplementary Information Fig. S2, Fig. S3), Austral-Antarctic species showed lower values of T_5 (F (1,12) = 16.93, p-value < 0.01) and higher values of T_{95} (F (1,12) = 13.25, p-value < 0.01) and DW (F (1,12) = 21.47, p-value < 0.01). The values of T_{50} did not differ between contingents (F (1,12) = 0.226, p-value = 0.64). Floristic contingents did not differ significantly for the cold assay (F (1,12) = 0.171, p-value = 0.69; Fig. 2).

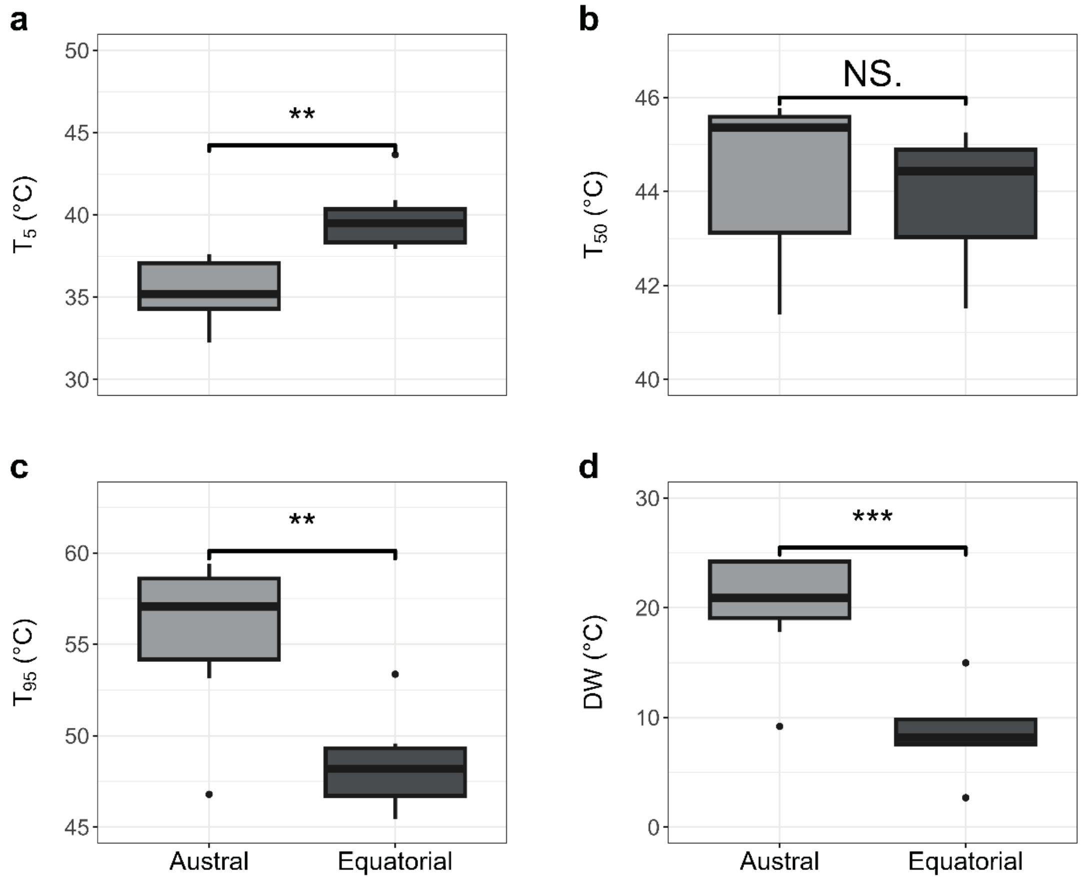

Fig. 1 Boxplots comparing physiological parameters of heat tolerance between the floristic contingent species. Annotation above boxplots represent the results of an Analysis of Variance: "**" = p-value < 0.01, "***" = p-value < 0.001, NS. = p-value > 0.05. Legend: a) T_5 – temperature of 95% of decline of F_v/F_m, b) T_{50} – temperature of 50% of decline of F_v/F_m, c) T_{95} – temperature of 5% of decline of F_v/F_m, d) DW – distance width between T_{95} and T_5.

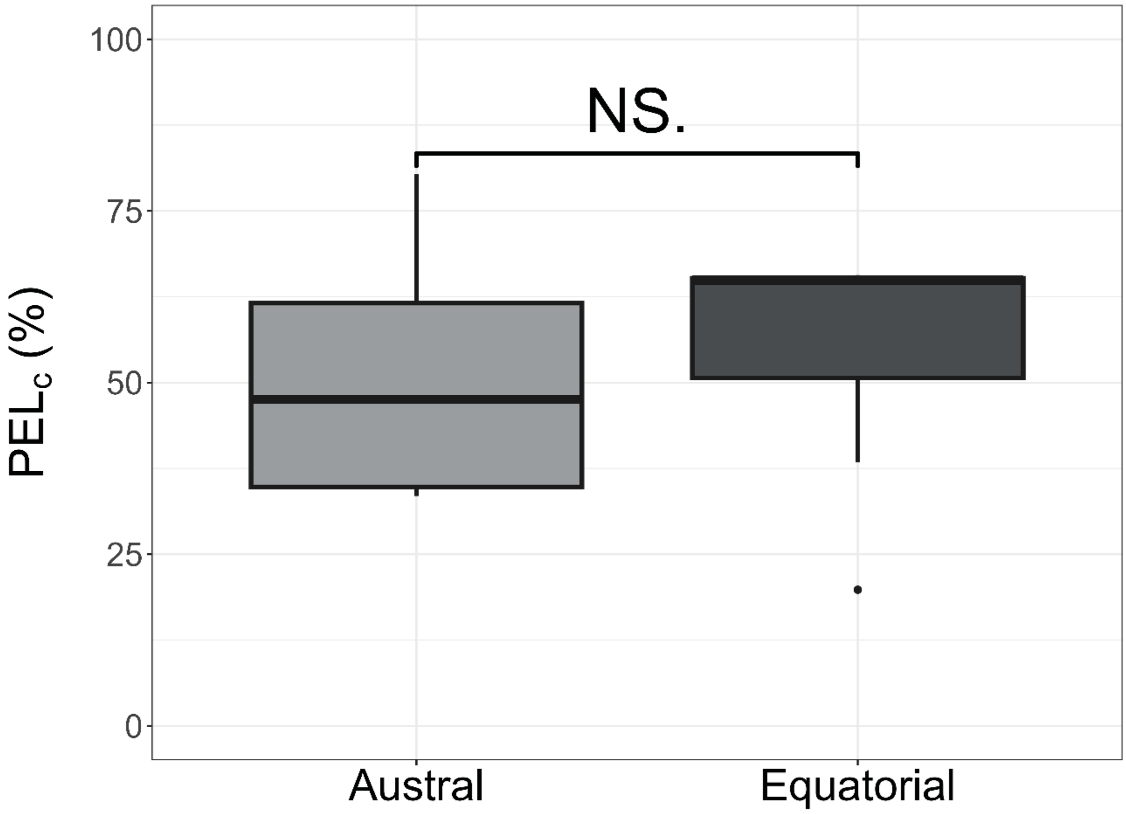

Fig. 2 Effective percentage of electrolyte leakage (PEL$_e$) comparison between the floristic contingent species. Annotation above boxplots represent the results of an Analysis of Variance: NS. = p-value > 0.05.

We found no evidence of trade-offs between heat and cold tolerances among species from different floristic contingents. The PCA shows that heat tolerance is associated with the first axis, which explains 56.4% of total variation, while the second axis is related to T_{50} (24.8% of variation explained; Fig. 3). The heat tolerance traits display patterns comparable to those identified in the previous ANOVA, with Austral-Antarctic species exhibiting higher DW and T_{95} values than Equatorial-Brazilian species, whereas the latter are more strongly associated with the T_5 loading (Fig. 1; Supplementary Information Fig. S4). Cold tolerance is only strongly associated with the third principal component axis (see Supplementary Information Table S3) and does not contribute to differentiating species either within or between contingents along the first two PCA axes.

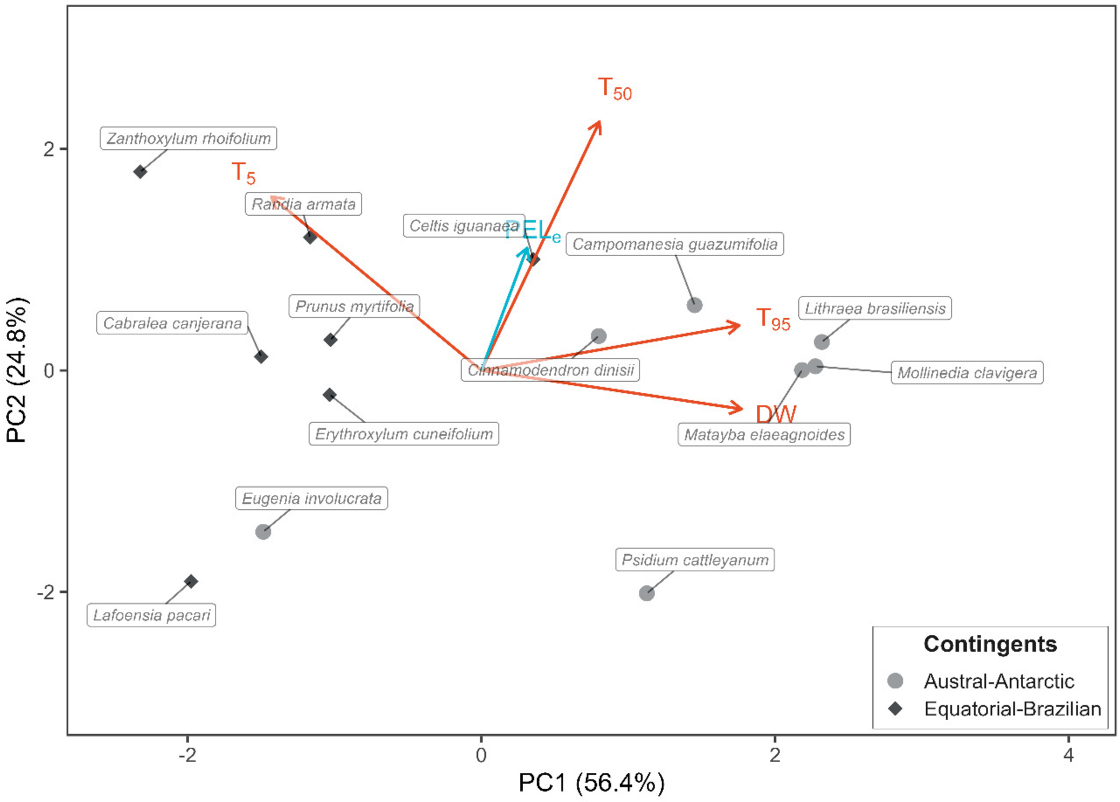

Fig. 3 Principal Component Analysis (PCA) biplot of the different tolerance metrics. The red arrows are heat tolerance metrics, and the blue arrow is the cold tolerance metric. Legend: PEL_e – effective percentage of electrolyte leakage, T_5 – temperature of 95% of decline of F_v/F_m, T_{50} – temperature of 50% of decline of F_v/F_m, T_{95} – temperature of 5% of decline of F_v/F_m, DW – distance width between T_{95} and T_5.

3.3.2 Trait networks

Thermotolerance and morphological traits were strongly correlated across species (Fig. 4, Supplementary Information Table S4). The highest positive significant correlation was between DW and T_{95} (r (12) = 0.955, p-value < 0.001), and the highest negative significant correlation was between DW and T_5 (r(12) = -0.879, p-value < 0.001). Between the morphological traits, L_{th} had the highest centrality (degree = 4, Fig. 4, Supplementary Information Table S4), being positively correlated with DW and PEL_e, and negatively with T_5, while SLA was only correlated with T_5. Both morphological traits were strongly correlated with each other. Only T_{95} and T_{50} were not correlated with morphological traits, only with other heat tolerance traits (Fig. 4). PEL_e did not show a significant correlation with any other thermotolerance traits.

We did not find correlations between physiological and morphological traits within each floristic contingent (Supplementary Information Fig. S5).

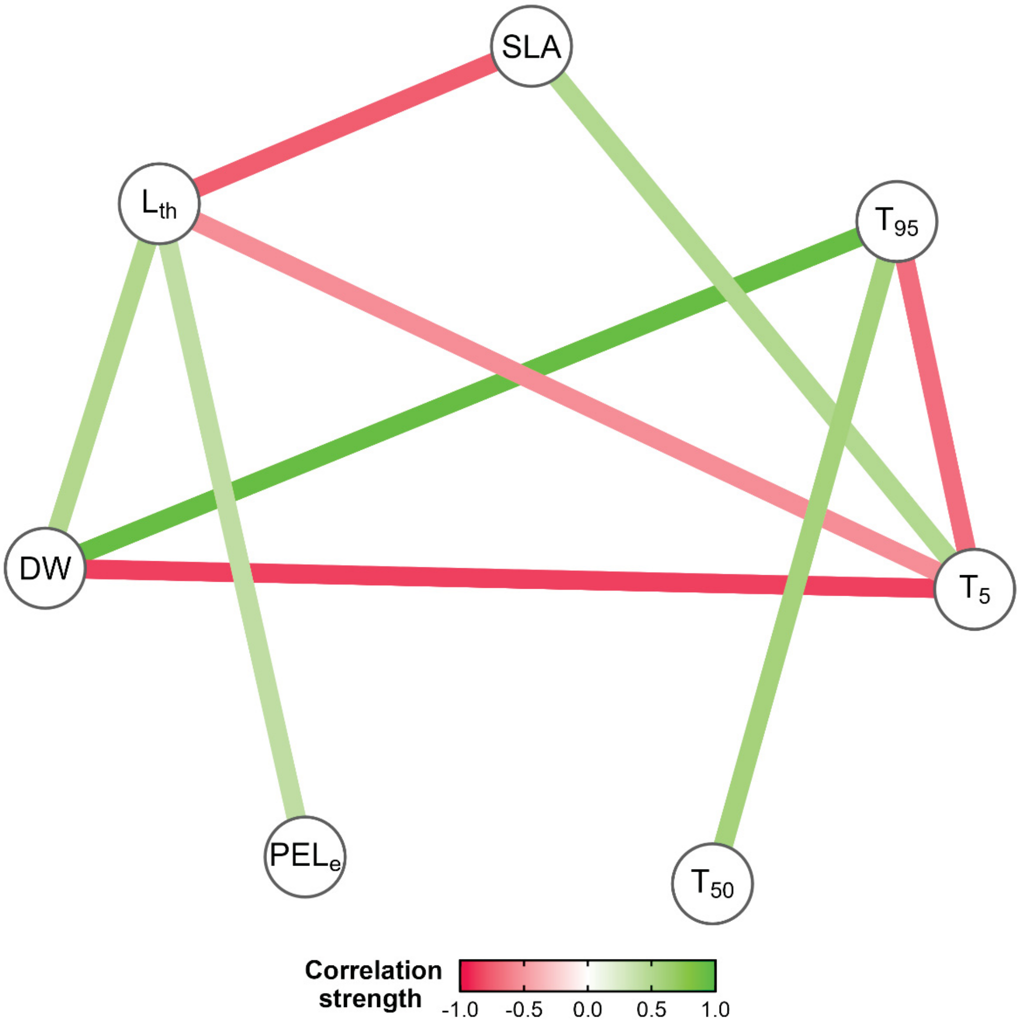

Fig. 4 Correlation network for all species studied. Correlation strength is represented by line colour. Only significant correlations ($p < 0.05$) are shown. For correlation coefficients, see Supplementary Information Table S4. Legend: L_{th} – leaf thickness, PEL_e – effective percentage of electrolyte leakage, SLA – specific leaf area, T_5 – temperature of 95% of decline of F_v/F_m, T_{50} – temperature of 50% of decline of F_v/F_m, T_{95} – temperature of 5% of decline of F_v/F_m, DW – distance width between T_{95} and T_5.

3.3.3 Relationships among thermotolerance and bioclimatic variables

We observed interaction between environmental variables and physiological measures. The random forest models succeed in accessing variable importance for DW, but not for PEL_e (Fig. 5). Heat tolerance model was mostly influenced by the maximum elevation of the species occurrence, followed by the potential evaporation (Fig. 5a). MTCM slightly corrupts model performance, thus returning negative values close to zero. The model corruption was observed for all variables in PEL_e models (Fig. 5b).

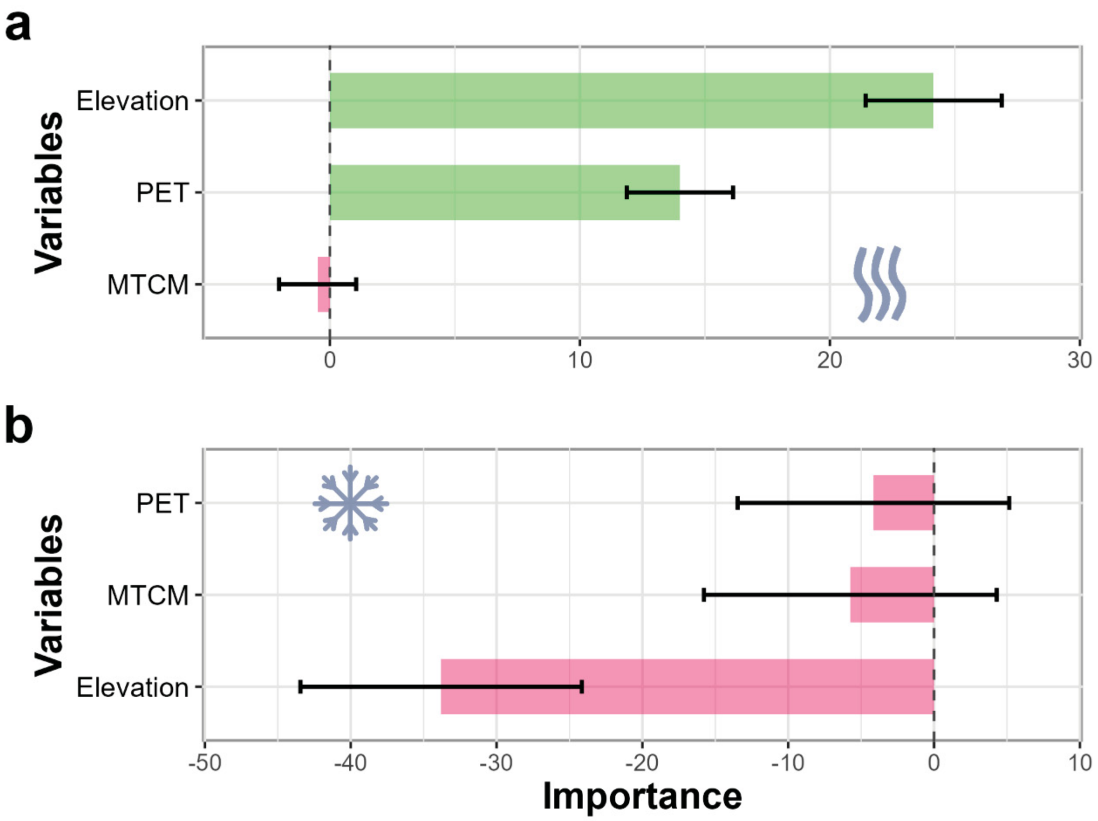

Fig. 5 Barplots representing the importance rank of climatic variables on heat (DW, plot a) and cold (PEL_e, plot b) tolerances of random forest models. Variable importance is calculated using the permutation method that shuffles each predictor variable and observes the impact on the model's performance. Error bars are the confidence interval at 5% and 95%. Legend: PET – potential evapotranspiration; MTCM – minimum temperature in the coldest month.

3.4 DISCUSSION

The biogeographical legacy of plants is perpetuated in them through their thermal tolerance and environmental preference. Our study is a pioneer in integrating ecophysiological responses to the biogeographic past of tree species from South America (but see Sklenář *et al.*, 2012 for a similar approach in the South American páramos). We highlighted a possible niche conservatism in the current lineages descended from both floristic contingent migrators through the assessment of physiological heat tolerance. The Austral-Antarctic representatives had lower values of T_5 and higher values of T_{95} than Equatorial-Brazilian species. This wide tolerance interval (here represented as DW) in the Austral-Antarctic species expresses heat sensitivity (Tiwari *et al.*, 2021). Although they may occupy today's areas with higher temperatures and mild precipitation regimes than their ancient lineages (Rezende *et al.*, 2017), their heat sensitivity could reflect an austral niche conservatism, also reflected by their bioclimatic niche (Rezende *et al.*, 2021). The opposite was observed for the lineages of the Equatorial-Brazilian contingent (i.e., a short tolerance interval that represents heat tolerance; Tiwari *et al.*, 2021), pointing to a tropical niche conservatism at the physiological scale (Wiens & Donoghue, 2004; Neves *et al.*, 2021). Hypotheses of niche conservatism are often tested at the spatial scale (i.e., species occurrence data) and through analyses of morphological traits (Simon & Pennington, 2012; Hawkins *et al.*, 2014; Harris *et al.*, 2022). Although physiological parameters are inherently more variable and context-dependent, certain patterns remain consistent with the predictions of niche conservatism (Araújo *et al.*, 2013; Pérez *et al.*, 2014). In the present study, the sensitivity of the photosynthetic apparatus tied to the niche conservatism hypotheses suggests limited potential for phenotypic plasticity in these essential physiological structures through evolution (Araújo *et al.*, 2013). Nevertheless, the absence of significant differences between the contingents in T_{50} values highlights similar thresholds of irreversible damage to the photosynthetic apparatus. Although T_5 values differed, they do not indicate a strong ecophysiological warning, but rather the onset of incipient damage (Winter, 2024). Our results suggest that species from both contingents may face comparable risks of loss of photosynthetic capacity under long-term exposure to extreme heat.

We found no evidence of differentiation between contingents for species freezing tolerance through electrolyte leakage (Fig. 2). One explanation for this finding is related to acclimatisation, the phenotypical plasticity driven by seasonal variance. Freezing tolerance is known to increase progressively until winter (Kreyling *et al.*, 2015; Sklenář *et al.*, 2023), an effect of slow changes in climatic conditions that trigger physiological mechanisms. This process involves metabolic and cellular adaptations, including changes in the lipid composition of the cell membrane (Uemura *et al.*, 2006) and accumulation of osmolytes to prevent desiccation (Thomashow, 1999). Our assays were conducted at the end of the southern hemisphere growing season, when plants had not yet entered the hardening process. Altogether, these mechanisms are not the same in different phenological types. Deciduous and evergreen species show different strategies to cope with freezing temperatures besides leaf lifespan (di Francescantonio *et al.*, 2020). Although not evaluated, our selected species do show different phenological strategies for foliage maintenance through seasons. Such strategies may mask the full potential of cold resistance in evergreen plants after acclimatisation (Cavender-Bares *et al.*, 2005).

Sapling thermotolerance was significantly correlated with structural traits. Leaf thickness was the trait with higher centrality, being positively correlated with PEL and DW, and negatively correlated with T_5. Thicker leaves are implied to buffer heat spikes in the mesophyll, controlling the leaf temperature (Leigh *et al.*, 2012). Sometimes inferred through the specific leaf area (Pérez-Harguindeguy *et al.*, 2013), several studies reported that thicker leaves are more tolerant to heat stress (Knight & Ackerly, 2003; Sastry & Barua, 2017; Slot *et al.*, 2021; da Silva & Rossatto, 2022). Although our results contradict the assumption that leaf thickness is positively associated with the critical temperature of photosynthetic damage (Leigh *et al.*, 2012), we report the positive relationship between plant thermal sensitivity (i.e., DW) and leaf structure. Such findings are not the same found in temperate forests (Münchinger *et al.*, 2023) but are supported by the findings in tropical dry forests (Sastry & Barua, 2017; Sastry *et al.*, 2018). While for freeze tolerance, PEL values being positively correlated with leaf structure was expected (Ladwig *et al.*, 2018; Bucher & Rosbakh, 2021), most likely regarding heat retention (Leigh *et al.*, 2012). In the field, these morphological traits are highly correlated with the degree of frost damage in plants (Bucher & Rosbakh, 2021; Araujo Frangipani *et al.*, 2021; de Antonio *et al.*, 2021).

The interaction between climate and thermotolerance was inconsistent across ecophysiological traits (Fig. 5). Heat tolerance, set here as the temperature width of photosynthetic damage, was highly influenced by maximum elevation and potential evapotranspiration. Elevation is known as a strong variable controlling plants' climatic tolerance (Slot *et al.*, 2021; Chaves *et al.*, 2024), while potential evapotranspiration may buffer heat effects on plants due to increased air moisture (Kibler *et al.*, 2023). The climatic variable mean temperature of the coldest month (MTCM) reduced model performance for predicting ecophysiological traits (Fig. 5). This weak correlation is consistent with findings for European tree species, where physiological tolerance did not account for species distributions (Visakorpi *et al.*, 2024). In addition to the acclimatisation issue mentioned above, it should be noted that physiological tolerances were measured in saplings, and the spatial bioclimatic niche is computed from adult occurrences. There is an evident ontogenetic shift of trait values in plants (Barton, 2023), which could influence different abiotic stress resistances from juveniles to mature plants (Vitasse *et al.*, 2014; Pavanetto *et al.*, 2024).

Our method used species occurrence data to estimate climatic tolerance among the studied tree species. It is useful to note that occurrence records are often biased to locations near universities and research centres, roads, and within protected areas (Zwiener *et al.*, 2021). Despite these biases, there is a debate regarding the validity of predicting species' abiotic niche with only correlative spatial data (Colwell & Rangel, 2009; Bocsi *et al.*, 2016). This concern arises from the possibility that physiological tolerance ranges may be broader than the climatic conditions reflected by its geographic distribution due to constraints of negative biotic interactions, such as competition or parasitism (Bocsi *et al.*, 2016; Bush *et al.*, 2018). Additionally, while it is important to consider intraspecific differences regarding physiological tolerance among populations (Chaves *et al.*, 2024), our multispecies approach focused on species-level potential adaptations (or exaptations) to the current climatic conditions in the subtropical Atlantic Forest. Under Gould and Lewontin's (1979) framework, we differentiate between true adaptations, traits that evolved under direct selection for their current thermal tolerance function, and exaptations, which are traits originally influenced by other selective pressures (e.g., drought resistance) that coincidentally confer thermal tolerance. Given our study's scale, we cannot precisely separate these evolutionary pathways. Nevertheless, we account for their combined effects through phenotypic plasticity across ontogenetic stages and populations, while acknowledging that current trait-environment relationships may reflect historical contingencies and/or contemporary adaptation (Ackerly, 2009).

In the face of a changing world, the phenotypic plasticity of populations may save them from local extinctions (Pearman *et al.*, 2010; Becklin *et al.*, 2016; Arnold *et al.*, 2019). In particular, the plasticity of physiological traits is key to plants’ migration to more suitable environments (Becklin *et al.*, 2016), a pattern recurrent in the past (Becklin *et al.*, 2014). Given our findings of niche conservatism, one could expect similar patterns of macroevolution among species of the same floristic contingent. The higher sensibility of the Austral-Antarctic contingent to heating temperatures may imply the retraction of temperate-origin species southwards and/or at higher elevations (Bergamin *et al.*, 2021, 2024; Osland *et al.*, 2021; Antão *et al.*, 2022). Nevertheless, further integrated studies are necessary to provide more reliable predictions of species vulnerability to climate change (Pacifici *et al.*, 2015; Foden *et al.*, 2019). Models of potential distribution are a common method to evaluate responses of species to future climatic scenarios (Anadón *et al.*, 2014; Bernardinis *et al.*, 2023; dos Santos Alves *et al.*, 2024), but their interpretation and refinement could be improved with physiological traits (Kearney & Porter, 2009; Morin & Thuiller, 2009), life-history traits (Foden *et al.*, 2013), demography (Keith *et al.*, 2008), and biotic interactions (Tylianakis *et al.*, 2008; Trivedi *et al.*, 2022). Here we also emphasise the importance of considering data from juvenile life stages of development and biogeographic information to improve predictions of future climate impacts (Cavender-Bares *et al.*, 2016; Pliūra *et al.*, 2019).

Although currently lineages may be distributed in large ranges and co-occur in distinct climatic conditions, the biogeographic history of their clade accounts for their realised thermal niche (Ackerly, 2009; Hawkins *et al.*, 2011; Sklenář *et al.*, 2012; Cavender-Bares *et al.*, 2016). Here, we described how the floristic contingents of tree species in the Atlantic Forest influenced their physiology through phylogenetic niche conservatism. Using parameters for both heating and freezing temperatures, species with equatorial biogeographic legacy showed higher heat tolerance. To resist higher temperatures, plants also showed positive correlations with leaf morphology and environmental variables of species distribution. Our method addressed a gap in the thermotolerance of trees within a biodiversity hotspot by incorporating elements of ecophysiology, functional traits, spatial niche differentiation, and phylogenetic conservatism. Further studies may enhance the understanding of climate change impacts on these species by addressing genetic diversity, range shifts, and ontogenetic variability. The future risk of biodiversity due to temperature oscillations can extend to a biogeographical legacy loss.

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3.5 SUPPORTING INFORMATION

Fig. S1 Location of the matrix plants of the saplings used for experimentation. Species were categorised based on their floristic contingent. Paraná state is highlighted in the upper left map. The insets illustrate site locations within the most densely sampled area.

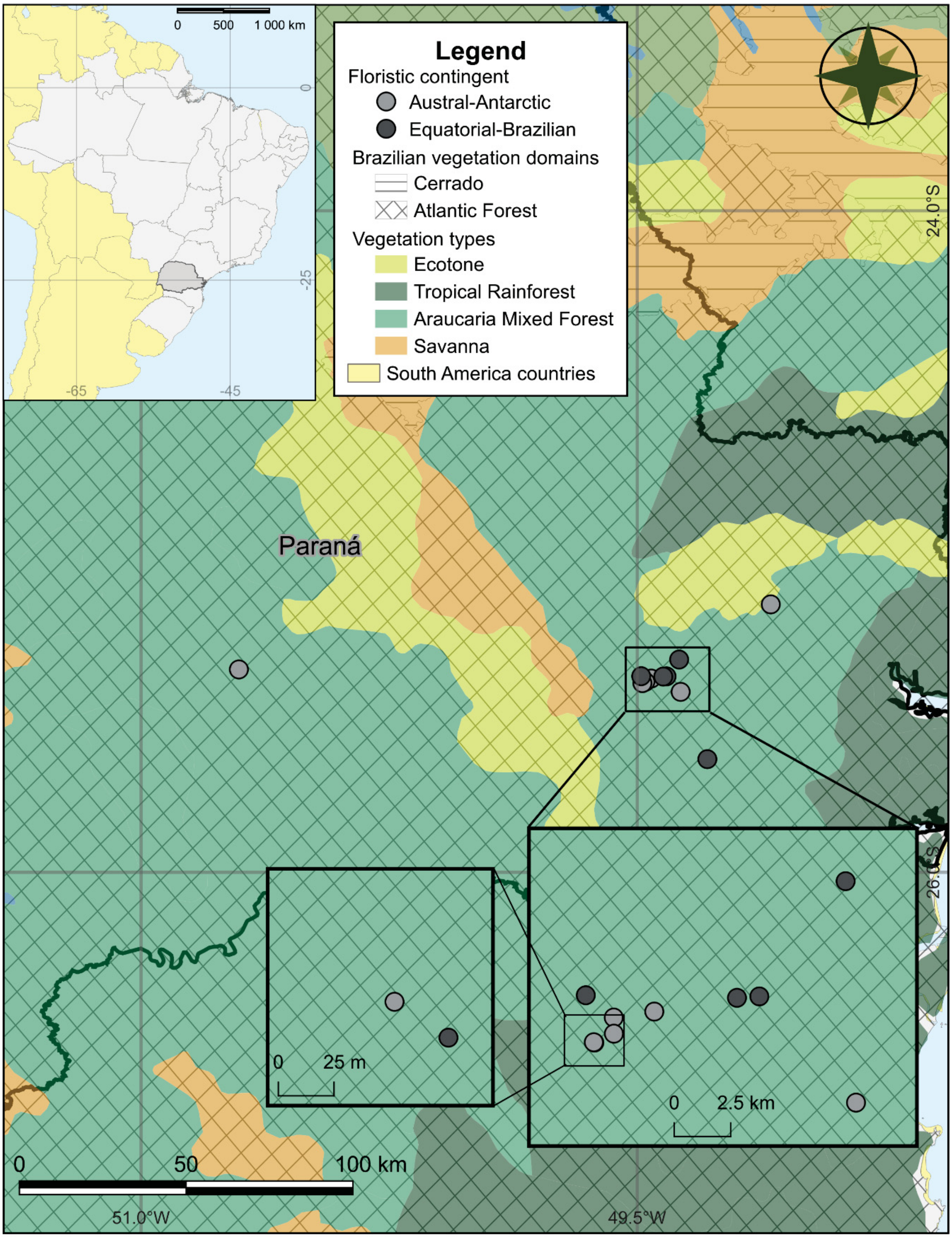

Fig. S2 The maximum quantum yield of photosystem II (F_v/F_m) as a function of temperature (°C) among seven tree species from the Austral-Antarctic floristic contingent, with three measurements per species (N = 3). The bold orange line illustrates the fitted curve using a four-parameter logistic model, and the transparent orange ribbon shows the 95% confidence interval. Vertical grey lines stand for physiological parameters: dotted - T_5 (temperature at 95% decline of F_v/F_m), solid - T_{50} (temperature at 50% decline), and dashed - T_{95} (temperature at 5% decline).

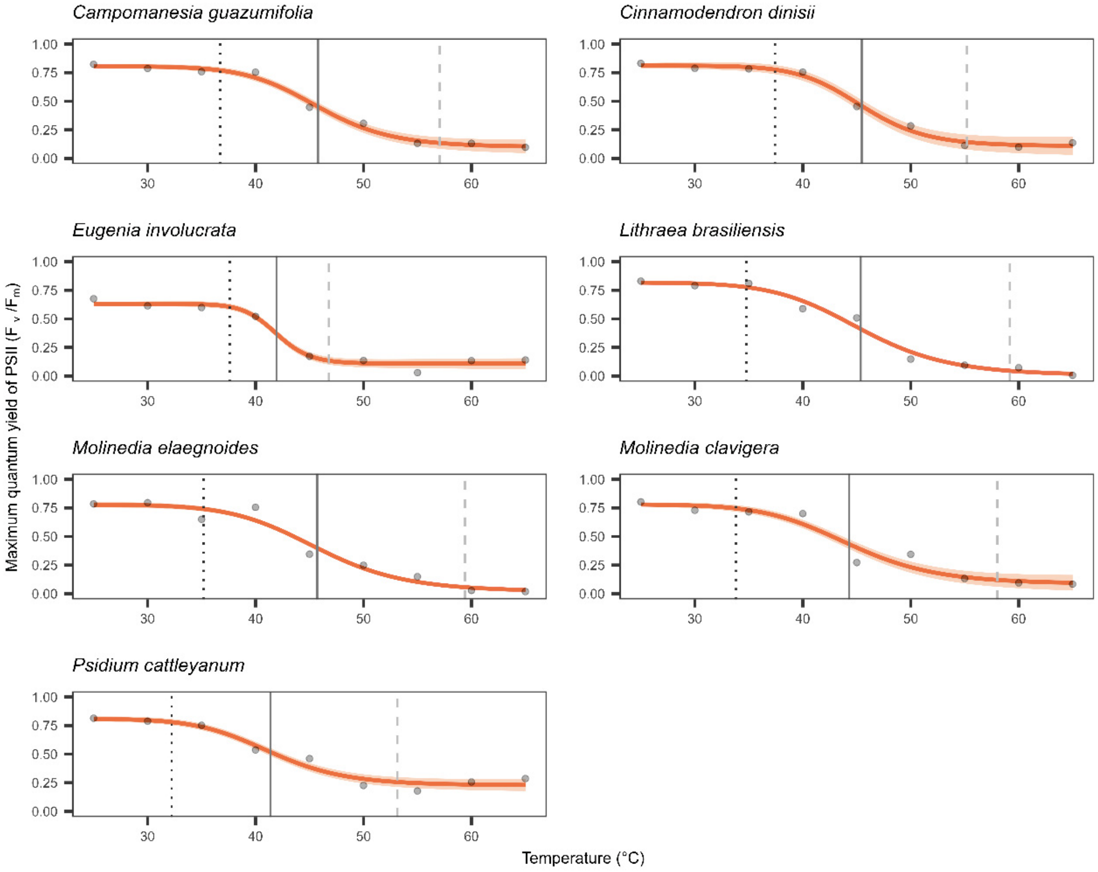

Fig. S3 The maximum quantum yield of photosystem II (F_v/F_m) as a function of temperature (°C) among seven tree species from the Equatorial-Brazilian floristic contingent, with three measurements per species (N = 3). The bold orange line illustrates the fitted curve using a four-parameter logistic model, and the transparent orange ribbon shows the 95% confidence interval. Vertical grey lines stand for physiological parameters: dotted - T_5 (temperature at 95% decline of F_v/F_m), solid - T_{50} (temperature at 50% decline), and dashed - T_{95} (temperature at 5% decline).

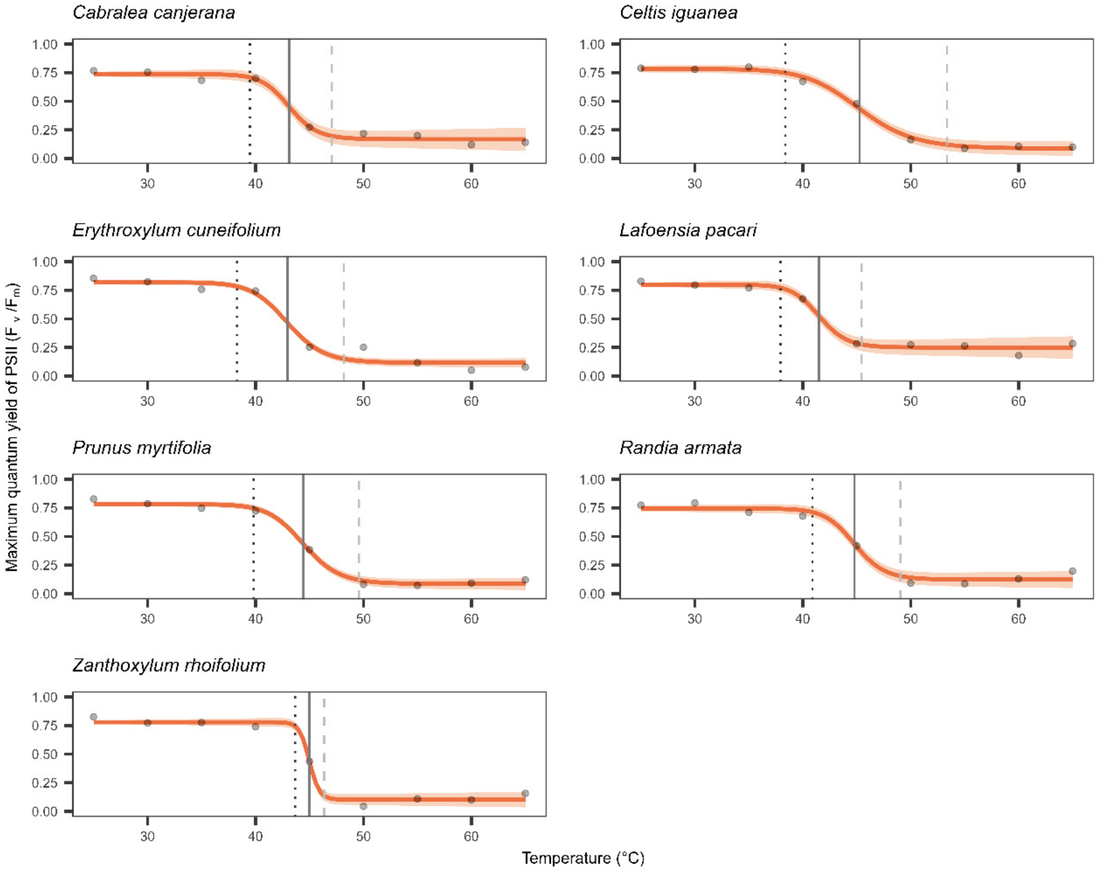

Fig S4 Comparison of the variation of a) axis 1 and b) axis 2 of the PCA of the thermal tolerance space between the floristic contingent species. Annotation above boxplots represent the results of an Analysis of Variance: “**” = p-value < 0.01, NS. = p-value > 0.05.

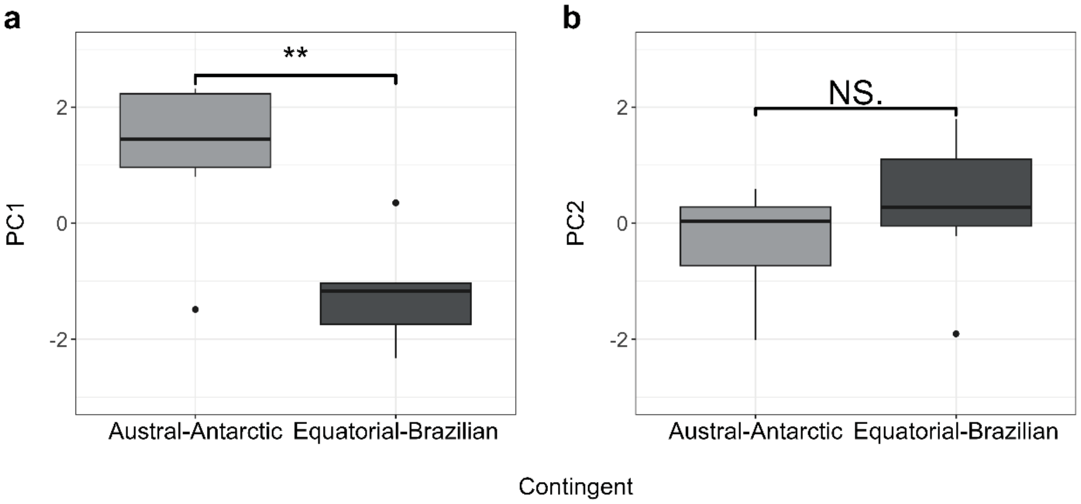

Fig S5 Correlation network for species studied in the (a) Austral-Antarctic and (b) Equatorial-Brazilian floristic contingents. Line colour represents correlation strength. Only correlations with $p < 0.05$ are shown. Legend: L_{th} – leaf thickness, PEL_e – effective percentage of electrolyte leakage, SLA – specific leaf area, T_5 – temperature of 95% of decline of F_v/F_m, T_{50} – temperature of 50% of decline of F_v/F_m, T_{95} – temperature of 5% of decline of F_v/F_m, DW – distance width between T_{95} and T_5.

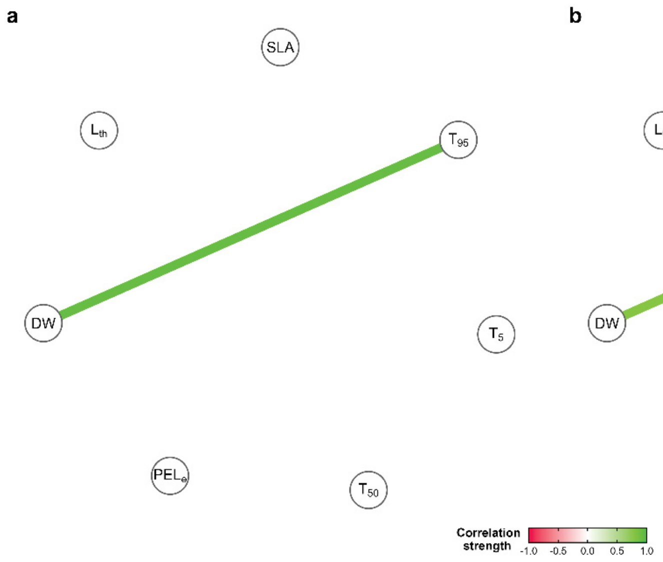

Table S1 Literature visited to classify the species available in the local nursery into floristic contingents.

SPECIES	FLORISTIC CONTINGENT	REFERENCE
Cabralea canjerana	Equatorial-Brazilian	Muellner, A.N. *et al.* (2006) *Molecular Phylogenetics and Evolution*, 40(1), 236–250. https://doi.or/10.1016/j.ympev.2006.03.001
Campomanesia guazumifolia	Austral-Antarctic	Lucas, E.J. *et al.* (2007) *TAXON*, 56(4), 1105–1128. https://doi.or/10.2307/25065906
Celtis iguanaea	Equatorial-Brazilian	Jin, J. *et al.* (2020) *Journal of Systematics and Evolution*, 58(4), 461–473. https://doi.or/10.1111/jse.12552
Cinnamodendron dinisii	Austral-Antarctic	Sanmartín, I., & Ronquist, F. (2004) *Systematic Biology*, 53(2), 216–243. https://doi.or/10.1080/10635150490423430
Erythroxylum cuneifolium	Equatorial-Brazilian	White, D.M. *et al.* (2019) *American Journal of Botany*, 106(1), 154–165. https://doi.or/10.1002/ajb2.1224
Eugenia involucrata	Austral-Antarctic	de Oliveira Bünger, M. *et al.* (2016) *Annals of Botany*, 118(7), 1209–1223. https://doi.or/10.1093/aob/mcw209
Lafoensia pacari	Equatorial-Brazilian	Inglis, P.W. *et al.* (2023) *Annals of Botany*, 132(2), 293–318. https://doi.or/10.1093/aob/mcad091
Lithraea brasiliensis	Austral-Antarctic	Mitchell, J.D. *et al.* (2022) *Brazilian Journal of Botany*, 45(1), 139–180. https://doi.or/10.1007/s40415-022-00793-5
Matayba elaeagnoides	Austral-Antarctic	Buerki, S. *et al.* (2011) Journal of Biogeography, 38(3), 531–550. https://doi.or/10.1111/j.1365-2699.2010.02432.x
Mollinedia clavigera	Austral-Antarctic	Renner, S.S. *et al.* (2010) Journal of Biogeography, 37(7), 1227–1238. https://doi.or/10.1111/j.1365-2699.2010.02319.x
Prunus myrtifolia	Equatorial-Brazilian	Chin, S.-W. *et al.* (2014) *Molecular Phylogenetics and Evolution*, 76, 34–48. https://doi.or/10.1016/j.ympev.2014.02.024
Psidium cattleyanum	Austral-Antarctic	Lucas, E.J. *et al.* (2007) *TAXON*, 56(4), 1105–1128. https://doi.or/10.2307/25065906

SPECIES	FLORISTIC CONTINGENT	REFERENCE
Randia armata	Equatorial-Brazilian	Manns, U. *et al.* (2012) *International Journal of Plant Sciences*, 173(3), 261–286. https://doi.or/10.1086/663971
Zanthoxylum rhoifolium	Equatorial-Brazilian	Appelhans, M.S. *et al.* (2018) *Molecular Phylogenetics and Evolution*, 126, 31–44. https://doi.or/10.1016/j.ympev.2018.04.013

Table S2 Species occurrence points, the total latitudinal and longitudinal variation among them, and bioclimatological variables extracted. Elevation is provided as the 95th percentile of occurrence in meters above sea level. Legend: AA – Austral-Antarctic; EB: Equatorial-Brazilian; MTCM - minimum temperature in the coldest month (°C, 5th percentile); PET – potential evapotranspiration (kg m^{-2} $month^{-1}$, 95th percentile).

Species	**Floristic contingent**	**Occurrences**	**Latitude var.**	**Longitude var.**	**Elevation**	**MTCM**	**PET**
Cabralea canjerana	EB	803	-9.6; -31.8	-38.5; -65.6	1349	7.95	113.03
Campomanesia guazumifolia	AA	412	-15.5; -31.6	-39.2; -58	1047.4	8.005	116.81
Celtis iguanaea	EB	1907	27.1; -36.7	-34.9; -109.1	1321.9	8.08	128.1
Cinnamodendron dinisii	AA	107	-23.5; -28.7	-45.8; -53.8	1066.1	7.21	100.52
Erythroxylum cuneifolium	EB	372	-13; -35.2	-42.8; -65.2	1113.2	7.505	123.33
Eugenia involucrata	AA	504	-13.2; -31.8	-39; -64.3	1276.5	7.75	115.595
Lafoensia pacari	EB	800	-3; -25.1	-41.4; -63.1	1081.15	11.15	138.935
Lithraea brasiliensis	AA	216	-22.4; -34.6	-46.8; -56.5	1051.6	6.325	105.1
Matayba elaeagnoides	AA	565	-14.7; -32.2	-42; -60.6	1028.85	7.65	113.08
Mollinedia clavigera	AA	204	-22.3; -28.7	-45.2; -54	1061.95	7.88	104.82
Prunus myrtifolia	EB	2099	-7.7; -33.8	-39; -60.8	1283.5	7.35	113.46
Psidium cattleyanum	AA	268	-8.2; -31.2	-35.2; -54.7	1015.8	8.05	108.545
Randia armata	EB	3071	19.9; -35.2	-34.9; -92.3	977.15	10.85	132.43
Zanthoxylum rhoifolium	EB	1438	8.1; -35.2	-34.9; -77	1161	7.65	124.615

Table S3 Loadings (i.e. correlation coefficients) between the different tolerance metrics and the first three axes of the two PCA axes

Tolerance metric	**PC1 (56.4%)**	**PC2 (24.8%)**	**PC3 (18.8%)**
Frost tolerance ($_e$PEL)	0.174	0.41	0.895
Heat tolerance (T_5)	-0.8	0.582	-0.143
Heat tolerance (T_{50})	0.45	0.832	-0.324
Heat tolerance (T_{95})	0.982	0.151	-0.11
Heat tolerance (DW)	0.991	-0.129	-0.018

Table S4 Correlation coefficients matrix of morphological and thermotolerance traits of the fourteen species studied. Significant correlations (i.e., $p < 0.05$ after permutation tests) are shown in bold. Legend: L_{th} – leaf thickness, PEL_e – effective percentage of electrolyte leakage, SLA – specific leaf area, T_5 – temperature of 95% of decline of F_v/F_m, T_{50} – temperature of 50% of decline of F_v/F_m, T_{95} – temperature of 5% of decline of F_v/F_m, DW – distance width between T_{95} and T_5.

	DW	**L_{th}**	**PEL_e**	**SLA**	**T_5**	**T_{50}**	**T_{95}**
DW	1.000						
L_{th}	**0.538**	1.000					
PEL_e	0.062	**0.530**	1.000				
SLA	-0.376	**-0.753**	-0.336	1.000			
T_5	**-0.879**	**-0.531**	0.007	**0.535**	1.000		
T_{50}	0.316	0.012	0.124	0.317	0.168	1.000	
T_{95}	**0.955**	0.495	0.134	-0.270	**-0.716**	**0.557**	1.000

Methods S1 Literature search of phylogeographic studies with ancestral area reconstruction.

Using a regional species list provided by Dvorak *et al.* (2025), we selected those with a tree life form, which resulted in 390 species (including subspecies). We consider their higher taxonomic categories (i.e., section, genus, tribe, and family) when researching phylogeographic studies.

We searched on Scopus and Google Scholar using a set of keywords with the following structure: ("biogeographic” OR “biogeography”) OR (“phylogeographic” OR “phylogeography") AND ("neotropical” OR “neotropics") AND taxonomic level (e.g., family, subfamily, genus, or section).

We included only those studies using phylogenetic information for ancestral area reconstruction in the literature selection. If a specific species was not available in an article, we included the article if any other closely related species was present (e.g., same section or same genus). We found 66 articles pointing to historic areas of the lineages of 245 species. Among those, we excluded the ones with results of undetermined or phylogenetically unclear areas (eight) and evidence of species introduction (one), resulting in 218 species available.

Methods S2 Evaluation of phylogenetic signal in plants' physiological thermal tolerances.

To decide whether our statistical analyses should account for the phylogenetic relationships among species, we investigated the presence of phylogenetic signal in their trait values.

We based our phylogenetic signal analysis on a comprehensive mega-phylogeny of vascular plants (Qian & Jin, 2016). For species not represented in this tree, we included closely related species by consulting the literature and utilising the ‘*bind.relatives’* and ‘*phylo.maker’* functions from the *V.PhyloMaker2* package (Jin & Qian, 2022). The binding was made using scenario 3, which sets up branch lengths for added taxa within a genus by evenly spacing the nodes between the dated nodes and tips inside the genus. It also introduces a missing species at the crown node of its genus. Next, we pruned the updated phylogeny to focus on our specific species.

We employed Pagel’s λ index to assess the phylogenetic signal (Pagel, 1999). To determine the presence of a phylogenetic signal for a particular trait, Pagel’s lambda should be close to 1, indicating that trait evolution aligns closely with what is expected under Brownian motion (Münkemüller *et al.*, 2012). The calculation was made using the ‘*phytools*’ package (Revell, 2024).

All values of Pagel’s λ were close to zero, revealing an absence of phylogenetic signal in the physiological traits among the species. Given this result, we omitted the phylogenetic factor in all analyses.

4 CONSIDERAÇÕES FINAIS

Considerando organismos que as plantas são sésseis, elas precisam de estratégias mais plásticas para prevenir e mitigar o estresse fisiológico, diferente de organismos móveis, que recorrem à termorregulação comportamental (BRADSHAW, 1965; HUEY; TEWKSBURY, 2009; MOLINA-MONTENEGRO; NAYA, 2012). Entre essas estratégias estão a regulação fisiológica, as estruturas morfológicas e os padrões fenológicos (CHAUDHRY; SIDHU, 2022; GRIME, 1977; KNIGHT; ACKERLY, 2003). Esta dissertação investigou esses mecanismos em diferentes níveis de organização biológica, com foco em espécimes juvenis, ressaltando a sensibilidade desse estádio ontogenético e a importância da resistência às intempéries climáticas para seu amadurecimento (BARTON, 2023), no contexto da Mata Atlântica — um bioma altamente ameaçado (MARQUES et al., 2021).

No primeiro capítulo, observamos como a estruturação morfológica das folhas se correlaciona com variáveis ambientais e com os impactos das geadas sobre arvoretas e arbustos em um gradiente campo-floresta. Complementando o que já se sabe sobre o impacto das geadas da região subtropical do Brasil em espécies isoladas em jardins comuns (ARAUJO FRANGIPANI; CRISTINA MÜLLER; ANAND, 2021), e as estratégias aquisitivas e conservativas na construção de características em mosaicos campo-floresta (KLIPEL et al., 2021; MARACAHIPES et al., 2018), demonstramos como essas estratégias estão correlacionadas ao impacto de geadas nesse tipo de sistema na região. Sabe-se como No Cerrado, estudos já mostraram como as geadas são um distúrbio determinante na construção das bordas nítidas entre florestas e ecossistemas abertos (BRANDO; DURIGAN, 2005; HOFFMANN et al., 2019; PILON et al., 2022), e que atributos funcionais estão diretamente relacionados com o impacto (DE ANTONIO; HOFFMANN; ROSSATTO, 2021; DE ANTONIO; SCALON; ROSSATTO, 2023). Em nosso estudo, o efeito foi menos pronunciado na Floresta com Araucária, possivelmente devido à filtragem ambiental que favorece espécies mais tolerantes ao frio (OLIVEIRA-FILHO et al., 2014) ou à intensidade moderada das geadas no ano da amostragem em campo. Como o estudo foi realizado em apenas um local, conclusões mais amplas exigiriam maior abrangência amostral. Contudo, diante das mudanças climáticas, é possível que os efeitos das geadas observados hoje sejam mais expressivos do que os provocados por eventos futuros (GULIZIA et al., 2022).

Esse tópico de efeito de geadas em mosaicos campo-floresta poderia ser mais aprofundado ao considerar diferentes formas de efeito estrutural na vegetação. Nosso estudo focou em formas lenhosas mais consolidadas (arvoretas e arbustos), mas seria enriquecido ao incluir estádios ontogenéticos mais jovens. Além da avaliação de folhagens, entender o impacto (ou ausência) das geadas na arquitetura, crescimento e mortalidade das plantas lenhosas que se estabelecem no campo (BOTHA; ARCHIBALD; GREVE, 2020).

No segundo capítulo, discutimos como a biogeografia histórica influencia a tolerância térmica atual das linhagens de plantas. Embora não seja o principal fator, as áreas de ocorrência de linhagens ancestrais influenciaram como clados atuais respondem fisiologicamente a extremos de temperatura. Esta abordagem inova ao discutir a hipótese de conservadorismo de nicho entre contingentes florísticos biogeográficos de maneira mais mecanística em plantas (ver KHALIQ et al., 2015 para animais). Verificamos a relação entre atributos morfológicos e fisiológicos, destacando a influência da espessura foliar na termotolerância (DA SILVA; ROSSATTO, 2022; GURVICH et al., 2002). Também avaliamos se condições climatológicas das áreas de ocorrência das espécies explicam suas respostas fisiológicas. Embora a altitude máxima contribuiu para a explicação da tolerância ao calor das espécies através de suas ocorrências, fatores adicionais (climáticos e bióticos) devem ser considerados para uma aproximação mais exata do nicho térmico das espécies (BOCSI et al., 2016; BUSH et al., 2018; COLWELL; RANGEL, 2009).

A tolerância fisiológica e a tolerância espacial devem ser usadas de forma complementar para prever o efeito de cenários climáticos futuros na distribuição das espécies e na diversidade biogeográfica do bioma, ampliando a discussão sobre ocorrência e nicho. Essa abordagem difere da usada por modelos de nicho com fisiologia incorporada (BUCKLEY et al., 2011; CUSTER et al., 2024) uma vez que não usa os limiares fisiológicos para refinar a predição de distribuição futura, mas considera esses parâmetros fisiológicos para entender padrões de migração e restrição de ocorrência.

Os resultados desta dissertação evidenciam como a temperatura é influente para as plantas além da perspectiva de desempenho individual. Além da avaliação do efeito da temperatura em atributos funcionais fisiológicos e morfológicos dos indivíduos, essa condição também afeta a distribuição das plantas em diferentes escalas espaciais. Em escala local, a ocorrência de fenômenos climáticos é capaz de moldar a estrutura de comunidades vegetais. Em escalas regionais, a temperatura modula a ocorrência das espécies, refletindo as preferências ambientais de linhagens ascendentes. Desta maneira, a temperatura emerge não apenas como um fator do nicho ecológico, mas como uma força estruturante em múltiplos níveis da organização ecológica. Ao propor essa integração de múltiplas abordagens e múltiplas escalas, esta dissertação oferece evidências empíricas relevantes e caminhos metodológicos aplicáveis ao monitoramento de plantas frente às mudanças climáticas.

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