



UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
Instituto de Biociências – Câmpus de Botucatu/Rio Claro
Seção Técnica de Pós-Graduação



**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL
(INTERUNIDADES)**

**RESPOSTAS FUNCIONAIS FOLIARES E GERMINAÇÃO DE
SEMENTES DE ESPÉCIES HERBÁCEO-ARBUSTIVAS DO
CERRADO**

PEDRO FIRME DA CRUZ JÚNIOR

Dissertação apresentada ao Instituto de Biociências, Câmpus de Rio Claro, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Biologia Vegetal, Interunidades entre o Instituto de Biociências do câmpus de Botucatu e Instituto de Biociências do câmpus de Rio Claro.

**RIO CLARO – SP
2025**



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“Realize that everything connects to everything else”.

Leonardo da Vinci

RESUMO

Ações antrópicas vêm causando alterações significativas nas dinâmicas de fogo e disponibilidade hídrica no Cerrado. O adensamento vegetacional, resultado da ausência de legislação e manejo do fogo, promove mudanças microambientais, como maior sombreamento e acúmulo de serapilheira. Este bioma sazonal e inflamável, sujeito a subprodutos do fogo como a fumaça, também enfrenta impactos relacionados à redução hídrica, como diminuição de precipitação e aumento de estiagens. Espécies herbáceo-arbustivas, que representam cerca de 80% da vegetação, são particularmente afetadas, mas pouco se sabe sobre suas respostas funcionais e processos de recrutamento sob este cenário. Este estudo analisou (i) como mudanças na luminosidade e serapilheira influenciam traços funcionais foliares e (ii) as respostas germinativas e pós-seminais de espécies herbáceo-arbustivas sob estresse hídrico e o possível efeito protetor da fumaça. O sombreamento emergiu como o principal fator determinante dos traços foliares, e após 2,5 anos, as comunidades apresentaram estratégias aquisitivas, como maior área foliar específica, menor espessura foliar e maiores teores de pigmentos fotossintéticos, favorecendo espécies resilientes e generalistas. Germinação e desenvolvimento inicial foram severamente afetados pelo estresse hídrico, mas a fumaça mostrou efeito protetor, mitigando parcialmente os impactos dos baixos potenciais hídricos e estimulando o estabelecimento. Conclui-se que as alterações antrópicas nos regimes de fogo e disponibilidade hídrica atuam como novos filtros ambientais, limitando o recrutamento e a persistência das espécies, evidenciando a necessidade de manejo adaptativo frente às alterações ambientais.

Palavras-chave: morfofisiologia foliar, filtros ambientais, recrutamento de espécies, comunidade não-arbórea, fumaça.

ABSTRACT

Anthropogenic activities have significantly altered fire dynamics and water availability in the Cerrado. Woody encroachment, driven by the lack of legislation and fire management, induces microenvironmental changes, including increased shading and litter accumulation. This seasonal and fire-prone biome, influenced by fire byproducts such as smoke, also faces impacts associated with water scarcity, including reduced precipitation and prolonged droughts. Ground-layer species, which comprise approximately 80% of the vegetation, are particularly affected; however, little is known about their functional responses and recruitment processes under these conditions. This study investigated (i) how changes in light availability and litter accumulation affect leaf functional traits and (ii) the germination and seedling responses of ground-layer species under water stress, as well as the potential protective role of smoke. Shading emerged as the primary driver of leaf traits, and after 2.5 years, the communities exhibited resource-acquisitive strategies such as higher specific leaf area, thinner leaf tissues, and increased photosynthetic pigment concentrations, favoring resilient and generalist species. Germination and seedling development were severely constrained by water stress, but smoke demonstrated a protective effect, partially mitigating the adverse impacts of low water potentials and promoting seedling establishment. These findings highlight that anthropogenic alterations to fire regimes and water availability impose new environmental filters, limiting species recruitment and persistence, and underscore the need for adaptive management strategies in the face of environmental changes.

Keywords: leaf morphophysiology, environmental filters, species recruitment, ground-layer community, smoke.

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INTRODUÇÃO GERAL

O Cerrado e seus desafios

Biomas savânicos são fortemente influenciados pela interação entre fatores climáticos, distúrbios e vegetações (Lehmann et al., 2011). Entre os principais fatores, destacam-se o fogo e a disponibilidade hídrica, que desempenham papéis centrais na manutenção das formações campestres e florestais (Bond et al., 2005; Ribeiro et al., 2021a; Kobziar et al., 2024). Estes elementos atuam como filtros, influenciando diretamente o recrutamento (Escobar et al., 2018; Ribeiro et al., 2021b), o crescimento (Loram-Lourenço et al., 2020) e a reprodução das espécies vegetais (Gawryszewski et al., 2020).

O fogo, elemento integrante da história evolutiva de biomas savânicos, atuou na seleção de espécies com diferentes estratégias de resiliência aos seus efeitos (Pausas e Parr, 2018). Estratégias adaptativas ao fogo já foram descritas para espécies de savanas africanas (Maurin et al., 2014), australianas (Burrows et al., 2010) e brasileiras (Simon e Pennington, 2012; Pilon et al., 2018; Cozin et al., 2024). Da mesma forma, a disponibilidade hídrica – sazonalidade e nível do lençol freático – desempenha um papel fundamental nas dinâmicas vegetacionais, permitindo a germinação e desenvolvimento de plântulas na estação chuvosa (Escobar et al., 2018) ou restringindo o estabelecimento de espécies típicas de savana em campos alagados (Ribeiro et al., 2021b), atuando como *drivers* da vegetação (Cordeiro et al., 2021).

O Cerrado, a savana mais biodiversa do mundo, possui um complexo vegetacional em mosaico, que originalmente se estende por cerca de 2 milhões de km², sendo o segundo maior bioma do Brasil em área ocupada (Ribeiro e Walter, 2008). Entretanto, no estado de São Paulo, o Cerrado sofreu uma redução drástica em sua extensão, perdendo 88,5% de sua área original (Kronka et al., 2005). Além disso, as áreas remanescentes estão sob ameaça devido a fatores antrópicos, como a ausência de estratégias de manejo do fogo (Durigan e Ratter, 2016), o uso intensivo da terra e as alterações na composição atmosférica (Ferreira et al., 2021).

Historicamente, o Brasil desenvolveu uma série de legislações voltadas à supressão do fogo (Durigan e Ratter, 2016). As diretrizes do *Código Florestal* (Lei nº 12.651/2012), ao restringirem o fogo em áreas de vegetação nativa, contribuíram para a visão generalista de que o fogo não ocorre naturalmente em áreas preservadas e que deve ser suprimido. Tais normas frequentemente desconsideram as especificidades ecológicas do Cerrado, como a dependência de regimes de fogo para a manutenção de sua biodiversidade (Durigan, 2020). Além disso, as

projeções do Painel Intergovernamental sobre Mudanças Climáticas (IPCC, 2023) indicam aumentos de temperatura e períodos de estiagem, além de reduções na precipitação, para o Brasil. Estas novas condições podem impor restrições hídricas a ambientes naturalmente úmidos e ameaçados, como as áreas úmidas do Cerrado (Durigan et al., 2022). Além disso, podem afetar dinâmicas de recrutamento fortemente dependentes da estação chuvosa (Escobar et al., 2018). Considerando o histórico e a importância da pirodiversidade e da disponibilidade hídrica para a formação e manutenção do Cerrado, bem como os impactos diretos e indiretos das ações antrópicas, as alterações nos regimes de fogo e nos ciclos hidrológicos colocam em risco as dinâmicas desse hotspot de biodiversidade.

Adensamento vegetacional no Cerrado

O fogo é um evento comum e natural que desempenha papel essencial na manutenção da estrutura e composição da vegetação (Antar et al., 2022; Dairel e Fidelis, 2024), a pirodiversidade atua como um filtro ambiental que restringe o recrutamento de espécies arbóreas, especialmente daquelas típicas de formações florestais (Hoffmann et al., 2009; Gawryszewski et al., 2020). A alta frequência de incêndios limita o estabelecimento e o desenvolvimento dessas espécies, favorecendo a preservação de formações vegetacionais abertas, como as savânicas (Maurin et al., 2014; Dantas et al., 2016).

A supressão do fogo, no entanto, permite o avanço de espécies arbóreas sobre fitofisionomias abertas (Durigan e Ratter, 2016), promovendo alterações significativas no microambiente. Mudanças como a redução da incidência luminosa (Pinheiro et al., 2016), o aumento no acúmulo de serapilheira (Paiva et al., 2015) e a diminuição da recarga hídrica no solo (Honda e Durigan, 2016), são capazes de impactar negativamente a biota local. Por exemplo, em comunidades de pequenos mamíferos (Furtado et al., 2021) e formigas (Neves et al., 2023), o adensamento vegetal resulta na redução da abundância e diversidade de espécies adaptadas a ambientes abertos, favorecendo espécies generalistas.

Além disso, as espécies herbáceo-arbustivas, que correspondem a até 80% das espécies vegetais no Cerrado, são as mais prejudicadas com o aumento no sombreamento ocasionado pelo adensamento florestal (Abreu et al., 2017). No entanto, tanto a redução da luminosidade quanto o aumento no acúmulo de serapilheira podem diminuir a germinação e a emergência de plântulas de espécies não-arbóreas, funcionando como um gargalo na dinâmica de regeneração (Pinheiro et al., 2022).

De forma similar, reduções na diversidade florística em formações originalmente campestres e savânicas já foram documentadas tanto a longo (Souza et al., 2022a; Trindade et

al., 2024) quanto a curto prazo (Pinheiro et al., 2022), acompanhadas por um processo de homogeneização, com comunidades formadas por espécies generalistas (Flake et al., 2021). Devido à alta demanda por luminosidade, muitas dessas espécies podem ser extintas nesse novo cenário (Pilon et al., 2021a), a menos que desenvolvam mecanismos morfofisiológicos capazes de otimizar a absorção de luz difusa (Pinheiro et al., 2018; Rossatto et al., 2018).

Respostas funcionais foliares à luminosidade e serapilheira

Além de ser um fator indispensável para o metabolismo vegetal, a luminosidade também tem grande influência no fenótipo, afetando a morfologia de tecidos, sobretudo aqueles associados à transpiração e fotossíntese (Rossatto e Kolb, 2010; 2012), promovendo plasticidade em espécies herbáceas (Gobbi et al., 2011) e lenhosas (Lauton et al., 2022). Essas alterações estruturais podem estar relacionadas a estratégias adaptativas aos fatores ambientais (Pinheiro et al., 2018), de forma que, sob ambientes sombreados (Mendonça et al., 2019) ou florestados (Carlos e Rossatto, 2017), caracteres mais mesomorfos e relacionados ao uso menos conservativo de recursos são observados como respostas (Valladares e Niinemets, 2008). Em conjunto com estas alterações morfoanatômicas, são relatadas alterações nos conteúdos de pigmentos, como os aumentos de clorofila *a*, *b* (Mendonça et al., 2020) e carotenoides (Rossatto et al., 2018), podendo este último auxiliar na captura de luminosidade (Valladares et al., 2000).

Por outro lado, o adensamento vegetacional não apenas reduz a incidência luminosa, mas também propicia o acúmulo de serapilheira (Pinheiro et al., 2016; Rossatto e Rigobelo, 2016). Uma vez que a porção foliar da camada de serapilheira atua como reservatório de nutrientes (Campos et al., 2008), o seu acúmulo sobre o solo, causado pela supressão do fogo, influencia diretamente na quantidade de nutrientes capaz de retornar ao solo (Belsky et al., 1989; Mordelet et al., 1993). Dessa forma, o aumento da disponibilidade nutricional pode contribuir para a formação de folhas com traços típicos de mesófitas (Boeger e Wisniewski, 2003; Miatto et al., 2016). Essa classificação, originalmente relacionada à disponibilidade hídrica, designa plantas adaptadas a ambientes que não enfrentam condições extremas de seca ou alagamento (Warning and Vahl, 1909). Além disso, o aumento desta camada está relacionado com o acréscimo nas taxas de transpiração foliar e condutância estomática, em conjunto com reduções no uso eficiente da água (Scalon et al., 2014).

Estratégias de recrutamento via sementes à sazonalidade climática em risco

A sazonalidade hídrica é uma característica marcante do Cerrado, com verões chuvosos e invernos secos (Alvares et al., 2013). Dessa forma, o processo de recrutamento neste ambiente é altamente dependente da estação chuvosa, período onde a saturação hídrica no solo pode atingir o seu máximo (Borghetti et al., 2024). A disponibilidade de água, fator crucial no processo germinativo (Baskin e Baskin, 2014), é responsável por reativar o metabolismo das sementes, possibilitando sua germinação (Marcus et al., 1966; Simon, 1984; Bradford, 1990; Bewley, 2013). Em condições de deficiência hídrica, muitas espécies apresentam redução na porcentagem de germinação (Hu et al., 2015) e na viabilidade das sementes (Souza et al., 2022), além de um aumento no tempo médio de germinação (Antunes et al., 2011).

A seca, um dos principais estresses abióticos que limitam o desenvolvimento inicial das plantas (Moles e Westoby, 2004), também induz alterações morfofisiológicas significativas em espécies vegetais de diferentes formas de vida (Eziz et al., 2017). Entre as respostas observadas em plantas arbóreas durante as fases iniciais de desenvolvimento destacam-se a diminuição da biomassa total (Chaïb et al., 2023), maior alocação de recursos para a produção de raízes (Bayen et al., 2021) e redução na estatura (Ferreira et al., 2015). Contudo, embora grande parte dos estudos sobre respostas morfofisiológicas à seca tenha focado em espécies herbáceas e arbustivas de interesse comercial (ver Tombesi et al., 2018; Nadeem et al., 2019; Cai et al., 2020), pesquisas envolvendo espécies típicas do Cerrado ainda são escassas (Ribeiro e Kolb, 2016; Melo et al., 2021; Souza et al., 2022).

Como consequência do início da estação chuvosa, propicia-se o cenário para a ocorrência de incêndios naturais no Cerrado (Miranda et al., 2009). Durante a transição entre as estações, o fogo queima a biomassa seca acumulada ao longo da estação seca (Ramos-Neto e Pivello, 2000). A queima da matéria vegetal gera subprodutos, como fumaça, carvão e cinzas, que interagem com os ecossistemas do Cerrado (Daibes e Borghetti, 2024). A fumaça, composta por uma ampla variedade de compostos orgânicos e inorgânicos, pode apresentar efeitos neutros, negativos ou positivos sobre parâmetros germinativos de espécies savânicas (Motta et al., 2024). Estudos recentes têm demonstrado que a fumaça pode estimular tanto a germinação quanto o desenvolvimento pós-seminal (Ramos et al., 2019), mesmo sob condições de estresse (Li et al., 2017; Singh et al., 2023). Desta forma, a fumaça e seus compostos são capazes de atuar através de diferentes mecanismos e níveis (genéticos e morfológicos), garantindo melhor adaptação ao estresse, com manutenção da homeostase vegetal (Aslam et al., 2024). Embora tais resultados sejam documentados apenas para espécies de interesse comercial (Moyo et al., 2022a, b), eles oferecem pistas sobre as

possíveis interações ecológicas em ambientes sazonalmente secos, especialmente aqueles sujeitos ao aumento na frequência de incêndios (Silva et al., 2024) e ao prolongamento de períodos de estiagem (Hofmann et al., 2021; IPCC, 2023).

Lacunas no estado da arte das dinâmicas no Cerrado e estruturação da dissertação

Embora o número de estudos sobre a comunidade não-arbórea do Cerrado – o estrato mais representativo deste bioma – tenha aumentado nos últimos anos (ver Pilon et al., 2021; Pinheiro et al., 2022; Souza et al., 2022; Motta et al., 2024), poucos trabalhos investigaram as respostas dessa comunidade em condições de campo simuladas e por períodos prolongados, considerando os novos filtros ambientais impostos pela invasão lenhosa e suas alterações microambientais (Pinheiro et al., 2022). Além disso, no que diz respeito aos parâmetros germinativos, apesar da influência reconhecida da sazonalidade hídrica e de subprodutos do fogo (e.g. fumaça) no recrutamento e nas dinâmicas do Cerrado (Ramos et al., 2017; 2019; Zironi et al., 2019; Daibes et al., 2019), pouquíssimos estudos enfocam as respostas de espécies de Cerrado ao déficit hídrico (Daibes et al., 2019), e não há, até o momento, estudos que avaliem conjuntamente a interação desses fatores.

Diante destas lacunas, este estudo visa não apenas contribuir para o avanço do conhecimento, mas também oferecer suporte para pesquisas futuras voltadas a este hotspot de biodiversidade. Assim, apresentamos nesta dissertação os resultados de experimentos realizados em campo e laboratório, organizados nos seguintes capítulos:

Capítulo 1. Woody encroachment and leaf functional traits of ground-layer savanna species

Neste capítulo, investigamos as respostas funcionais foliares da comunidade não-arbórea do Cerrado diante da simulação de condições promovidas pelo adensamento vegetacional. Foram avaliados 20 parâmetros morfofisiológicos coletados antes e após 2,5 anos de simulação em campo, com o objetivo de caracterizar o conjunto de traços funcionais da comunidade inicial e final, bem como o nível de resposta fenotípica às novas condições ambientais.

Capítulo 2. Combining the effects of water stress and smoke on seed recruitment

Neste capítulo, avaliamos as respostas germinativas e pós-seminais da comunidade não-arbórea do Cerrado ao longo de um gradiente de redução do potencial osmótico, bem como o potencial *efeito protetor* da fumaça nessas condições. Para isso, conduzimos experimentos em laboratório, manipulando ambos os fatores de forma conjunta e isolada, com

o objetivo de analisar não apenas as respostas ao déficit hídrico, mas também a capacidade da fumaça em mitigar os efeitos do estresse hídrico.

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CAPÍTULO 1. Woody encroachment and leaf functional traits of ground-layer savanna species

ACEITO NO PERIÓDICO FLORA: MORPHOLOGY, DISTRIBUTION, FUNCTIONAL ECOLOGY OF PLANTS

ABSTRACT

Woody encroachment is a widespread phenomenon affecting savanna and grassland ecosystems. The filters imposed by encroachment promote floristic changes in the ground-layer community, decreasing species richness, and providing species replacement. However, functional aspects are still poorly understood, especially the role of leaf anatomical traits. Thus, our aim was to investigate the leaf traits changes in the ground-layer community of a Cerrado area in response to changes in litter and light, caused by woody encroachment. Through a field experiment, we simulated higher amounts of litter biomass and increased shading of the plant community for 2.5 years. We measured leaf tissue thickness, specific leaf area (SLA), pigments content and photosynthetic parameters, such as carbon assimilation ($A_{\max}$), stomatal conductance (g_s) and quantum efficiency of photosynthesis (α). Our results showed that shading is the main factor altering leaf functional traits, with the post-shading ground-layer community exhibiting more acquisitive resource-use strategies such as reduced leaf tissue thickness, higher SLA, and higher pigment content compared to plants under noencroached conditions. However, these leaf responses were not sufficient to ensure the maintenance of higher photosynthetic performance ($A_{\max}$, g_s and α) in the study species. Therefore, we demonstrate that the studied leaf functional traits of the ground-layer community are insufficient to ensure persistence and diversity in this new scenario. With the change in species composition promoted by encroachment, some shade-tolerant and generalist plants exhibit responses that contribute to their survival and occurrence.

Keywords: shading; litter; photosynthetic pigments; carbon assimilation; leaf tissue thickness.

1. Introduction

Natural wildfires shape the structure and composition of savanna vegetation across the globe (Staver et al. 2011; Rosan et al. 2019). Therefore, fire presence maintains the proper open physiognomy of savannas (Maurin et al. 2014). However, under fire suppression, savannas - especially in South America – tend to become denser or even be turned into forest formation due to encroachment by typical forest species (Pinheiro and Durigan, 2009; Hoffmann et al. 2009; Stevens et al. 2017). This process, known as woody encroachment, alters different microenvironmental factors. Therefore, a decrease in understory light incidence has been observed (Pinheiro et al. 2016), along with an increase in litter deposition (Paiva et al. 2015; Rossatto and Rigobelo, 2016). The persistence of typical savanna species will depend on the extent of their ability to adjust to the new environmental conditions imposed by encroached environments, especially the ground-layer species. As they require greater luminosity, many ground-layer species may disappear in this new scenario (Pilon et al. 2021a), as has been shown by some studies carried out in natural conditions (Pilon et al. 2021b; Pinheiro et al. 2016) and in field-manipulated experiments (Pinheiro et al. 2022; Raymundo et al. 2023; Wierzchowski and Lehmann, 2022).

Light irradiance plays a significant role in plant phenotype, affecting leaf structure, and traits associated with physiological aspects, such as transpiration and photosynthesis (Rossatto and Kolb, 2010; 2012; Lauton et al. 2022). Under shading, morphoanatomical adaptations can be observed in different leaf traits, such as a lower thickness of the mesophyll (Barbosa et al. 2022), palisade parenchyma (Th  roux-Rancourt et al. 2023), and adaxial surface epidermis (Pinheiro *et al.* 2018), lower stomatal density (Rossatto and Kolb, 2010) and a larger stomata size (Ara  jo et al. 2023). The specific leaf area (SLA), the ratio of leaf area to leaf dry mass corresponding, may also have higher values in plants under shade (Zhang et al. 2022; Andrade et al. 2024) or in forest environments (Carlos and Rossatto, 2017). Reduction in leaf tissue thickness and increase in leaf area can justify this response (Pinheiro et al. 2018, Torres-Leite et al. 2019). Furthermore, with these responses, higher chlorophyll levels provide more effective use of the scarce sunlight that reaches the understory (Valladares and Niinemets, 2008). In contrast, typical plants of sunny environments exhibit higher carotenoid content, as these pigments mitigate photoinhibition by dissipating excess energy (Frank and Cogdell, 1996). Additionally, ground-layer species may possess high photosynthetic activity, with higher rates of transpiration, stomatal conductance,

and carbon assimilation when compared with understory species from encroached environments (Carlos and Rossatto, 2017; Rossatto et al. 2018).

The advancement of woody plants besides altering the light availability, also leads to increased litter accumulation (Pinheiro et al. 2016; Rossatto and Rigobelo, 2016) because forest species naturally have higher leaf area indexes, thus being able to deposit a higher amount of leaf in savanna soils (Geiger et al. 2011). This soil-surface layer is formed by the deposition of different plant aerial organs (Freire et al. 2010) and is considered a nutrient reservoir in the environment (Campos et al. 2008; Zhang et al. 2023). Thus, the increase in litter accumulation and the consequent increase in nutrient availability can also lead to a leaf morphoanatomical change, resulting in more mesomorphic characteristics, such as higher SLA values (Boeger and Wisniewski, 2003; Miatto et al. 2016).

In this study, we aimed to evaluate the leaf functional responses of the ground-layer community of a Brazilian Cerrado site under the new environmental conditions caused by woody encroachment. Through experimental shading and litter manipulation, we assessed the leaf functional traits related to the survival response of the ground-layer community in this new dense environment. These results will help to understand the morphophysiological strategies of the ground-layer community under shading and litter conditions, separately and together over 2.5 years of simulation. We hypothesize that: (1) the ground-layer community under shading treatments will present leaf functional traits oriented towards acquisitive resource-use strategies, such as thinner tissue thickness, lower stomatal density, and higher SLA values; (2) Similarly, the ground-layer community under litter will also present mesomorphic morphoanatomical traits, but more subtly; (3) Although the ground-layer community will demonstrate functional responses, such as increases in photosynthetic pigments, we anticipate a reduction in photosynthetic efficiency in both the photochemical stage and carbon assimilation, ultimately hindering its persistence under dense conditions, and potentially leading to a decrease in species by the end of the experiment.

2. Materials and methods

2.1 Study area

This study was conducted in the Assis Ecological Station (AES – Fig. 1A), located in the state of São Paulo, municipality of Assis (22°33'2" S and 50°21'27" W). AES bears some Cerrado physiognomies as typical cerrado (*cerrado sensu stricto* – savanna physiognomy), dense cerrado, forested cerrado (*cerradão*), and wet grasslands (Pinheiro and Durigan, 2009). AES

vegetation is about 60 years under fire suppression, in this way, forest species advanced over savanna physiognomies, promoting vegetational encroachment (Pinheiro and Durigan, 2009). However, some remnants of cerrado *sensu stricto* are still present in AES. The climate is classified as Cfa according to Köppen classification (Alvares et al. 2013), with rainfall concentrated in the summer and an average annual rainfall of 1,400 mm. According to Soil Taxonomy, the AES exhibits different soil types, being primarily composed of Hapludox (oxisols) and Quartzipsamment (entisols) in cerrado *sensu stricto* (Assis et al. 2011), and Hapludox and Kandiodox (oxisols) in *cerradão* areas (Juhász et al. 2006; Assis et al. 2011).

2.2 Environmental characterization and field experiment

To characterize shading and litter conditions of the encroached areas in AES (*cerradão*; Fig 1B), was measured the understory photosynthetic active radiation (PAR) at 30 haphazardly points with a PAR sensor coupled to a CI-110-24P-ID plant canopy imager (CID Bioscience Inc, Camas, WA, USA). PAR was obtained between 8 a.m. and 12 p.m. Furthermore, litter accumulation was estimated in 30 1 x 1 m plots randomly distributed using a graduated ruler. Encroached areas showed an average PAR of $46.93 \pm 2.31 \mu\text{mol m}^{-2} \text{s}^{-1}$ and a litter layer with an average thickness of $3.93 \pm 1.42 \text{ cm}$ (Pinheiro et al. 2022).

Before the start of the field experiment, a preliminary sampling of the community was conducted (January 2017 – T_i). The characterization of the ground-layer community involved identifying the species and collecting a fully expanded leaf from the third or fourth node, as well as measuring photosynthetic parameters and pigment concentration (detailed methodological information is provided in the following section). Between July 2017 and January 2020 (T_f), an experiment with light and litter manipulation was carried out to simulate the environmental changes produced by woody encroachment in the remaining AES cerrado *stricto sensu* areas (no encroached) (Fig. 1C). The experiment consisted of four treatments: control (CT – no encroached conditions), shading (S), litter (L), and shading plus litter (S+L). To simulate shading like encroached areas understory, the S and S+L plots were covered with shade screens (70%) allowing a light incidence near $40\text{-}50 \mu\text{mol m}^{-2} \text{s}^{-1}$. On the other hand, the plots of L and S+L received litter previously collected in encroached areas till they reached a 4 cm litter layer. The treatments were distributed in 10 blocks, each with four treatments (Fig. 1A and C). Each treatment was simulated in a 1m² plot so that they would be randomly assigned between plots. The blocks and their respective plots were monitored monthly to maintain the shading and thickness of the litter conditions - experimental design used by Pinheiro et al. (2022) illustrated in Fig. 1.

2.3 Morphoanatomical parameters and photosynthetic pigments content

The ground-layer community in the 10 blocks was analyzed for leaf pigment content. For leaf morphoanatomical analysis, five blocks were chosen (see Table S1 and S2 for the number of species in each treatment). A representative individual of each species in the blocks was sampled, with a fully expanded, injury-free leaf from the third or fourth node collected before (T_i) and after 2.5 years (T_f) of the field experiment. The same material was used for both morphoanatomical analyses and photosynthetic pigment content assessment.

Specific leaf area (SLA) was obtained through the ratio between the leaf area (1 cm^2 leaf discs) and the corresponding leaf dry mass obtained after over-drying at 70°C for 3 days. To obtain leaf cross-sections, segments of the mid-leaf section, including the main vein, were rehydrated and placed in a 2% KOH solution (Smith and Smith, 1942). Subsequently, they were dehydrated in ethanol series and included in historesin Leica[®] (De Paula et al. 2019). Transversal sections of $8\mu\text{m}$ were stained with Toluidine Blue (O'Brien et al. 1964) and mounted in Entellan[®]. We measured epidermis thickness on both surfaces, palisade parenchyma, spongy parenchyma, and total leaf thickness 10 times for each parameter in each individual (sample). Paradermic sections were obtained by dissociating 1cm^2 leaf segments in a (1:1) glacial acetic acid and hydrogen peroxide solution (Kraus and Arduin, 1997). Each epidermis of the abaxial surface was washed with distilled water, stained with 1% Safranin, and mounted in glycerin. We measured the length of the guard cells and the equatorial diameter of 30 stomata from each individual. We also obtained the stomatal density from three random fields for each individual. The measurements obtained for each anatomical parameter per individual were averaged, avoiding pseudoreplicates. Images were obtained in a Zeiss[®] microscope, Axio Scope A1 model, coupled to an AxioCam and the AxioVision software. For the cross-sections and SLA, we used 266 samples, while for the paradermal sections, we used 240 samples (see Table S1 and S2). The difference in the number of samples was due to the limitation of leaf material for some species.

To measure leaf pigments content (chlorophylls *a* and *b*, and carotenoids), 1 cm^2 leaf discs were obtained, which were immediately placed in amber tubes containing 2 ml of dimethylformamide (Porra et al. 1989). Then, the tubes were placed in a refrigerator ($\pm 10^\circ\text{C}$ in the dark) to extract leaf pigment for 48 hours (Wellburn, 1994). The chlorophylls and carotenoid concentration were determined according to the equations proposed by Wellburn (1994) after reading absorbance at 667, 647, and 480 nm in a Quimis spectrophotometer, model Q898-DRMS.

2.4 Photosynthetic responses

To verify the photosynthetic performance across treatments, we selected 11 ground-layer plants, which were representative and abundant species in the field experimental plots. The selected species were: *Andira humilis* Mart. ex Benth. (Fabaceae), *Banisteriopsis stellaris* (Griseb.) B.Gates (Malpighiaceae), *Byrsonima intermedia* A.Juss. (Malpighiaceae), *Campomanesia adamantium* (Cambess.) O.Berg (Myrtaceae), *Chromolaena oxylepis* (DC.) R.M.King & H.Rob. (Asteraceae), *Cerradicola decumbens* (Benth.) L.P.Queiroz (Fabaceae), *Mascagnia cordifolia* (A.Juss.) Griseb. (Malpighiaceae), *Miconia fallax* DC. (Melastomataceae), *Moquiniastrum barrosoae* (Cabrera) G. Sancho (Asteraceae), *Rhynchospora exaltata* Kunth (Cyperaceae) and *Serjania lethalis* A.St.-Hil. (Sapindaceae). However, for gas exchange analyses, the species *C. decumbens* and *M. barrosoae* were not found in sufficient numbers (a minimum of three individuals), and therefore, were not included.

For the photochemical parameters analysis, rapid light curves were done (White and Critchley, 1999) using MINI-PAM-II equipment, Walz, Effeltrich, Germany (Software WinControl 3.0). The measurements were taken between 8 and 12 a.m. Leaves were acclimated to the dark for a minimum of 30 minutes, using the aluminum clips provided with the MINI-PAM-II. Twelve light intensity levels ($0 - 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) were used to create the curve. The following parameters were obtained from the curve: α = quantum efficiency of photosynthesis, ETR_{max} = maximum electronic transport rate, and I_k = light saturation point of photosynthesis (Jassby and Platt, 1976).

Carbon assimilation (A_{max}), transpiration (E), and stomatal conductance (g_s) on a leaf area basis were assessed in the selected species with a LcProSD gas exchange analyzer (ADC BioScientific Ltd., Hoddesdon, UK). Data were obtained with ambient CO_2 concentration (404-434 ppm). The light intensity used was between 1,300 and 1,400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which is sufficient to saturate the photosynthetic apparatus of most ground-layer savanna species (Rossatto et al. 2018). Water use efficiency (WUE) was obtained by the ratio between A_{max} and g_s .

2.5 Statistical analysis

The data were tested for normality and homoscedasticity (Shapiro-Wilk and Levene tests, respectively). When necessary, data were transformed into $\log_{10}$ (spongy parenchyma, chlorophylls a , carotenoids, ETR_{max} , A_{max} , and g_s). For leaf morphoanatomical analyses

(tissue thickness, SLA, and stomatal parameters), the Cerrado ground-layer community identified and collected before (T_i) the simulation of woody encroachment conditions was considered as the control (CT). To compare the leaf traits across the different treatments (CT, S, L, and S+L), we performed a two-way ANOVA, using the most parsimonious models. Tukey's test was applied for multiple comparisons. We adopted a significance level of 5%. All analyses were performed using R software (version 4.3.0) (R Development Core Team, 2023).

3. Results

3.1 Morphoanatomical parameters and pigment concentration

The morphoanatomical traits and pigment concentration of the ground-layer community were mainly affected by shading (Table 1). Litter had minor effects on leaf traits (Table 1). Changes were noted in the community profile (Table S1 and S2) and leaf functional traits from the initial community (T_i - cerrado *sensu stricto*) to the community at the end of the experiment under woody encroachment conditions (Fig. 2-5). The number of species of the ground-layer community decreased from 30 at T_i to 22 at T_f in the shading treatment and from 32 at T_i to 22 at T_f in the shading plus litter treatment (Table S1 and S2).

Regarding leaf tissues thickness, the ground-layer community when exposed to shading (S and S+L) showed lower values for the epidermis of the adaxial surface (Fig. 2A). Despite showing the isolated effect of shading (Table 1), the thickness of the abaxial epidermis surface was not affected by any treatment (Fig. 2B; Table S3). In the photosynthetic tissues, the palisade parenchyma showed lower values in shading treatments (S and S+L) compared to unshading treatments (Fig. 2C), whereas the spongy parenchyma was not affected by any treatment (Fig. 2D). Total leaf thickness showed interaction between the factors (Table 1), and thinner leaves were found in the community after shading (S and S+L treatments) compared to the leaves of the cerrado *sensu stricto* community (before exposure to shading conditions, CT treatment) (Fig. 2E and Fig. 3). Higher values of SLA were observed under shading (S; Fig. 2F) and litter (L; Fig. 2F) compared to the leaves of the CT treatment; moreover, despite no interaction between the factors (Table 1), the community in the S+L treatment exhibited the highest SLA values among the treatments (Fig. 2F). Additionally, with the persistence and/or establishment of plastic species (i.e. *Banisteriopsis stellaris*, *Rhynchospora albiceps*, *Miconia albicans* and *Byrsonima intermedia*), it was possible to observe anatomical leaf changes before (T_i) and after 2.5 years (T_f) of simulation of shading encroachment conditions (Fig. 3).

Table 1. Results of two-way ANOVA for the effects of shading, litter, and their interaction on morphoanatomical traits and pigment concentration of the Cerrado ground-layer community. *F*: F statistic; *P*: significance level. n.s.: not significant.

Parameter	Shading		Litter		Shading x Litter	
	F	P	F	P	F	P
Adaxial surface epidermis	15.57	< 0.01	0.12	n.s.	0.72	n.s.
Abaxial surface epidermis	5.72	< 0.05	0.11	n.s.	2.38	n.s.
Palisade parenchyma	56.81	< 0.001	0.94	n.s.	2.28	n.s.
Spongy parenchyma	3.11	n.s.	3.71	n.s.	0.04	n.s.
Total leaf thickness	7.98	< 0.05	1.82	n.s.	5.59	< 0.05
Specific leaf area	39.13	< 0.0001	9.05	< 0.01	0.15	n.s.
Stomatal density	0.74	n.s.	2.89	n.s.	3.56	n.s.
Length of guard cell	0.71	n.s.	5.86	< 0.05	6.96	< 0.05
Stomatal equatorial diameter	0.80	n.s.	4.97	< 0.05	7.40	< 0.05
Chlorophyll <i>a</i> – Ca	45.93	< 0.001	1.77	n.s.	1.00	n.s.
Chlorophyll <i>b</i> – Cb	23.80	< 0.001	1.16	n.s.	0.04	n.s.
Total carotenoids	47.87	< 0.001	2.14	n.s.	0.23	n.s.
Ratio Ca: Cb	16.25	< 0.001	0.70	n.s.	1.24	n.s.

Stomatal density was not affected by any treatment (Fig. 4A). The length of guard cells and the equatorial diameter of stomata showed an interaction between the factors (Table 1). However, shorter and narrower stomata were observed only in the ground-layer community after the shading treatment (S; Fig. 4B and C). The concentrations of chlorophylls *a* and *b*, as well as carotenoids, were significantly higher in the ground-layer community under shading treatments (S and S+L), whereas the Ca: Cb ratio was lower under shading treatment (S; Fig. 5D).

3.2 Photosynthetic responses

Similar to the morphoanatomical parameters and pigment content, the photosynthetic responses were mainly affected by shading (Table 2). Species under shading treatments (S and S+L) did not achieve a photochemical performance like the control and exhibited lower values of α , I_k , and ETR_{max} (Fig. 6A-C). Gas exchange parameters followed the same pattern, with shading treatments (S and S+L) presenting lower values of A_{max} , g_s , and E than control (Fig 6D-E, E data not shown). The WUE was not affected by any treatment (Fig. 6F).

Table 2. Results of two-way ANOVA for the effects of shading, litter, and their interaction on photosynthetic responses of Cerrado ground-layer selected species. *F*: F statistic; *P*: significance level; n.s.: not significant; α = quantum efficiency of photosynthesis; ETR_{max} = maximum electron transport rate; I_k = light saturation point of photosynthesis; A_{max} = carbon assimilation; g_s = stomatal conductance; *E* = transpiration, WUE = Water use efficiency.

Parameter	Shading		Litter		Shading x Litter	
	F	P	F	P	F	P
α	35.65	< 0.001	0.72	n.s.	1.29	n.s.
ETR_{max}	41.21	< 0.001	5.65	< 0.05	4.54	< 0.05
I_k	12.00	< 0.01	0.82	n.s.	0.14	n.s.
A_{max}	231.24	< 0.001	0.93	n.s.	0.0014	n.s.
g_s	57.37	< 0.001	1.36	n.s.	0.339	n.s.
<i>E</i>	67.41	< 0.001	0.056	n.s.	0.0024	n.s.
WUE	0.19	n.s.	0.99	n.s.	0.08	n.s.

4. Discussion

Our expectations were confirmed since shading was the main factor driving plasticity and changes in the leaf functional responses of the savanna ground-layer community under simulated woody encroachment conditions. In contrast, litter had a minor influence on such traits. Leaf changes related to shading include reduced total leaf thickness and certain tissues thickness, such as epidermis and palisade parenchyma, higher SLA values, higher chlorophylls, and carotenoid concentration, and a reduction in the ratio of chlorophyll *a* and *b*. All these changes are linked to a greater tolerance to shading (Valladares and Niinemets, 2008). However, these adjustments were insufficient to sustain photosynthetic efficiency for both photosynthetic processes: photochemical and gas exchange; which may explain the loss of species and the shift in the ground-layer community in this new scenario.

The community under shading exhibited more mesomorphic traits, such as a thinner adaxial epidermis surface. Under a natural encroachment gradient, a similar pattern of mesomorphism was found in environments with higher tree cover in both non-arboreal plants (Pinheiro et al. 2018) and tree species (Ariano et al. 2022). The epidermis has some ecological functions, such as light interception and protection against high irradiance (Karabourniotis et al. 2021) that may explain the greater thickness of the surface epidermis of the ground-layer community exposed to high irradiances in control conditions. Furthermore, we found more developed palisade parenchyma in the ground-layer community in the control condition (cerrado *sensu stricto*). Sun leaves commonly present larger cells and/or many layers of palisade parenchyma (Rossatto and Kolb, 2010; Wyka et al. 2022). Columnar cells of this

photosynthetic tissue effectively distribute light to the chloroplasts (Vogelmann and Martin, 1993). Therefore, this xeromorphic pattern for sun leaves would be a way to obtain higher photosynthetic rates (Th  roux-Rancourt et al. 2023).

Under shading, thinner leaves have been observed in some tree species (Rossatto and Kolb, 2010; Mendon  a et al. 2020), palms (Barbosa et al. 2022), and non-arboreal plants (Pinheiro et al. 2018; Andrade et al. 2024) typical of the Cerrado. However, based on a larger number of species, our results showed that the ground-layer community of cerrado *sensu stricto* exhibits insufficient morphophysiological changes to tolerate imposed shading. When analyzing a set of 11 species, we observed that photosynthetic performance is highly impacted, which may explain the loss of species and the change in the community profile. The encroached community composed of shade-tolerant and generalist species, exhibited morphophysiological changes capable of improving the capture of diffuse light, such as lower values for total leaf thickness and higher chlorophyll contents (Valladares et al. 2002). Thinner leaves may reflect the reduced thickness of the adaxial epidermis surfaces, along with diminished development of the palisade parenchyma.

Additionally, the community under shading showed higher SLA values, which assists in the diffuse light absorption in forested physiognomies (Valladares and Niinemets, 2008). Conversely, under high light intensity, the cerrado *sensu stricto* community (control) exhibited lower SLA values, a characteristic related to water use efficiency (Craufurd et al. 1999; Hoffmann et al. 2005), indicating a conservative strategy in this environment (Maracahipes et al. 2018). Thus, the morphoanatomical profile, observed in the community after simulated encroachment conditions allows resilient and generalist species to exploit shaded environments (Freitas et al. 2022).

Nevertheless, the higher SLA values in the litter treatment can be caused by a higher leaf area or a lower leaf tissue density, as the community under this treatment showed no differences in the thickness of any tissues compared to the control community. Highly correlated with seasonality (Ribeiro et al. 2022), litter decomposition influences nutrient cycling (Tonello et al. 2022), potentially increasing the available carbon and phosphorus concentrations in the soil in the short term (Rossatto and Rigobelo, 2016). Thus, larger leaves or leaves with less dense tissue (Witkowski and Lamont, 1991) can be observed in scenarios with higher nutritional availability (Boeger and Wisniewski, 2003; Witkowski and Lamont, 1991).

Contrary to our expectations, the ground-layer community in shading conditions did not show differences in stomatal density. Nevertheless, the ground-layer community in

shading had stomata with smaller guard cells, both in length and width, compared to the control community (cerrado *sensu stricto*). In environments subject to seasonality or high light intensity, smaller stomata in higher densities can respond quickly to environmental changes (Drake et al. 2013; Nunes et al. 2022), ensuring a better response against water loss (Pireda et al. 2019), which is considered a xeromorphic trait (Shields, 1950). However, smaller stomata, not accompanied by higher densities, may contribute to the low stomatal conductance and photosynthetic responses reported here for the species under shading conditions.

Regarding physiological parameters, higher chlorophyll concentrations were observed under shading conditions. High chlorophyll contents are widely recognized as a strategy for optimizing light use where light is a limited resource (Valladares and Niinemets, 2008). Leaf nitrogen allocation between the production of photosynthetic pigments and enzymes involved in the photosynthetic biochemical phase represents a trade-off between achieving high photosynthetic rates under abundant light and enhancing performance under low light conditions by reducing the light compensation point (Henry and Aarssen, 1997; Walters and Reich, 1996). In fact, despite possessing higher chlorophyll content, the study ground-layer community did not achieve high carbon assimilation rates when exposed to shading conditions. The increased carotenoid content in response to shading could also be interpreted as a mechanism for enhancing light capture, as carotenoids serve as accessory pigments (Havaux et al. 1998; Valladares et al. 2000).

Despite undergoing morphophysiological changes in response to shading, plants under these conditions could not achieve a photochemical performance similar to plants in control. Decreases in the photosynthesis quantum yield, electron transport rate, and minimum irradiance for saturation in plants subjected to shading treatments underscore this shortfall. Ground-layer species in forested savanna exhibit leaves with higher values of SLA, and chlorophyll *b*, but lower photosynthetic responses (Rossatto et al. 2018) when compared with typical savanna - a pattern also observed in our results. Despite these physiological plasticity responses, these decreases may have contributed to the low carbon assimilation in these plants, potentially jeopardizing their carbon balance (Rossatto et al. 2018).

Although some species can reduce their light compensation point as a trade-off (Kitajima, 1994), typical savanna species may not achieve their light compensation points in forested environments (Rossatto et al. 2018). Two of the 11 species selected for photosynthetic responses (*Cerradicola decumbens* and *Moquiniastrum barrosoae* - both from savanna physiognomies) were excluded from the gas exchange analysis, as they exhibited

aboveground biomass loss under shaded treatment. This aerial loss may be related to the limitations of the photosynthetic apparatus in forested environments. Under shading, ground-layer species may allocate a smaller proportion of assimilates to belowground biomass, prioritizing the allocation to the formation of photosynthetic branches (Solofondranohatra et al. 2021). Furthermore, acquisitive resource-use strategies, such as investment in leaf area and photosynthetic pigment content, may contribute to the carbon reserve exhaustion maintained by lower carbon assimilation rates (Kitajima and Myers, 2008). Nevertheless, studies at the plant level are necessary for a better understanding of reserve allocation and carbon balance.

In a study conducted in encroached wetland areas of the Cerrado, it was reported that ground-layer communities are strongly affected by woody encroachment, leading to species selection and converging into a community characterized by more acquisitive leaf traits (Trindade et al. 2024). This can potentially reduce the functional diversity of the initial community (Amaral et al. 2021). In fact, the floristic composition before and after simulated woody shading conditions reveals not only the elimination of species typically found in grasslands and savannas, e.g. *Axonopus pressus* (graminoid), *Peltaea polymorpha* (forb), and *Lepidaploa chamissonis* (shrub), but also their replacement by generalist or typically forest species, e.g. *Erythroxylum cuneifolium* (shrub), *Mascagnia cordifolia* (climber), and *Psychotria hoffmannseggiana* (shrub) – see Table S1 and S2. The negative impacts, in particular, on the ground-layer species in the community, showed a reduction of about 26% and 31% for the shading and shading plus litter treatment, respectively. Therefore, our findings align with other studies that indicate that typical ground-layer Cerrado plants are rarely encountered in areas that have undergone woody encroachment (Abreu et al. 2017; Pinheiro et al. 2016) and that they tend to disappear rapidly under field experimental shading (Pinheiro et al. 2022), thus more attention should be given on restoration processes of encroached areas, taken into account these morphophysiological responses (Carlucci et al. 2020).

5. Conclusion

In environmental changes caused by encroachment, especially for light availability, shading emerges as the main filter for selecting species and leaf functional traits. After shading conditions, the ground-layer community exhibits leaf traits characteristics of acquisitive resource-use strategies, such as higher SLA and photosynthetic pigments content, as well as lower photosynthetic responses that contribute, at least in the short term, to their

persistence under woody encroachment. However, these adjustments are insufficient to maintain the carbon balance and species diversity in the ground-layer community of cerrado *sensu stricto* in encroached areas.

CRedit authorship contribution statement

Pedro F. Cruz Júnior: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Luiz F. S. Pinheiro:** Conceptualization, Resources, Investigation, Methodology, Writing – review & editing. **Davi R. Rossatto:** Conceptualization, Methodology, Writing – review & editing. **Rosana M. Kolb:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. Supplementary materials

Table S1. Number of individuals per species across different treatments at the beginning of the experiment and after 2.5 years used in cross-sectional and specific area analyses.

Table S2. Number of individuals per species across different treatments at the beginning of the experiment and after 2.5 years used in paradermal section analyses.

Table S3. *Post-hoc* contrasts significance values for the interaction between treatments for the leaf functional traits of ground-layer community of Cerrado

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Figure and figure captions

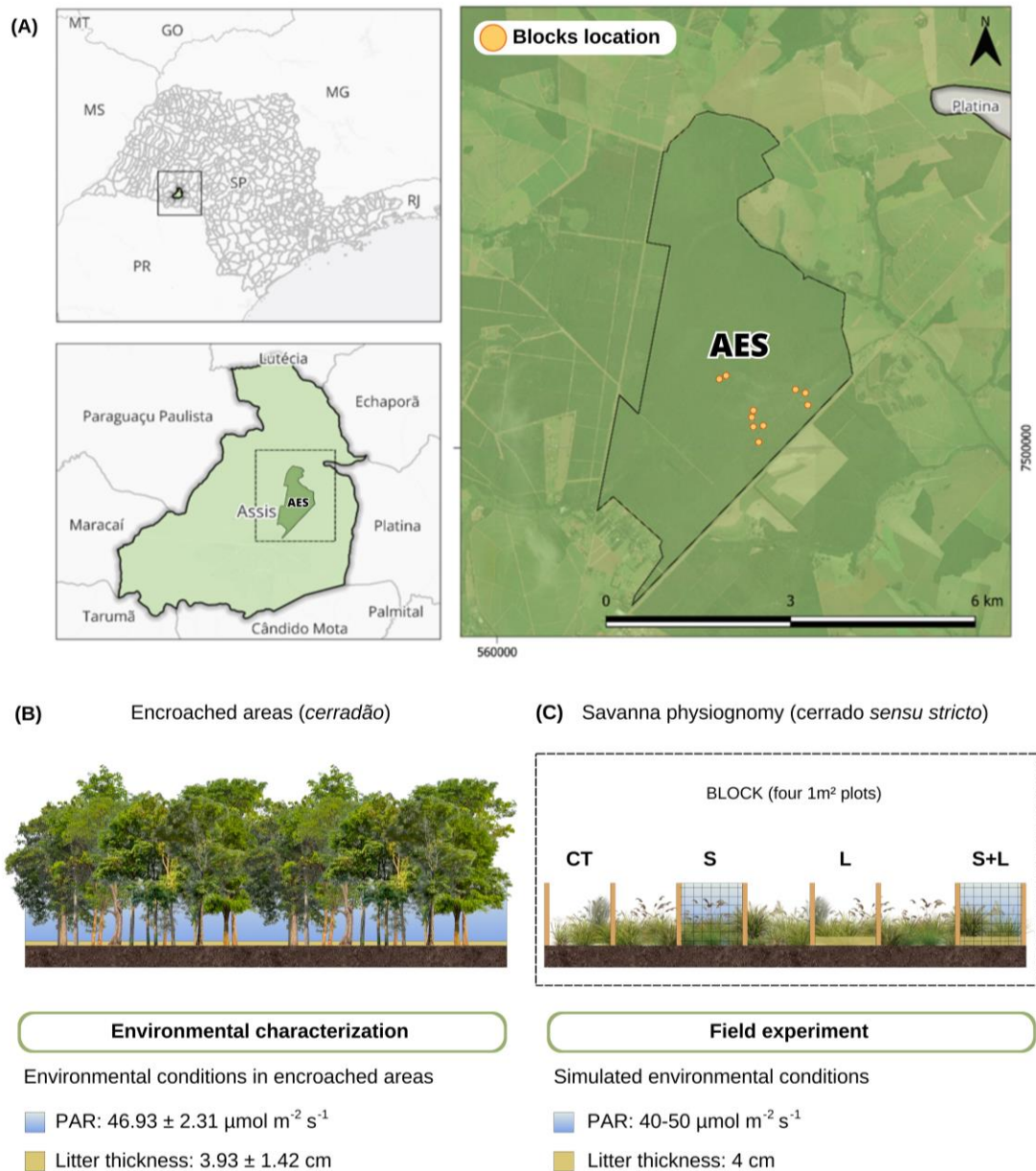


Fig. 1. Study area (A) and design of the simulation experiment for decreased light and litter accumulation conditions (encroached conditions - B) in a remnant *cerrado sensu stricto* area (C). AES: Assis Ecological Station; CT: control treatment (no encroached conditions); L: litter treatment; S: shading treatment; S+L: shading plus litter treatment; PAR: photosynthetically active radiation.

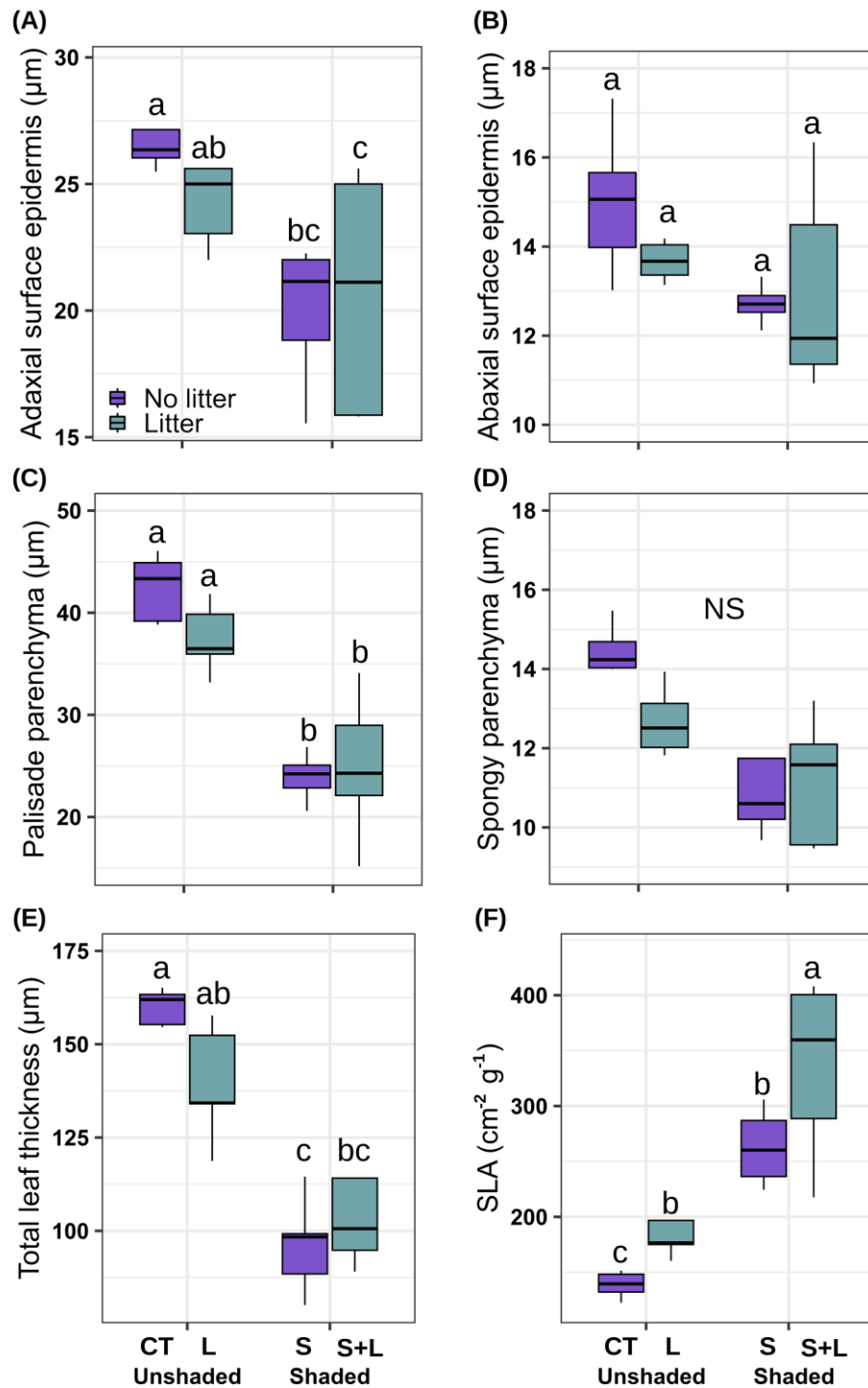


Fig. 2. Comparative of the morphoanatomical traits of Cerrado ground-layer community before (CT – cerrado *sensu stricto*) and after 2.5 years of simulation of woody encroachment shading (S), litter (L), and shading plus litter (S+L) conditions. Boxplots represent the values of the lower and upper limits, medians and quartiles ($n=5$). Distinct letters represent statistical differences according to Tukey's test ($P < 0.05$). SLA: specific leaf area, NS: not significant.

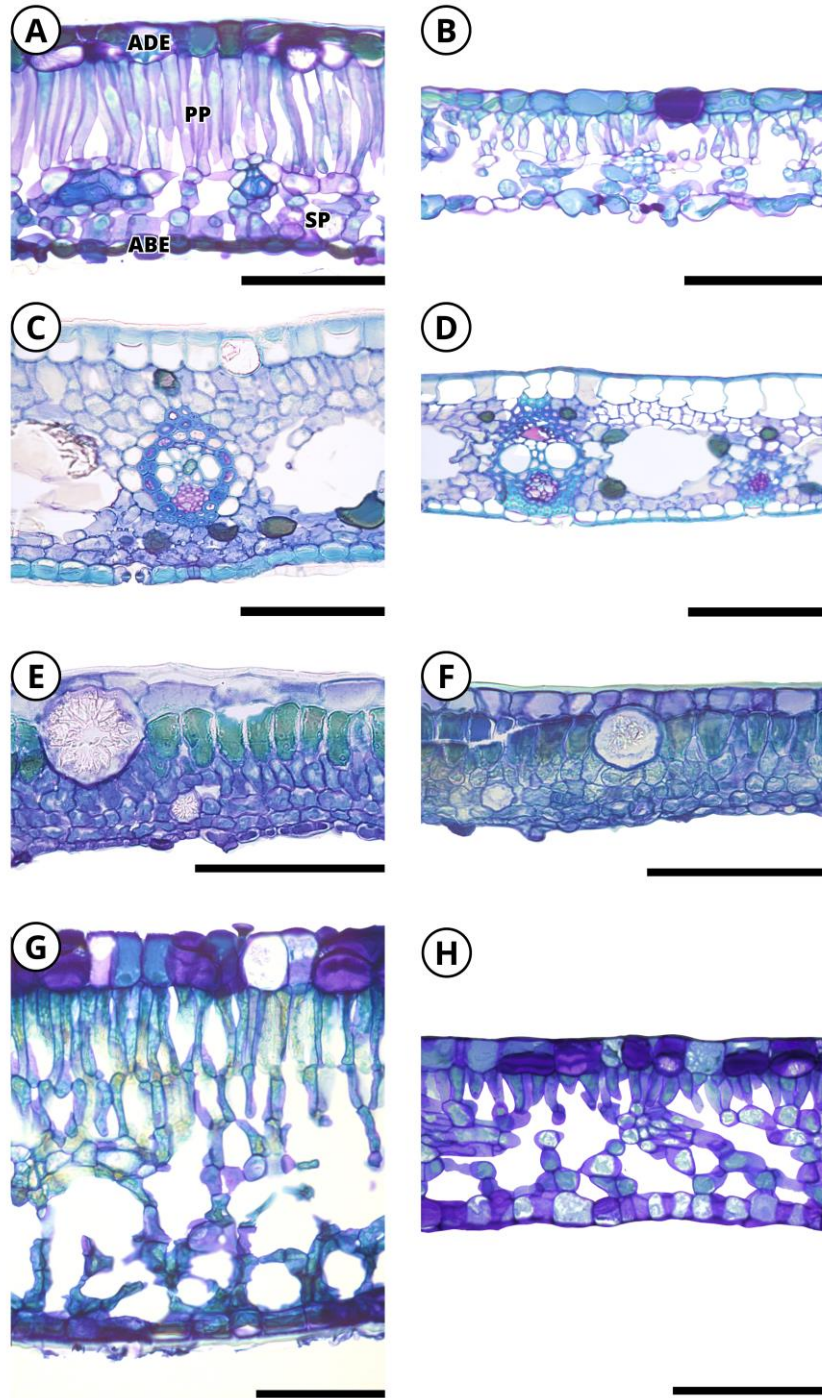


Fig. 3. Cross sections of the leaf blade of plastic species from the Cerrado before (T_i = A, C, E, and G) and after 2.5 years (T_f) of simulation of woody encroachment shade (B, D, F, H). A and B: *Banisteriopsis stellaris*; C and D: *Rhynchospora exaltata*; E and F: *Miconia albicans*; G and H: *Byrsonima intermedia*. ABE: abaxial epidermis; ADE: adaxial epidermis; PP: palisade parenchyma; SP: spongy parenchyma. Scale bar: 100 μm .

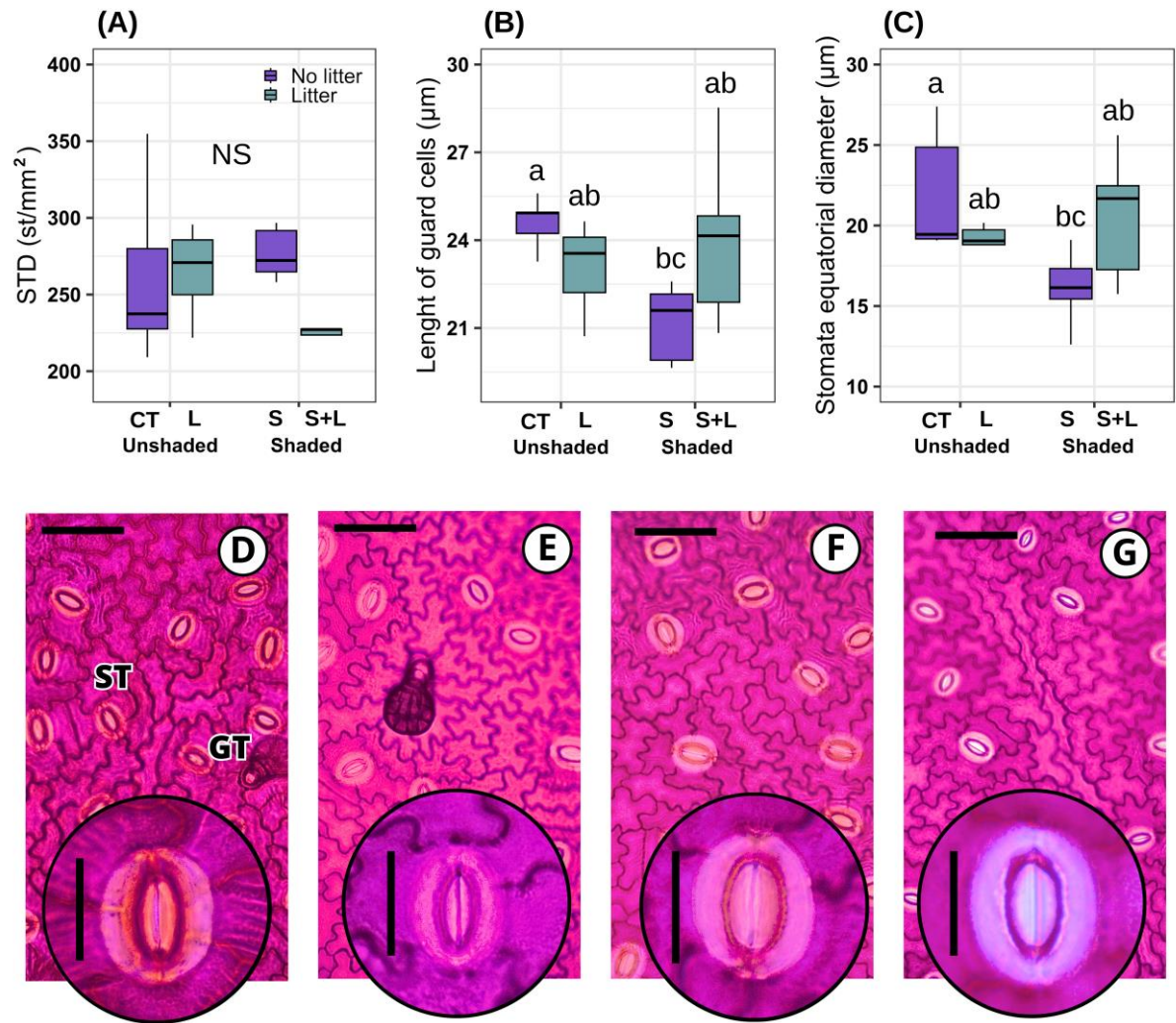


Fig. 4. Comparative of the stomatal parameters (A-C) of Cerrado ground-layer community before (CT, cerrado *sensu stricto* - D) and after 2.5 years of simulation of woody encroachment shading (S - E), litter (L - F), and shading plus litter (S+L - G) conditions. Boxplots represent the values of the lower and upper limits, medians and quartiles (n=5). Distinct letters represent statistical differences according to Tukey's test ($P < 0.05$). GT: Glandular trichome; STD: Stomatal density; ST: Stomata, NS: not significant. Scale bar: 100 µm for the general view and 20 µm for the detail of the stomata. D-G: paradermic sections of *Serjania lethalis*.

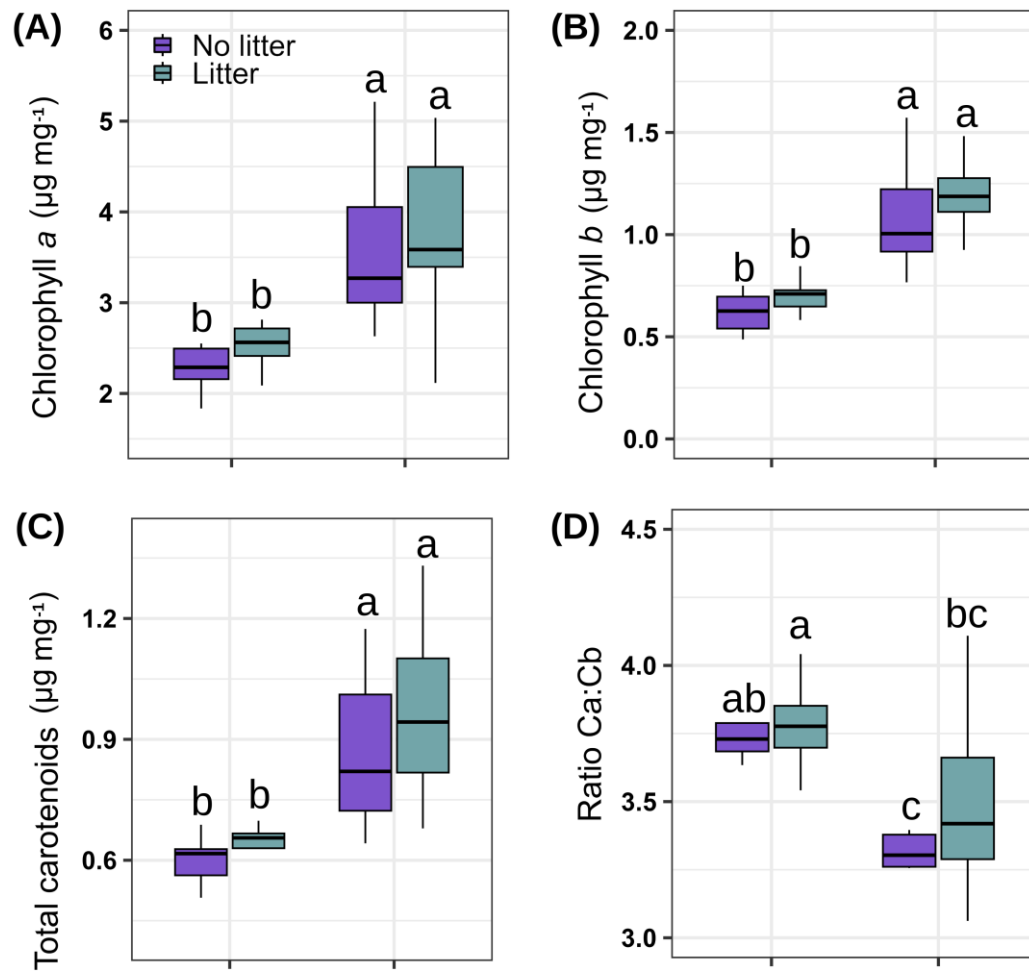


Fig. 5. Comparative of the chlorophyll and carotenoid concentration of Cerrado ground-layer before (CT, cerrado *sensu stricto*) and after 2.5 years of simulation of woody encroachment shading conditions. Boxplots represent the values of the lower and upper limits, medians and quartiles ($n=10$). Distinct letters represent statistical differences according to Tukey's test ($P < 0.05$).

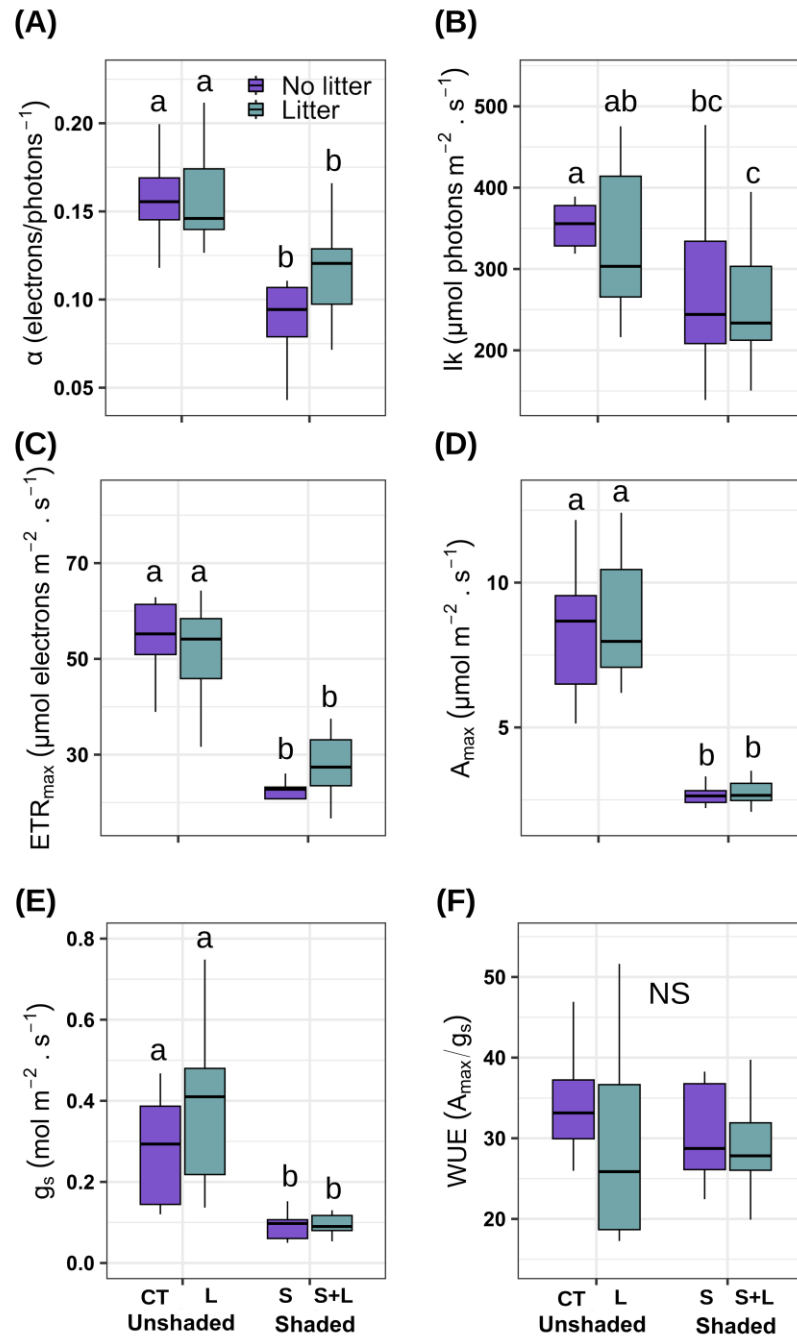


Fig. 6. Comparative of chlorophyll fluorescence and carbon assimilation traits of Cerrado ground-layer selected species under 2.5 years of control (CT) and simulation of woody encroachment shading (S), litter (L), and shade plus litter (S+L) conditions. Boxplots represent the values of the lower and upper limits, medians and quartiles (n=9-11). Different letters represent significant differences according Tukey's test ($P < 0.05$). Legend: α = quantum efficiency of photosynthesis; $ETR_{\max}$ = maximum electron transport rate; I_k = light saturation point of photosynthesis; $A_{\max}$ = carbon assimilation; g_s = stomatal conductance; WUE: water use efficiency.

Woody encroachment and leaf functional traits of ground-layer savanna species

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Supplementary material

Table S1. Number of individuals per species across different treatments at the beginning of the experiment (T_i) and after 2.5 years (T_f) used in cross-sectional and specific area analyses. L: litter treatment plots; GF: growth form; PSG: physiognomies of species occurrence; S: shading treatment plots; S+L: shading plus litter treatment plots.

Family/Species	GF	PSG	S		L		S+L	
			T _i	T _f	T _i	T _f	T _i	T _f
ANACARDIACEAE								
<i>Anacardium humile</i> A.St.-Hil.	subshrub	grasslands/savanna	1	0	0	0	0	0
ANNONACEAE								
<i>Annona coriacea</i> Mart.	shrub	grasslands	0	1	3	1	1	1
<i>Duguetia furfuracea</i> (A.St.-Hil.) Saff.	shrub	grasslands/savanna	0	0	0	0	1	1
APOCYNACEAE								
<i>Blepharodon bicuspidatum</i> E.Fourn.	climber	savanna/forest	1	0	0	1	0	0
<i>Forsteronia pubescens</i> A.DC.	climber	grasslands/savanna/forest	0	0	0	1	0	0
<i>Temnadenia violacea</i> (Vell.) Miers	climber	grasslands/savanna	0	0	0	1	0	0
ASTERACEAE								
<i>Baccharis crispa</i> Spreng.	forb	grasslands	1	0	0	1	1	0
<i>Bidens segetum</i> Mart. ex Colla	subshrub	savanna/forest	0	0	0	1	0	0

<i>Chromolaena oxylepis</i> (DC.) R.M.King & H.Rob.	shrub	grasslands/savanna	2	0	1	0	2	0
<i>Chrysolaena obovata</i> (Less.) Dematt.	forb	grasslands/savanna	0	0	0	1	0	0
<i>Lepidaploa chamissonis</i> (Less.) H.Rob.	shrub	grasslands/savanna	0	0	0	0	2	0
<i>Lessingianthus bardanoides</i> (Less.) H.Rob.	shrub	grasslands/savanna	0	0	1	1	0	0
<i>Moquiniastrum barrosoae</i> (Cabrera) G. Sancho	shrub	grasslands/savanna	1	1	4	3	1	0
BIGNONIACEAE								
<i>Anemopaegma arvense</i> (Vell.) Stellfeld ex de Souza	subshrub	grasslands/savanna	1	1	0	0	0	0
<i>Cuspidaria pulchella</i> (Cham.) K.Schum.	climber	forest	3	0	1	0	2	0
COMMELINACEAE								
<i>Commelina erecta</i> L.	forb	grasslands/savanna	0	0	0	0	1	1
CYPERACEAE								
<i>Fimbristylis</i> sp.	graminoid	-	0	0	0	1	0	0
<i>Rhynchospora exaltata</i> Kunth	graminoid	forest	3	3	5	5	5	5
DILLENIACEAE								
<i>Davilla elliptica</i> A.St.-Hil.	shrub	grasslands/savanna/forest	0	0	0	1	1	0
ERYTHROXYLACEAE								
<i>Erythroxylum campestre</i> S.St.-Hil.	shrub	grasslands/savanna	1	0	2	2	1	0
<i>Erythroxylum cuneifolium</i> (Mart.) O.E.Schulz	shrub	grasslands/savanna/forest	1	3	1	3	1	0
<i>Erythroxylum pelleterianum</i> A.St.-Hil.	shrub	grasslands/savanna/forest	0	0	1	0	0	0
EUPHORBIACEAE								
<i>Microstachys hispida</i> (Mart. & Zucc.) Govaerts	forb	grasslands/savanna/forest	0	0	0	0	1	0
FABACEAE								
<i>Andira humilis</i> Mart. ex Benth.	subshrub	grasslands/savanna	1	1	1	1	1	1

<i>Chamaecrista desvauxii</i> (Collad.) Killip var. <i>desvauxii</i>	forb/subshrub	grasslands/savanna	0	0	1	2	0	0
<i>Chamaecrista flexuosa</i> (L.) Greene	forb/subshrub	grasslands/savanna	0	0	0	1	1	0
<i>Eriosema campestre</i> Benth.	subshrub	grasslands	1	0	0	0	0	0
Fabaceae sp.	subshrub	-	2	0	2	0	1	0
<i>Cerradicola decumbens</i> (Benth.) L.P.Queiroz	subshrub	grasslands/savanna	0	0	0	2	0	0
<i>Mimosa xanthocentra</i> Mart.	subshrub	grasslands/savanna	0	0	1	1	0	0
<i>Senna rugosa</i> (G.Don) H.S.Irwin & Barneby	shrub	grasslands/savanna/forest	1	0	0	0	0	0
LAMIACEAE								
<i>Hyptis campestris</i> Harley & J.F.B. Pastore	forb	grasslands/savanna	3	0	2	3	3	0
MALPIGHIACEAE								
<i>Banisteriopsis stellaris</i> (Griseb.) B.Gates	shrub/climber	grasslands/savanna	2	4	4	5	2	1
<i>Banisteriopsis variabilis</i> B.Gates	climber	savanna/forest	0	0	0	1	0	0
<i>Byrsonima intermedia</i> A.Juss.	shrub	grasslands/savanna	2	2	5	5	5	0
<i>Mascagnia cordifolia</i> (A.Juss.) Griseb.	climber	forest	2	2	2	3	2	3
MALVACEAE								
<i>Pelteia polymorpha</i> (A.St.-Hil.) Krapov. & Cristóbal	forb	grasslands/savanna	1	0	1	1	2	0
MELASTOMATACEAE								
<i>Leandra aurea</i> (Cham.) Cogn	shrub	grasslands/gallery forest	0	0	1	1	0	1
<i>Miconia albicans</i> (Sw.) Triana	shrub	grasslands/savanna/forest	0	1	2	2	1	0
<i>Miconia fallax</i> DC.	shrub	grasslands/savanna	3	1	1	3	3	0
<i>Miconia paucidens</i> DC.	shrub	forest	0	1	0	0	0	0
MENISPERMACEAE								
<i>Cissampelos ovalifolia</i> DC.	subshrub	grasslands/savanna	1	0	0	0	1	1
MYRTACEAE								

<i>Campomanesia adamantium</i> (Cambess.) O.Berg	shrub	grasslands/savanna	2	1	1	2	1	2
<i>Eugenia anomala</i> D.Legrand	subshrub	grasslands/savanna	0	0	0	1	0	0
<i>Eugenia dysenterica</i> (Mart.) DC.	shurb	grasslands/savanna	0	0	0	1	0	0
<i>Myrcia bella</i> Cambess.	shurb	grasslands/savanna/forest	0	0	0	1	0	0
POACEAE								
<i>Axonopus pressus</i> (Nees ex Steud.) Parodi	graminoid	grasslands/savanna	3	0	3	3	2	0
<i>Panicum sellowii</i> Nees	graminoid	forest	0	0	0	0	0	1
<i>Urochloa decumbens</i> (Stapf) R.D.Webster	graminoid	grasslands/savanna/forest	0	0	0	1	0	0
RUBIACEAE								
<i>Borreria verticillata</i> (L.) G.Mey	subshrub	grasslands/savanna	0	0	1	1	1	0
<i>Palicourea rigida</i> Kunth	shrub	grasslands/savanna	0	0	1	1	0	0
<i>Psychotria hoffmannseggiana</i> (Willd. ex Schult.) Müll.Arg.	shrub	forest	0	1	0	1	0	1
SAPINDACEAE								
<i>Serjania lethalis</i> A.St.-Hil.	climber	forest	1	1	1	2	3	1
SMILACACEAE								
<i>Smilax campestris</i> Griseb	climber	grasslands/savanna	0	0	2	2	2	1
VERBENACEAE								
<i>Lippia origanoides</i> Kunth	subshrub	grasslands/savanna	3	0	1	3	2	0

Table S2. Number of individuals per species across different treatments at the beginning of the experiment (T_i) and after 2.5 years (T_f) used in paradermal section analyses. L: litter treatment plots; GF: growth form; PSG: physiognomies of species occurrence; S: shading treatment plots; S+L: shading plus litter treatment plots.

Family/Species	GF	PSG	S		L		S+L	
			T _i	T _f	T _i	T _f	T _i	T _f
ANNONACEAE								
<i>Annona coriacea</i> Mart.	shrub	grasslands	1	0	1	0	0	0
<i>Duguetia furfuracea</i> (A.St.-Hil.) Saff.	shrub	grasslands/savanna	0	0	0	0	0	1
APOCYNACEAE								
<i>Forsteronia pubescens</i> A.DC.	climber	grasslands/savanna/forest	0	0	0	1	0	0
<i>Temnadenia violacea</i> (Vell.) Miers	climber	grasslands/savanna	0	0	0	1	0	0
ASTERACEAE								
<i>Baccharis crispa</i> Spreng.	forb	grasslands	0	1	1	0	0	0
<i>Chromolaena oxylepis</i> (DC.) R.M.King & H.Rob.	shrub	grasslands/savanna	2	1	1	3	1	1
<i>Chrysolaena obovata</i> (Less.) Dematt.	forb	grasslands/savanna	0	0	0	1	0	0
<i>Lepidaploa chamissonis</i> (Less.) H.Rob.	shrub	grasslands/savanna	1	0	0	2	1	0
<i>Lessingianthus bardanoides</i> (Less.) H.Rob.	shrub	grasslands/savanna	0	0	0	1	0	0
<i>Moquiniastrum barrosoae</i> (Cabrera) G. Sancho	shrub	grasslands/savanna	0	0	2	1	1	1
BIGNONIACEAE								
<i>Anemopaegma arvense</i> (Vell.) Stellfeld ex de Souza	subshrub	grasslands/savanna	1	0	0	0	0	0
<i>Cuspidaria pulchella</i> (Cham.) K.Schum.	climber	forest	3	0	0	3	2	0
CYPERACEAE								
<i>Rhynchospora exaltata</i> Kunth	graminoid	forest	2	3	4	3	3	2
DILLENIACEAE								
<i>Davilla elliptica</i> A.St.-Hil.	shrub	grasslands/savanna/forest	0	0	0	1	0	1

ERYTHROXYLACEAE

<i>Erythroxylum campestre</i> S.St.-Hil.	shrub	grasslands/savanna	0	0	2	4	1	0
<i>Erythroxylum cuneifolium</i> (Mart.) O.E.Schulz	shrub	grasslands/savanna/forest	0	1	0	1	2	1
<i>Erythroxylum pelleterianum</i> A.St.-Hil.	shrub	grasslands/savanna/forest	0	0	1	0	0	0

EUPHORBIACEAE

<i>Microstachys hispida</i> (Mart. & Zucc.) Govaerts	forb	grasslands/savanna/forest	0	0	1	0	0	0
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FABACEAE

<i>Andira humilis</i> Mart. ex Benth.	subshrub	grasslands/savanna	1	2	2	1	0	1
<i>Chamaecrista desvauxii</i> (Collad.) Killip var. <i>desvauxii</i>	forb/subshrub	grasslands/savanna	0	0	1	3	0	0
<i>Chamaecrista flexuosa</i> (L.) Greene	forb/subshrub	grasslands/savanna	0	0	0	1	0	0
<i>Eriosema campestre</i> Benth.	subshrub	grasslands	1	0	0	1	0	0
Fabaceae sp.	subshrub	-	1	0	0	0	0	0
<i>Cerradicola decumbens</i> (Benth.) L.P.Queiroz	subshrub	grasslands/savanna	0	0	0	3	0	1
<i>Senna rugosa</i> (G.Don) H.S.Irwin & Barneby	shrub	grasslands/savanna/forest	0	1	0	0	0	0

LAMIACEAE

<i>Hyptis campestris</i> Harley & J.F.B. Pastore	forb	grasslands/savanna	2	2	1	4	0	0
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MALPIGHIACEAE

<i>Banisteriopsis stellaris</i> (Griseb.) B.Gates	shrub/climber	grasslands/savanna	2	2	4	3	2	2
<i>Byrsonima intermedia</i> A.Juss.	shrub	grasslands/savanna	4	2	5	5	4	2
<i>Mascagnia cordifolia</i> (A.Juss.) Griseb.	climber	forest	3	3	2	2	3	3

MALVACEAE

<i>Peltaea polymorpha</i> (A.St.-Hil.) Krapov. & Cristóbal	forb	grasslands/savanna	1	0	1	1	2	0
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MELASTOMATACEAE

<i>Leandra aurea</i> (Cham.) Cogn	shrub	grasslands/gallery forest	0	0	0	0	0	1
<i>Miconia albicans</i> (Sw.) Triana	shrub	grasslands/savanna/forest	1	3	0	1	2	1
<i>Miconia fallax</i> DC.	shrub	grasslands/savanna	1	1	3	3	1	1

MENISPERMACEAE

<i>Cissampelos ovalifolia</i> DC.	subshrub	grasslands/savanna	0	0	1	1	1	2
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MYRTACEAE

<i>Campomanesia adamantium</i> (Cambess.) O.Berg	shrub	grasslands/savanna	0	1	0	4	2	0
<i>Eugenia anomala</i> D.Legrand	subshrub	grasslands/savanna	0	0	0	1	0	0
<i>Eugenia dysenterica</i> (Mart.) DC.	shrub	grasslands/savanna	1	0	0	0	0	0
Myrtaceae sp.	-	-	0	0	1	0	0	0

ORCHIDACEAE

<i>Epidendrum secundum</i> Jacq.	forb	grasslands/savanna	0	0	1	0	0	0
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POACEAE

<i>Axonopus pressus</i> (Nees ex Steud.) Parodi	graminoid	grasslands/savanna	2	0	1	5	4	0
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RUBIACEAE

<i>Borreria verticillata</i> (L.) G.Mey	subshrub	grasslands/savanna	0	0	1	0	1	0
<i>Palicourea rigida</i> Kunth	shrub	grasslands/savanna	1	0	1	0	0	0
<i>Psychotria hoffmannseggiana</i> (Willd. ex Schult.) Müll.Arg.	shrub	forest	0	1	0	1	0	1

SAPINDACEAE

<i>Serjania lethalis</i> A.St.-Hil.	climber	forest	2	2	1	4	1	1
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SAPOTACEAE

<i>Pradosia brevipes</i> (Pierre) T.D. Penn.	subshrub	grasslands/savanna	0	1	0	0	0	0
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SMILACACEAE

<i>Smilax campestris</i> Griseb	climber	grasslands/savanna	0	0	1	3	2	3
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VERBENACEAE

<i>Lippia origanoides</i> Kunth	subshrub	grasslands/savanna	2	1	0	2	2	0
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<i>Lippia lupulina</i> Cham.	subshrub	grasslands/savanna	0	0	0	0	1	0
INDETERMINATE								
Indeterminate	-	-	0	1	0	0	0	0

Table S3. *Post-hoc* contrasts significance values (*P*-values according to Tukey's test) for the interaction between treatments (CT, S, L and S+L) for the leaf functional traits of ground-layer community of Cerrado. CT = control; L = litter; S = shading; S+L = shading plus litter; -: result of two-way ANOVA not significant.

Parameter	CT-S	CT-L	CT – S+L	S - L	S – S+L	L – S+L
Adaxial surface epidermis	0.0052	0.9856	0.0342	0.0877	0.9856	0.0052
Abaxial surface epidermis	0.9872	0.1166	0.2548	0.4827	0.1166	0.9872
Palisade parenchyma	< 0.0001	0.7690	0.0001	0.0012	0.7690	< 0.0001
Spongy parenchyma	-	-	-	-	-	-
Total leaf thickness	0.2313	0.0001	0.0010	0.0036	0.0534	0.5451
Specific leaf area	0.0360	< 0.0001	< 0.0001	0.1382	< 0.0001	0.0360
Stomatal density	-	-	-	-	-	-
Length of guard cell	0.0475	0.5694	0.9654	0.4187	0.1129	0.8324
Stomatal equatorial diameter	0.0420	0.3963	0.8866	0.5568	0.1576	0.8068
Chlorophyll a (Ca)	< 0.0001	0.5487	< 0.0001	0.0024	0.5487	< 0.0001
Chlorophyll b (Cb)	< 0.0001	0.2942	< 0.0001	0.0004	0.2942	< 0.0001
Total carotenoids	< 0.0001	0.4679	< 0.0001	0.0024	0.4679	< 0.0001
Ratio Ca: Cb	0.0015	0.8367	0.1345	0.0068	0.8367	0.0015
Quantum efficiency of photosynthesis	< 0.0001	0.8320	0.0042	0.0001	0.8320	< 0.0001
Maximum electron transport rate	< 0.0001	0.9190	< 0.0001	< 0.0001	0.0978	< 0.0001
Light saturation point of photosynthesis	0.0066	0.8012	0.0181	0.2841	0.8012	0.0066
Carbon assimilation	< 0.0001	0.7696	< 0.0001	< 0.0001	0.7696	< 0.0001
Stomatal conductance	< 0.0001	0.6505	0.0004	< 0.0001	0.6505	< 0.0001
Transpiration	< 0.0001	0.9952	< 0.0001	< 0.0001	0.9952	< 0.0001
Water use efficiency	-	-	-	-	-	-

**CAPÍTULO 2. Combining the effects of water stress and smoke on seed
recruitment**

SERÁ SUBMETIDO NO PERIÓDICO ANNALS OF BOTANY

ABSTRACT

Background and Aims

In savanna ecosystems, water availability and pyrodiversity shape plant structure and diversity. In the Brazilian savanna (Cerrado), factors such as water seasonality and fire cues, like smoke, interact and influence species recruitment. This study investigates the germinative and post-germinative responses of Cerrado ground-layer species to a gradient of water potential reduction, both independently and in combination with liquid smoke. It assesses the effects of these factors on treatment and post-stress recovery, while exploring the potential protective role of smoke under stress conditions.

Methods

Different concentrations of polyethylene glycol 6000, both isolated and in combination with varying concentrations of smoke water (1% and 2.5%), were used to assess the responses of 15 Cerrado ground-layer species in germination chambers (25°C). Effect sizes were used to analyze the data.

Key Results

The reduction of water potential was the primary factor negatively impacting both germination and post-germination responses. While species-specific, smoke had a protective effect on seed germination and early seedling development under moderate water deficit. During recovery, most species were able to recover from stressful conditions or maintain seed viability.

Conclusions

Both water potential reduction and smoke influence the germination and early development of ground-layer species. Results show that the same factor (e.g., smoke) can have varying effects depending on the developmental stage. In all cases, species demonstrated tolerance to stressful conditions without loss of seed viability, reducing and arresting germination during water deficiency, and also being capable of entering secondary dormancy after stress.

Keywords: Savanna, germination, seedling formation, abiotic stress, recovery, fire cues, secondary dormancy.

Introduction

In tropical savannas, plant establishment and distribution are strongly influenced by the interaction between climatic factors and disturbances such as drought and fire (Bond, 2008; Holdo and Nippert, 2022). These ecosystems are shaped by seasonal rainfall patterns and pyrodiversity (Lehmann *et al.* 2011), creating challenging conditions for seed germination and establishment (Hoffmann *et al.* 2004). Among these, water deficit and fire cues, such as smoke, can play a critical role in regulating germination (Souza *et al.* 2022; Motta *et al.* 2024). However, germination responses to the interaction between drought stress and fire cues remain poorly understood, particularly for ground-layer plant species, which have been much less studied. Understanding how these factors influence germination dynamics is crucial to unraveling the mechanisms driving plant establishment under the current and future climates of savanna ecosystems.

Water availability is a major limiting factor for seed germination and seedling establishment (Baskin and Baskin, 2014). Drought directly compromises germination by reducing germinability, prolonging the mean germination time (Ribeiro and Kolb, 2016), and even decreasing seed viability (Bhatt *et al.* 2022; Souza *et al.* 2022). In seasonal environments, many species disperse their seeds at the end of the rainy season, and seed traits that enhance resilience to seasonal drought have evolved (Silveira *et al.* 2011). Such as seed dormancy during dispersal (Ramos *et al.* 2017; Escobar *et al.* 2018), which prevents uncontrolled germination during seasonal transitions (Escobar *et al.* 2021). Given that the seed-to-seedling transition is intrinsically linked to seasonality (Escobar *et al.* 2018), projected droughts in tropical savannas are likely to impact recruitment dynamics in dry and wet ecosystems (Souza *et al.* 2022). In Neotropical savannas, the predicted shifts in hydrological cycles and rising temperatures over the coming decades (Alves *et al.* 2021) are already being observed, with reductions in rainfall regimes (Hofmann *et al.* 2023) and groundwater recharge rates (Ferraz *et al.* 2024), because of the most severe droughts recorded in the last 700 years (Stríkis *et al.* 2024).

Simultaneously, pyrodiversity plays a pivotal role in species survival, reproduction, and recruitment in fire-prone environments (Pausas and Keeley, 2023; Kobziar *et al.* 2024). Fire, which has been integral to the evolutionary history of savannas for over 10 million years (Simon *et al.* 2009), has shaped plant communities

with distinct adaptive mechanisms, such as underground storage structures (Leal *et al.* 2024), soluble carbohydrate storage (e.g. fructans) (Moraes *et al.* 2016), and protected buds (Cozin *et al.* 2024), enabling rapid resprouting after fires. Other adaptations include accelerated flowering post-fire (Pilon *et al.* 2018; Fidelis and Zironi, 2021; Fidelis *et al.* 2022) and the breaking of physiological seed dormancy triggered by temperature fluctuations (19–55°C, simulating fire opening gaps; Dairel and Fidelis, 2020) or exposure to smoke (Ramos *et al.* 2019; Zironi *et al.* 2019; Motta *et al.* 2024).

Since the 1990s, smoke and its organic compounds have been widely studied for commercial (De Lange and Boucher, 1990; Kępczyński and Kępczyńska, 2023), conservation and restoration (Hodges *et al.* 2022), and ecophysiological purposes (Motta *et al.* 2024). This fire cue, derived from the combustion of plant material, contains over 70 organic and inorganic substances (Baldwin *et al.* 1994). Among these, butenolides such as karrikins (KAR) are recognized for their stimulatory role in germination in fire-prone (Flemmatti *et al.* 2004; Light *et al.* 2009; Çatav *et al.* 2024) and non-fire-prone environments (Daws *et al.* 2007; Ferraz *et al.* 2013). Beyond this stimulation, smoke compounds may also exert a protective effect under stress conditions (Banerjee *et al.* 2019), enhancing not only germination rates (Commander *et al.* 2009) but also seedling vigor (Moyo *et al.* 2022).

Thus, various benefits have been attributed to smoke. When applied to lettuce achenes, it triggered multiple genetic and metabolic responses, including the upregulation of stress-related and ABA-associated genes (Soós *et al.*, 2009). Acting as phytohormones, smoke-derived molecules (e.g., KAR) can modulate the production of antioxidant enzymes such as ascorbate peroxidase and glutathione reductase, which play key roles in neutralizing reactive oxygen species (ROS) (Cembrowska-Lech and Kępczyński, 2016; Ahmad *et al.*, 2021). Furthermore, positive effects on the balance of photosynthetic pigments under stress conditions have been reported, preventing pigment concentration reductions in plants treated with smoke (Waheed *et al.*, 2016).

Additionally, even under stress conditions, smoke can stimulate plant growth, leading to increased organ length and biomass accumulation (Otori *et al.*, 2020). By influencing genetic and morphophysiological pathways, smoke presents the potential to activate multiple stress-alleviation mechanisms (Aslam *et al.*, 2024), including responses to suboptimal temperature (Jain *et al.*, 2006) and water availability (Ghebrehiwot *et al.*, 2008). While these effects have been explored in commercial crops,

their potential remains largely uninvestigated in native plant species, representing a critical gap in the ecophysiological understanding of biodiversity hotspots such as the Brazilian savanna (Cerrado) (Myers et al., 2000).

Given the increasing water deficit in fire-prone environments, this study evaluates the effects of a water potential gradient combined with different smoke-water concentrations on ground-layer species, the most diverse and representative stratum of the Cerrado. We aim to address the following questions: (i) How do Cerrado ground-layer species respond to reduced water potential? (ii) To what extent does smoke influence these responses under water stress, and at which developmental stages? (iii) Does smoke facilitate recovery after stress?

We hypothesize that reduced water potential negatively impacts germinative and post-germinative parameters. However, we expect smoke-water to mitigate water stress by stimulating germination and seedling development. This protective effect should thus enhance recovery after stress. Our findings could provide insights into ecophysiological responses, with implications for species recruitment under projected drought conditions, resilience to drought events, and possible strategies for the management, conservation, and restoration of this climate-vulnerable hotspot through seeding.

Materials and Methods

Study area and diaspore collection

The diaspores for the experiments were collected at the Assis Ecological Station (22°33'20"S and 50°20'27"W) and the Santa Bárbara Ecological Station (24°48'S and 49°13'W), both located in the state of São Paulo, Brazil (Figure S1 and Table S1). The diaspores (Figure S2) were obtained from at least five random individuals to ensure genetic variability. All collected seeds were screened under a stereomicroscope to discard empty seeds. Fifteen species were selected (Table 1), with the criteria being abundant in seed production and the representativeness of their family in floristic surveys of the Cerrado, prioritizing more representative families such as Poaceae and Asteraceae, among others (Meira Neto *et al.* 2007; Rossatto *et al.* 2008; Mendonça *et al.* 2008). To facilitate comprehension, the dispersal units of all species will be henceforth referred to as seeds.

Table 1. Ground-layer study species. Growth form according to Durigan *et al.* (2018). AES: Assis Ecological Station; PSG: physiognomies of species occurrence; SBES: Santa Bárbara Ecological Station; SCD: Site and collection date; ST: Storage.

Species	Family	Growth form	PSG	SCD	ST (months)
<i>Andropogon leucostachyus</i> Kunth	Poaceae	Graminoid	Grasslands/Savanna (Dry)	AES; Jan/2023	3
<i>Aristida megapotamica</i> Spreng.	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; Apr/ 2023	16
<i>Baccharis sessiliflora</i> Vahl	Asteraceae	Subshrub	Grasslands/Savanna (Dry)	SBES; Apr/ 2023	7
<i>Ctenium polystachyum</i> Balansa	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; Mar/ 2023	14
<i>Eragrostis leucosticta</i> Nees ex Döll	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; Apr/2023	9
<i>Gymnopogon spicatus</i> (Spreng.) Kuntze	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; May/2023	15
<i>Hyptis campestris</i> Harley & J.F.B.Pastore	Lamiaceae	Forb	Grasslands/Savanna (Dry)	AES; May/2023	< 1
<i>Lepidaploa chamissonis</i> (Less.) H.Rob.	Asteraceae	Subshrub	Grasslands/Savanna (Dry)	SBES; Apr/2023	< 1
<i>Loudetia flammida</i> (Trin.) C.E.Hubb.	Poaceae	Graminoid	Grasslands/Forest (Wet)	AES; Apr/ 2023	15
<i>Loudetiopsis chrysothrix</i> (Nees) Conert	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; Mar/2023	3
<i>Miconia chamissois</i> Naudin	Melastomataceae	Shrub	Grasslands/Forest (Wet)	AES; Mar/2023	1
<i>Mimosa dolens</i> var. <i>rigida</i> (Benth.) Barneby	Fabaceae	Subshrub – Shrub	Grasslands/Savanna (Dry – Wet)	SBES; May/2023	9
<i>Paspalum cordatum</i> Hack.	Poaceae	Graminoid	Grasslands (Wet)	SBES; Jan/2023	6
<i>Pterocaulon rugosum</i> (Vahl) Malme	Asteraceae	Subshrub	Grasslands (Dry)	SBES; Apr/2023	6
<i>Sorghastrum minarum</i> (Nees) Hitchc.	Poaceae	Graminoid	Grasslands/Savanna (Dry)	SBES; Mar/2023	3

Smoke water treatments and osmotic potentials

For the experiments, each treatment consisted of four replicates with 25 seeds each, totaling 100 seeds per species. The seeds were placed on a double layer of filter paper in 90 x 15 mm Petri dishes and incubated in germination chambers at a constant temperature of 25°C, with a photoperiod of 12 hours of white light (60 $\mu\text{mol m}^2 \text{s}^{-1}$). However, for larger seeds (>3 mm), 110 x 110 x 35 mm germination boxes were used.

To simulate the water deficit gradient, different concentrations of polyethyleneglycol 6000 (PEG 6000) were applied, following the specifications of Villela, Doni Filho, and Sequeira (1991) (see Table S1 for PEG 6000 mass). PEG 6000 was dissolved in distilled water and aqueous solutions of commercial SW (Regen® SMOKEMASTER™) at concentrations of 1 and 2.5%. The seeds were subjected to 12 treatments: Control (distilled water); SW 1%; SW 2.5%; -0.3; -0.6; and -0.9 MPa (the last three treatments used PEG diluted in distilled water, SW 1%, and SW 2.5%) – see Figure S3.

Each Petri dish received 5 mL of the corresponding treatment solution, while the germination boxes received 11.7 mL, a volume proportional to the base area compared to the Petri dishes. The containers were sealed with plastic film (polyvinyl chloride) in both cases. Every 15 days, all treatments had their solutions replaced to maintain the desired concentrations and osmotic potentials. Germination was monitored daily for 30 days. Seeds were considered germinated when the radicle (or coleorhiza for grasses) protruded >1 mm. The germination percentage (G%) was calculated according to the equation

$$G\% = \frac{n_g \times 100}{T_{si}}$$

Where: n_g = the number of seeds germinated, and T_{si} = total number of seeds at the start of the experiment. The mean germination time (MGT) was calculated using the equation:

$$MGT = \frac{\Sigma (n_g \times n_d)}{T_f}$$

Where: n_g = the number of seeds germinated per day; n_d = the number of days since the start of the test, and T_f = the total number of seeds germinated by the end of the experiment. Furthermore, from the onset of the experiments, both seedling formation percentage (S%) and mean seedling formation time (MSFT) were assessed. In addition to the potential seedlings formed during the initial 30 days, an additional 30 days were allocated exclusively to evaluate the formation of seedlings from seeds that had previously germinated

in their respective treatments; solutions were also replaced every 15 days. Grass seedlings were considered formed when the second leaf expanded (>1 mm), while for eudicotyledons, when the aerial organ (epicotyl > 1 mm in height) emerged – see Figure S3. The S% was calculated using the equation:

$$S\% = \frac{n_f \times 100}{T_{sgi}}$$

Where: n_f = the number of seedlings formed and T_{sgi} = the total number of seeds that had germinated at the start of the experiment. The MSFT was calculated using the following equation:

$$MSFT = \frac{\sum (n_s \times n_d)}{T_f}$$

Where: n_s = number of seedlings formed per day; n_d = number of days since the beginning of the test, and T_f = total number of seedlings formed by the conclusion of the experiment.

Recovery treatment

The ungerminated seeds, excluding the control treatment, were rinsed with distilled water and subjected to a recovery treatment. For each replicate ($n \geq 1$) with ungerminated seeds ($n \geq 1$) from the treatments, a double layer of filter paper was used in 90x15 mm Petri dishes or 110x110x35 mm germination boxes. At this stage, both containers received only distilled water and were placed in germination chambers at a constant temperature of 25°C, with a photoperiod of 12 hours of white light ($60 \mu\text{mol m}^{-2} \text{s}^{-1}$). As in the previous treatments, 30 days were allocated for germination monitoring and a total of 60 days for seedling development and formation (Figure S3). The same criteria used in the treatments were applied to consider a seed as germinated (radicle or coleorhiza > 1 mm) and a seedling as formed (epicotyl or second leaf > 1 mm).

Seed viability

The ungerminated seeds at the end of the recovery experiments (or control treatment) were analyzed using a 1% solution of 2,3,5-triphenyl-2H-tetrazolium chloride (see Table S1). The seeds were incubated in the dark at 25°C for 72 hours. Following this period, they were

dissected using a scalpel, inspected under a stereomicroscope, and classified as viable (embryos exhibited a pink hue) or non-viable (no color change in the embryo) – see Figure S3.

Data analyses

To assess the effects of water potential reduction and smoke on germinative and post-germinative responses, effect size analyses were performed using the log response ratio (LRR) as a measure of composition. The LRR was calculated using the following equation:

$$LRR = \ln \left(\frac{R_t}{R_{ct}} \right)$$

Where: R_s = response observed under treatment or recovery, and R_{ct} = response observed in the respective control.

Effects size was calculated using *escalc* function from the R package METAFOR (version 4.6.0; Viechtbauer, 2024). A constant (0.1) was added before calculating the LRR to prevent issues with zero values in the means. Negative LRR values (–;–) indicate reduced responses under treatment or recovery conditions compared to control, while positive values (+;+) denote increased. Confidence intervals that include zero (–;+) indicate no significant difference between treatment/recovery and control responses. All analyses were performed using R software (version 4.3.0) (R Development Core Team, 2023).

Results

Germination responses to water deficit and smoke

All species exhibited reduced germination parameters under water deficit conditions, particularly at more reduced osmotic potentials. Conversely, the effects of smoke varied, ranging from negative to neutral or positive, with species-specific responses observed (Fig. 1). Among the 15 species, three (20%) showed enhanced germination in response to both smoke concentrations (1% and 2.5%) (Fig. 1A). Notably, while *Eragrostis leucosticta* and *Loudetia flammida* exhibited increased G% under both concentrations, *Sorghastrum minarum* was stimulated only under SW 1% (Fig. 1A).

Additionally, 20% of the species experienced reductions in G% under SW 1%, increasing to 40% under SW 2.5%. While both concentrations negatively affected the G% of *Baccharis sessiliflora*, *Gymnopogon spicatus*, and *Paspalum cordatum*, only the higher concentration adversely impacted this parameter in *Aristida megapota*, *Lepidaploa*

chamissonis, and *Pterocaulon rugosum*. For MGT, reductions were observed in 20% of the species, but only under SW 2.5% (Fig. 1B). Conversely, increases in MGT were noted in $\cong$ 33% of the species under SW 1%, rising to 40% under SW 2.5% (Fig. 1B).

Under the less negative osmotic potential (-0.3 MPa) without smoke, 60% of the species exhibited reductions in G% (Fig. 2A). However, under the same osmotic potential with smoke, this percentage dropped to $\cong$ 47% under SW 1% and $\cong$ 33% under SW 2.5%; moreover, *E. leucosticta* showed increased G% at both SW concentrations, while *Loudetia flammida* displayed a similar response only at SW 1% (Fig. 2A). For MGT, 12 out of 15 species (80%) exhibited increases under -0.3 MPa, rising to $\cong$ 87% with SW 1% and remaining at 80% with SW 2.5% (Fig. 2B). Although *Gymnopogon spicatus* showed increased MGT under treatment without SW, no differences were detected compared to the control when combined with SW, regardless of concentration (Fig. 2B). Conversely, the opposite pattern was observed for *Hyptis campestris* and *Miconia chamissois*, which exhibited increased MGT when exposed to SW (Fig. 2B).

At the moderate osmotic potential tested (-0.6 MPa) without SW, 14 out of 15 species ($\cong$ 93%) showed reduced G% (Fig. 3A). However, with smoke exposure, this percentage decreased to 80% at SW 2.5% and $\cong$ 73% at SW 1%. Within the -0.6 MPa potential, while *Lepidaploa chamissonis* exhibited improved germination under SW 1% (not differing from the control), similar responses were observed for *Miconia chamissois* and *Sorghastrum minarum* at both SW concentrations (Fig. 3A). Regarding MGT, except for *Gymnopogon spicatus* under the isolated -0.6 MPa potential treatment, all species were negatively affected regardless of SW presence or concentration (Fig. 3B). Under the most negative osmotic potential (-0.9 MPa), all species except *Eragrostis leucosticta* experienced reduced %G, irrespective of SW presence or concentration (Fig. 4A). For MGT, only *Andropogon leucostachyus* showed no changes under SW 1%, while all other species were negatively affected irrespective of SW presence or concentration (Fig. 4B).

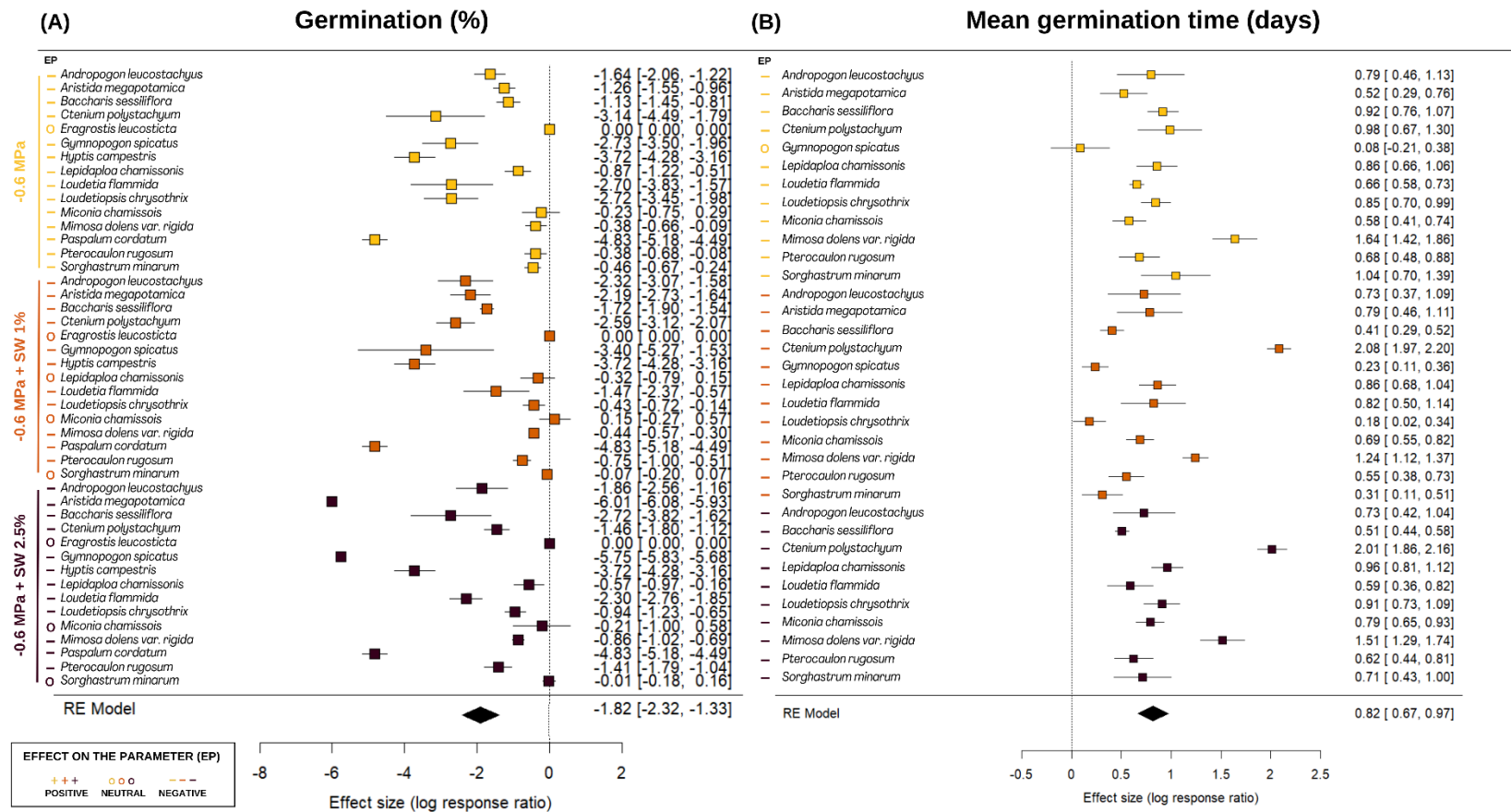


Figure 3. Effect size of osmotic potential, independently and in combination with varying concentrations of smoke water (SW), on germination responses (**A** and **B**) of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

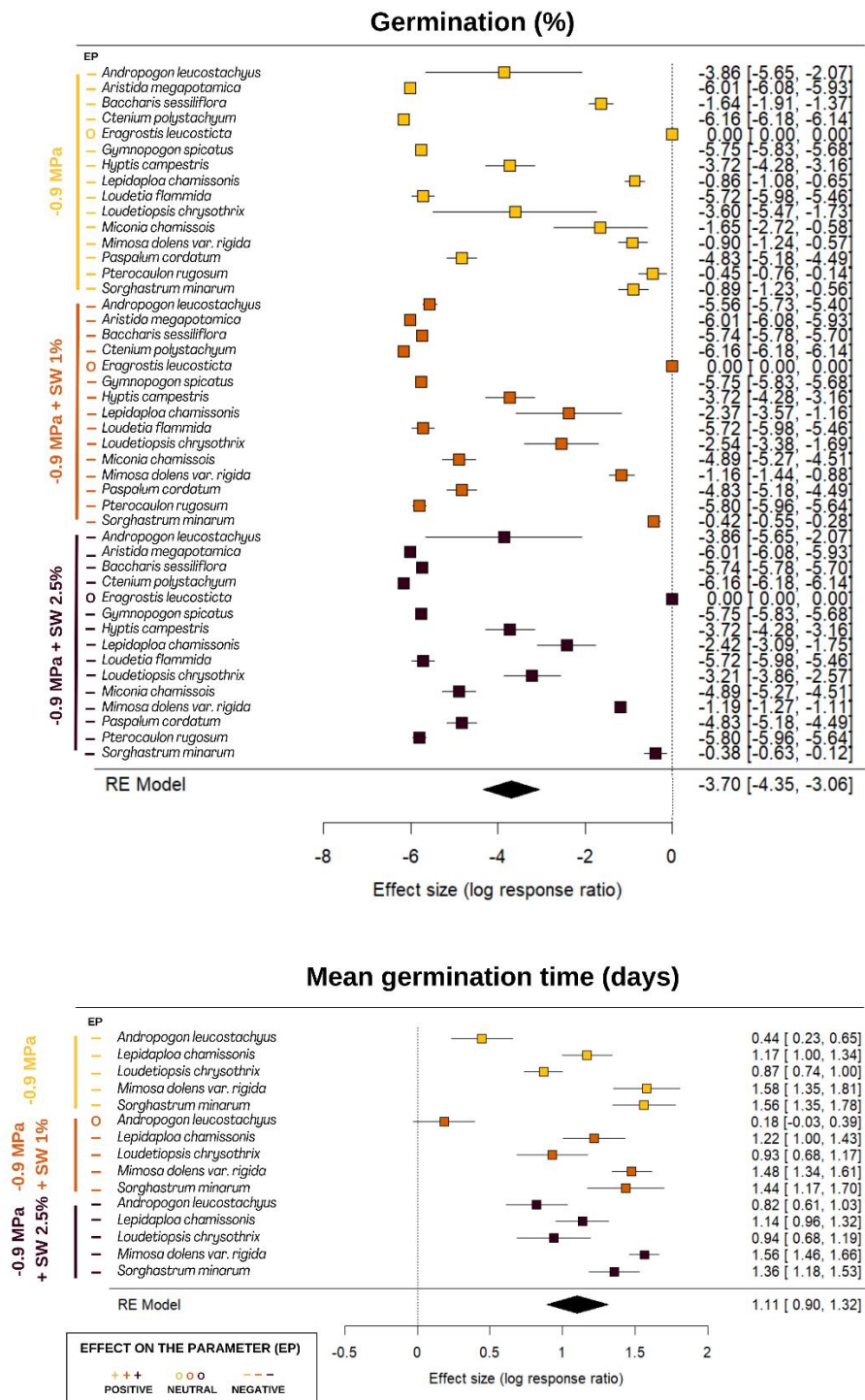


Figure 4. Effect size of osmotic potential, independently and in combination with varying concentrations of smoke water (SW), on germination responses (**A** and **B**) of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

Post-germination responses to water stress and smoke

Similar to germination parameters, S% was negatively affected by water deficit. In the absence of water stress, smoke negatively impacted S% in 4 out of the 14 evaluated species ($\cong 28\%$), increasing to 9 ($\cong 64\%$) under SW 2.5% (Fig. 5A). *Lepidaploa chamissonis* was the only species to show an increase in S% under SW 2.5% (Fig. 5A). For MSFT, increases were observed in $\cong 31\%$ and $\cong 46\%$ of the species under SW 1% and 2.5%, respectively (Fig. 5B). Both concentrations negatively affected MSFT in *Aristida megapotamica*, *Ctenium polystachyum*, *Pterocaulon rugosum*, and *Sorghastrum minarum* (Fig. 5B). Under SW 2.5%, increases in MSFT were observed only in *Lepidaploa chamissonis* and *Miconia chamissois* (Fig. 5B). In contrast, *Loudetiopsis chrysothrix* benefited under the highest concentration, showing a reduction in MSFT (Fig. 5B).

Under water stress, at the less negative osmotic potential (-0.6 MPa), reductions in S% were observed across all treatments, regardless of SW presence or concentration (Fig. 6). However, while 9 out of the 14 evaluated species ($\cong 64\%$) showed reductions under the isolated osmotic potential, this increased to 11 ($\cong 78\%$) and 10 ($\cong 71\%$) under SW 1% and 2.5%, respectively (Fig. 6A). *Ctenium polystachyum* was the only species to benefit from smoke, and this effect was observed only under SW 1% (Fig. 6A). For MSFT, 9 out of the 12 evaluated species (75%) exhibited increases in this parameter (Fig. 6B).

With further reduction in osmotic potential (-0.6 MPa), only *Andropogon leucostachyus*, *Lepidaploa chamissonis*, and *Loudetiopsis chrysothrix* did not show a decrease in S% under the isolated treatment (Fig. 7A). Conversely, smoke consistently had a negative effect on S% across all species, regardless of its concentration (Fig. 7A). Similar trends were observed for MSFT, where, except for *Lepidaploa chamissonis*, which was unaffected by the isolated osmotic potential, all species exhibited increases in MSFT values (Fig. 7B). Under the most negative osmotic potential (-0.9 MPa), all species showed reductions in S%, irrespective of smoke presence or concentration (Fig. 8). Due to the high number of species with inhibited S% at this potential or with data available from only two replicates, MSFT could not be assessed.

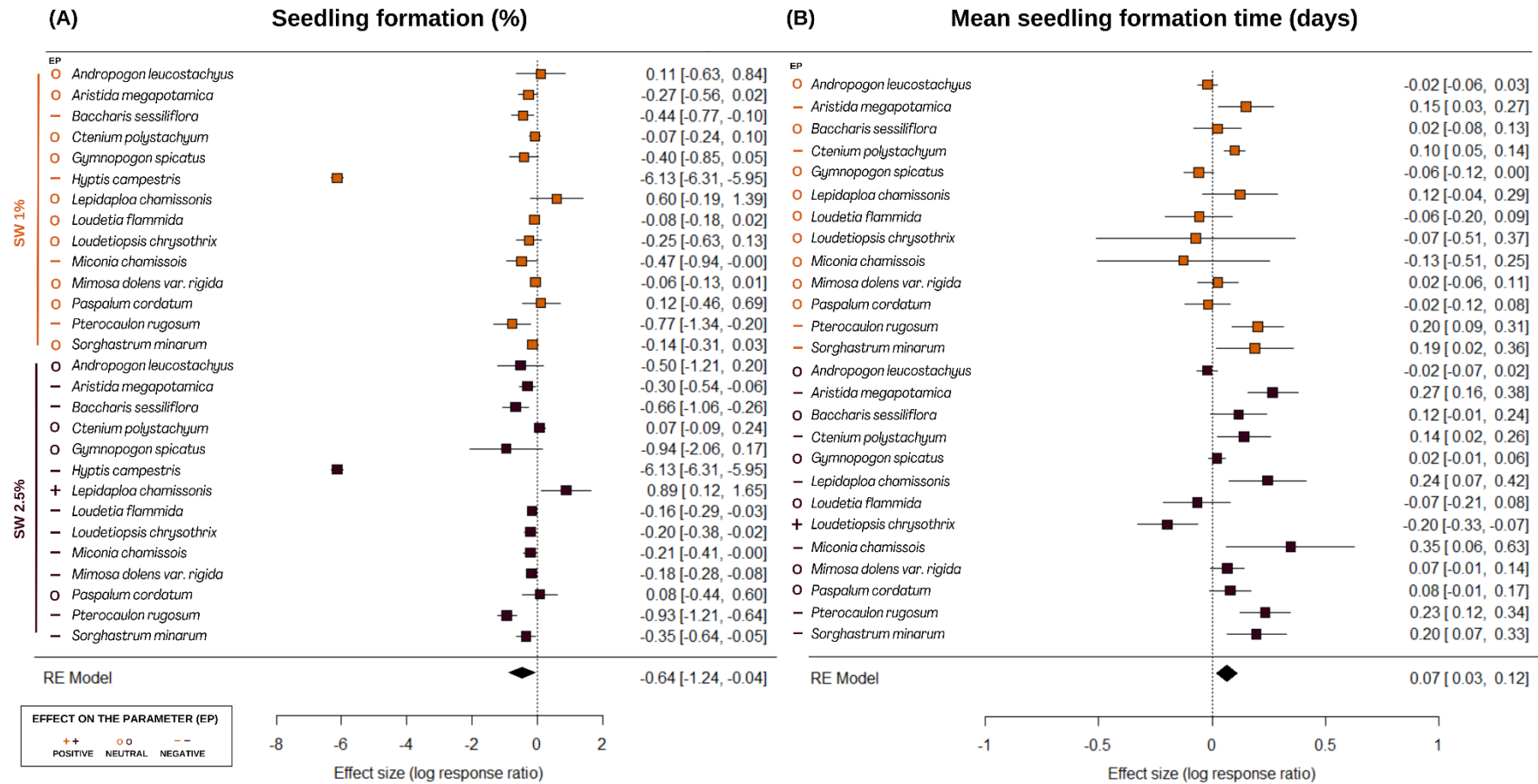


Figure 5. Effect size of varying concentrations of smoke water (SW), on post-germinative responses (**A** and **B**) of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

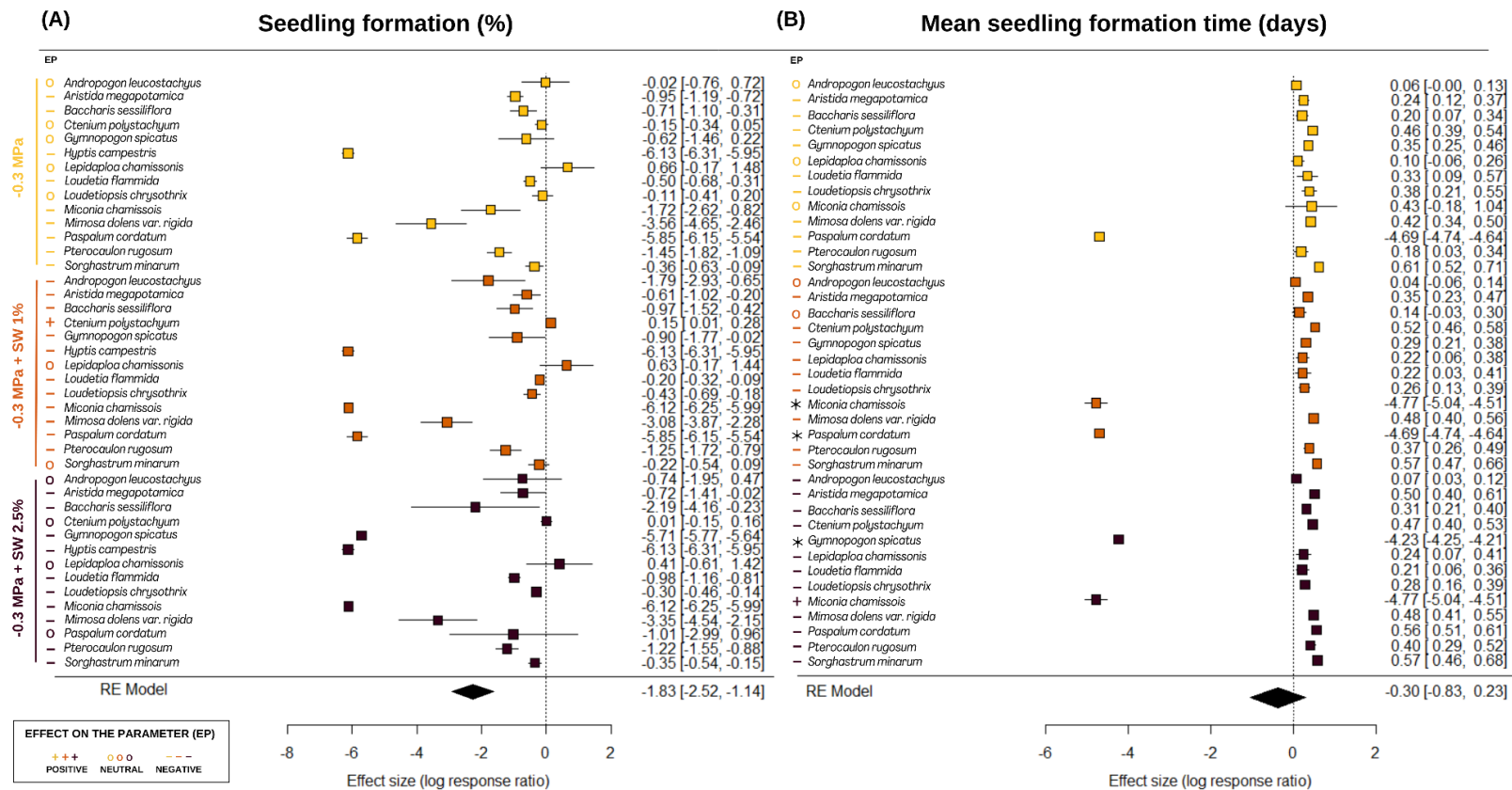


Figure 6. Effect size of osmotic potential, independently and in combination with varying concentrations of smoke water (SW), on post-germinative responses (**A** and **B**) of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals. *: with only 1-2 replicates.

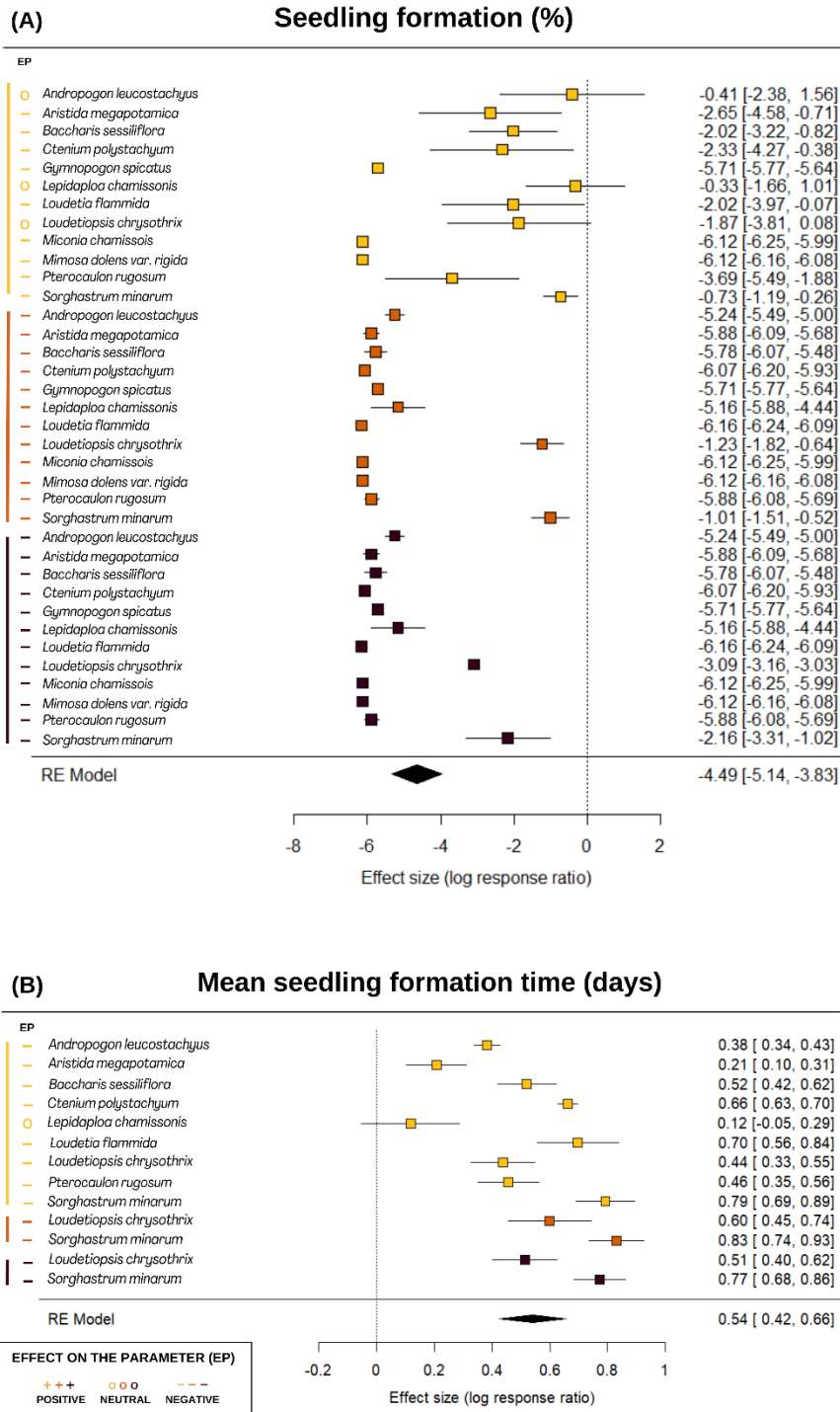


Figure 7. Effect size of osmotic potential, independently and in combination with varying concentrations of smoke water (SW), on post-germinative responses (**A** and **B**) of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

Seedling formation (%)

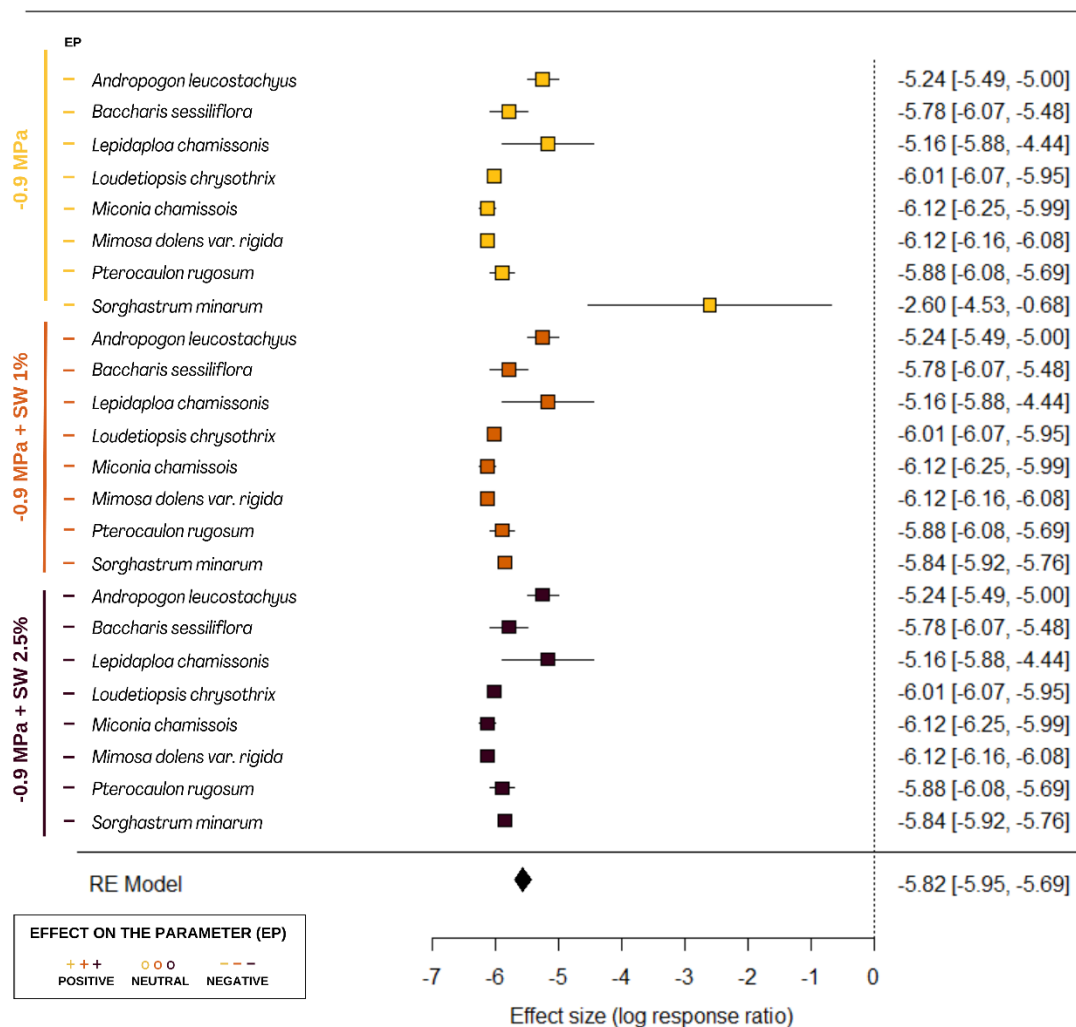


Figure 8. Effect size of osmotic potential, independently and in combination with varying concentrations of smoke water (SW), on post-germinative response of Cerrado ground-layer species. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

Post-stress germination responses

Similar to the parameters observed in the treatments, recovery responses varied according to osmotic potentials and smoke concentrations. Furthermore, at the end of the initial 30 days of treatment, although ungerminated, the *Mimosa dolens* var. *rigida* seeds were excluded from the recovery phase due to fungal contamination. A similar issue occurred with the seeds of *Andropogon leucostachyus* in treatments involving isolated SW. In the recovery of treatments solely involving smoke, 10 out of the 13 evaluated species ($\cong 77\%$) exhibited reduced germinability under the SW 1% treatment, increasing to 11 species ($\cong 85\%$) under SW 2.5% (Fig. 9A). While seeds of *Baccharis sessiliflora* and *Sorghastrum minarum* did not show reduced G% after exposure to SW 1%, higher concentrations negatively impacted both species (Fig. 9A). Conversely, the opposite pattern was observed in *Loudetia flammida* (Fig. 9A). Regarding MGT, *Aristida megapotamica* and *Gymnopogon spicatus* displayed shorter germination times for seeds exposed to SW 1% (Fig. 9B). In contrast, only *Baccharis sessiliflora* exhibited increased MGT values under both SW concentrations (Fig. 9B). Null or positive effects on MGT were observed in four out of five species ($\cong 80\%$) across both SW concentrations (Fig. 9B).

For seeds originating from less negative water deficits, the same proportion of species in each treatment, 6 out of 14 ($\cong 43\%$), exhibited reduced G%, regardless of SW presence or concentration (Fig. 10A). However, responses were species- and concentration-specific. While *Baccharis sessiliflora* showed no difference compared to the control under recovery from -0.3 MPa, prior exposure to smoke at this osmotic potential reduced G% in both SW concentrations (Fig. 10A). Conversely, *Loudetiopsis chrysothrix* exhibited stimulated G% recovery under -0.3 MPa with SW 1%, whereas *Eragrostis leucosticta* demonstrated increased G% during recovery from isolated osmotic potential and -0.3 MPa with SW 2.5% (Fig. 10A). In recovery from -0.3 MPa, irrespective of SW, *Andropogon leucostachyus* showed increased G% (Fig. 10A). Regarding MGT, three species (*Baccharis sessiliflora*, *Eragrostis leucosticta*, and *Lepidaploa chamissonis*) experienced longer germination times during recovery from the isolated osmotic potential (Fig. 10B). However, under -0.3 MPa with SW, the number of negatively affected species dropped to one and two, respectively (Fig. 10B). Notably, while *Lepidaploa chamissonis* exhibited increased MGT during recovery from isolated -0.3 MPa, reductions were observed under both SW treatments (Fig. 10B).

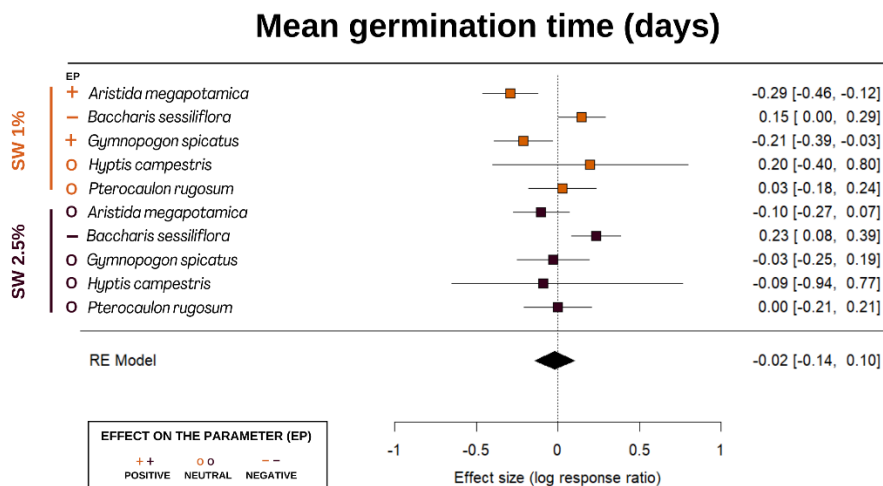
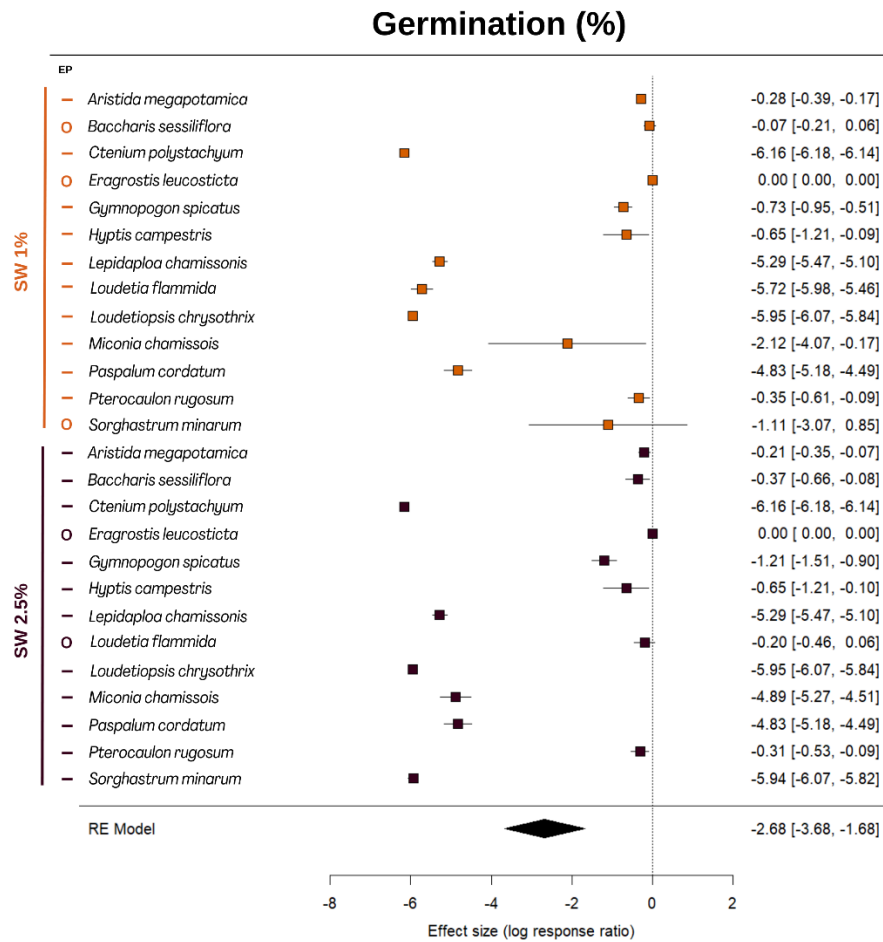


Figure 9. Effect size of smoke water (SW) on germination responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

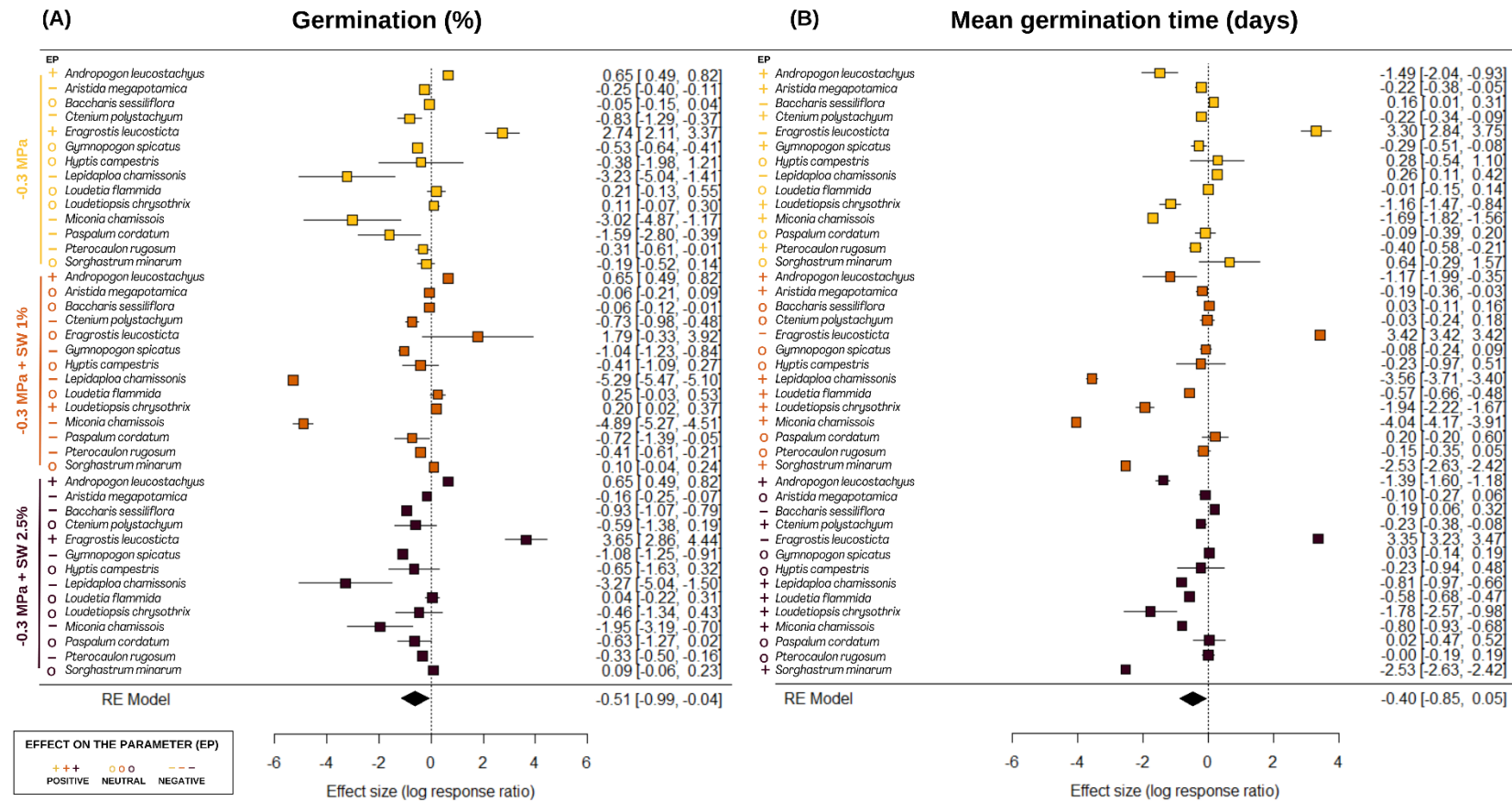


Figure 10. Effect size of osmotic potential and smoke water (SW) on germination responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

Similarly to recovery from isolated -0.3 MPa, during recovery from -0.6 MPa, $\cong 43\%$ of species showed reduced G% across treatments (Fig. 11A). Among these, *Lepidaploa chamissonis*, *Miconia chamissois*, and *Pterocaulon rugosum* exhibited reduced G% in recovery across all treatments involving -0.6 MPa, regardless of SW presence (Fig. 11A). As observed in -0.3 MPa recovery, *Andropogon leucostachyus* displayed increased G% across all treatments under -0.6 MPa (Fig. 11A). Regarding MGT, during recovery from isolated -0.6 MPa, only *Eragrostis leucosticta* exhibited increased values, whereas other species either germinated faster or showed no significant differences compared to the control (Fig. 11B). Under -0.6 MPa with SW, the number of species exhibiting increased MGT rose to three across both SW concentrations (Fig. 11B). Despite these differences, $\cong 57\%$ of species showed reduced MGT across all treatments, independent of SW (Fig. 11B).

During recovery from the isolated -0.9 MPa, only four out of 14 species exhibited reduced G% (Fig. 12A). In treatments with SW 1% and 2.5% at the same osmotic potential, three and five species, respectively, were negatively impacted (Fig. 12A). Moreover, as SW concentration increased, the percentage of species benefiting in G% declined from $\cong 28\%$ under isolated -0.9 MPa to $\cong 21\%$ and $\cong 14\%$ under SW 1% and 2.5%, respectively. Regardless of SW presence, *Andropogon leucostachyus* and *Eragrostis leucosticta* exhibited increased G% compared to the control (Fig. 12A). MGT increases were observed across all treatments under -0.9 MPa recovery. However, while only two species exhibited increased MGT under isolated -0.9 MPa, three and one out of 14 species were negatively impacted under SW 1% and 2.5%, respectively (Fig. 12B). Conversely, regarding positively affected species, 50% of species under isolated -0.9 MPa exhibited reduced MGT, while $\cong 64\%$ and $\cong 35\%$ also benefited under SW 1% and 2.5%, respectively, with species-specific responses across treatments (Fig. 12B).

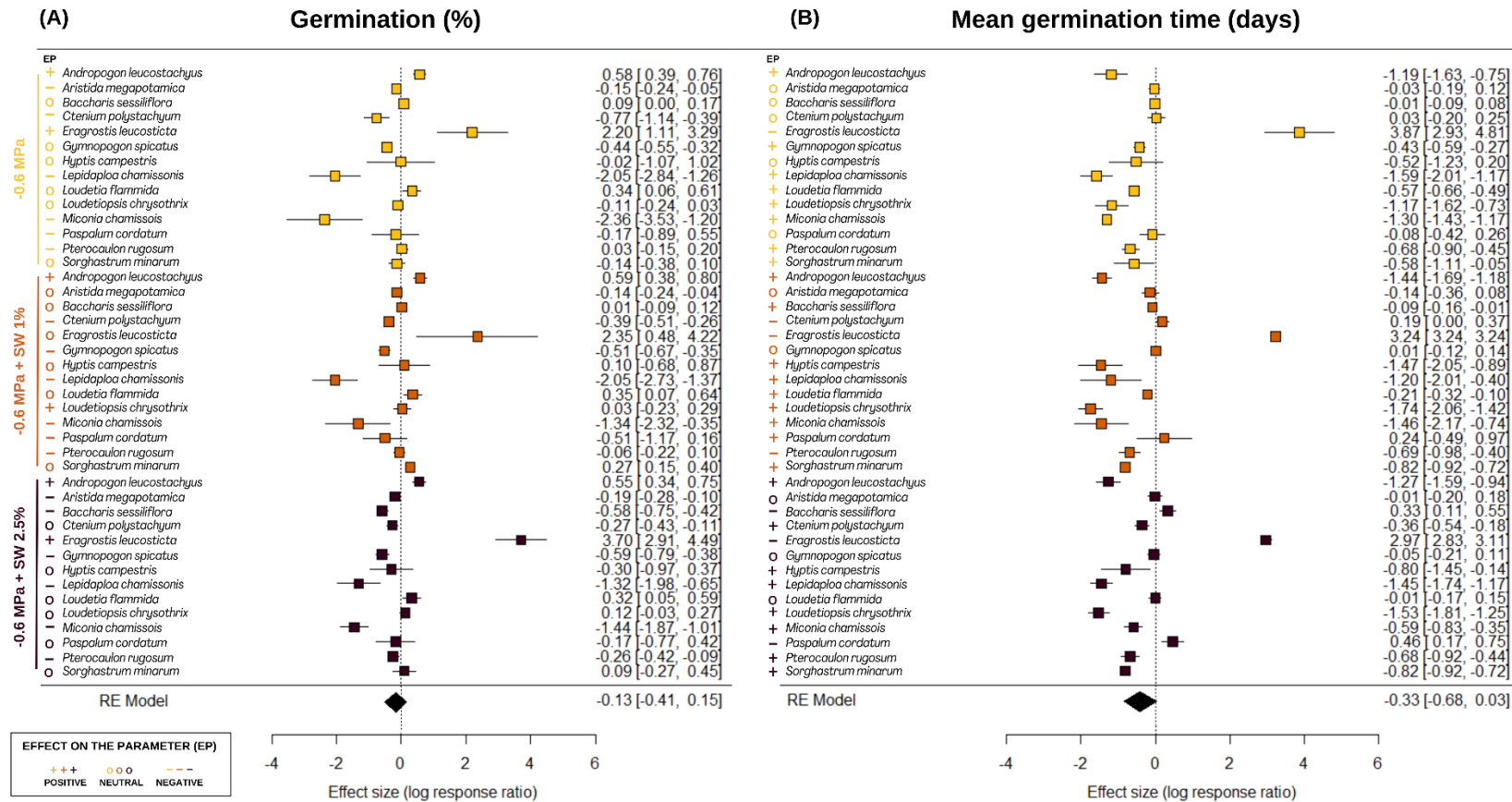


Figure 11. Effect size of osmotic potential and smoke water (SW) on germination responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

Post-germination responses post-stress

For seedling formation during recovery from isolated smoke treatments, reductions were observed in two of the eight species under SW 1%, rising to four under SW 2.5% (Fig. 13A). In both SW concentrations, reductions in S% were observed for *Pterocaulon rugosum* during recovery (Fig. 13A). Additionally, only seeds of *Sorghastrum minarum* exposed to SW 1% exhibited increased S% during recovery (Fig. 13A). However, an increase in MSFT was observed exclusively for *S. minarum* under the same condition (Fig. 13B). Positive effects on S% were observed only for two evaluated species, *Ctenium polystachyum* and *Loudetia flammida*, during recovery from SW 1% and 2.5%, respectively (Fig. 13B).

Regarding S% recovery under -0.3 MPa, three out of 12 species (25%) showed reductions, increasing to five species ($\cong 42\%$) in SW treatments (Fig. 14A). Only *Lepidaploa chamissonis* exhibited increased S% during recovery from isolated -0.3 MPa (Fig. 14A). For MSFT, only *Aristida megapotamica* and *Lepidaploa chamissonis* showed increased seedling formation time during recovery from isolated osmotic potential (Fig. 14B). However, seeds of *Aristida megapotamica* exposed to SW treatments at both concentrations did not differ in MSFT compared to the control (Fig. 14B). For smoke treatments, only *Gymnopogon spicatus* showed an increase in MSFT under both concentrations (Fig. 14B). Positive effects on MSFT were observed in four out of 11 species ($\cong 36\%$) during recovery from isolated potential, four out of nine species ($\cong 44\%$) under SW 1%, and five out of 11 ($\cong 45\%$) under SW 2.5% (Fig. 14B).

During recovery from -0.6 MPa osmotic potential, a higher percentage of species showed negative impacts on S% in treatments involving SW (Fig. 15A). While $\cong 23\%$ (two species) of the evaluated species under isolated osmotic potential exhibited reductions in S%, this percentage rose to $\cong 46\%$ in treatments where seeds were exposed to SW (Fig. 15A). Among negatively affected species, *Ctenium polystachyum* and *Gymnopogon spicatus* showed reductions regardless of SW presence (Fig. 15A). Positive effects on S% during recovery from -0.6 MPa treatments were observed only for *Andropogon leucostachyus* and *Paspalum cordatum* after exposure to SW 1% (Fig. 15A).

Regarding MSFT, only *Gymnopogon spicatus* and *Miconia chamissois* showed increased formation time during recovery from isolated -0.6 MPa and under SW 2.5% (Fig. 15B). Additionally, only *Miconia chamissois* exhibited increased MSFT during

recovery from -0.6 MPa with SW 1% (Fig. 15B). On the other hand, the percentage of species showing reduced MSFT increased with the presence and concentration of SW (Fig. 15B). While $\cong 15\%$ of species benefited from recovery post-stress under isolated -0.6 MPa, the percentage rose to $\cong 33\%$ and $\cong 38\%$ during recovery from -0.6 MPa with SW 1% and 2.5%, respectively (Fig. 15B). *Loudetia flammida* and *Loudetiopsis chrysothrix* formed seedlings faster than the control during recovery from all -0.6 MPa treatments (Fig. 15B).

For recovery from the most negative potential, a higher percentage of species showed reductions in S% under -0.9 MPa with SW 2.5% (Fig. 16A). While $\cong 23\%$ (three out of 13) of species exhibited reductions in seedling formation under isolated -0.9 MPa and SW 1%, this value increased to $\cong 54\%$ with SW 2.5% (Fig. 16A). Furthermore, no species exposed to the most negative osmotic potential showed increased S% during recovery compared to the control (Fig. 16A). Conversely, $\cong 46\%$ (six out of 13) of species exhibited reduced MSFT during recovery from isolated -0.9 MPa and SW 1%, decreasing to $\cong 42\%$ under SW 2.5% (Fig. 16B). Additionally, *Miconia chamissois* showed increased MSFT during recovery from all treatments involving -0.9 MPa osmotic potential (Fig. 16B).

Viability

Despite the negative impacts on germination parameters in the treatments and recovery, no osmotic potential or SW concentration, isolated or combined, affected the viability of ungerminated seeds for any species (Fig. S4).

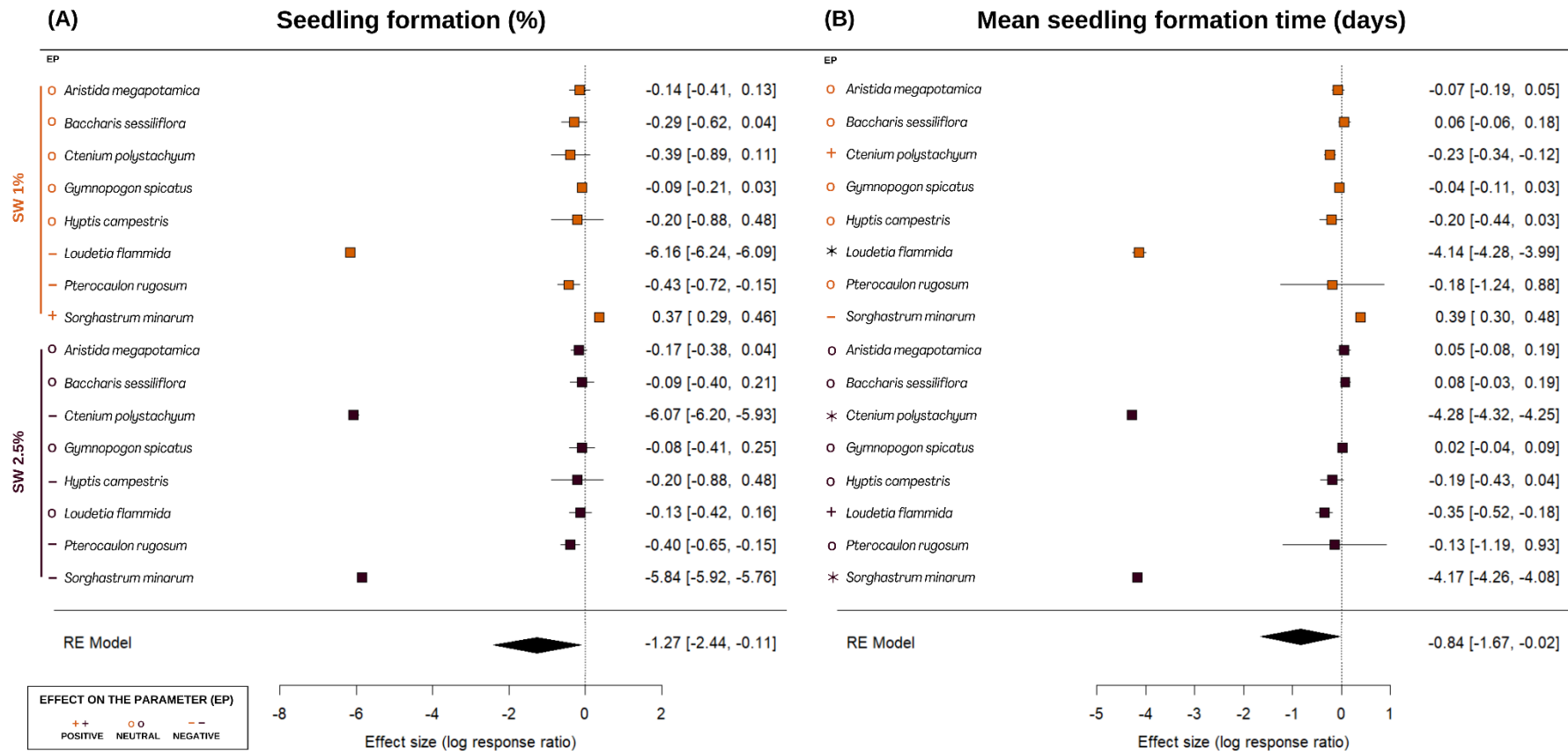


Figure 13. Effect size of smoke water (SW) on post-germinative responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals. *: with only 1-2 replicates.

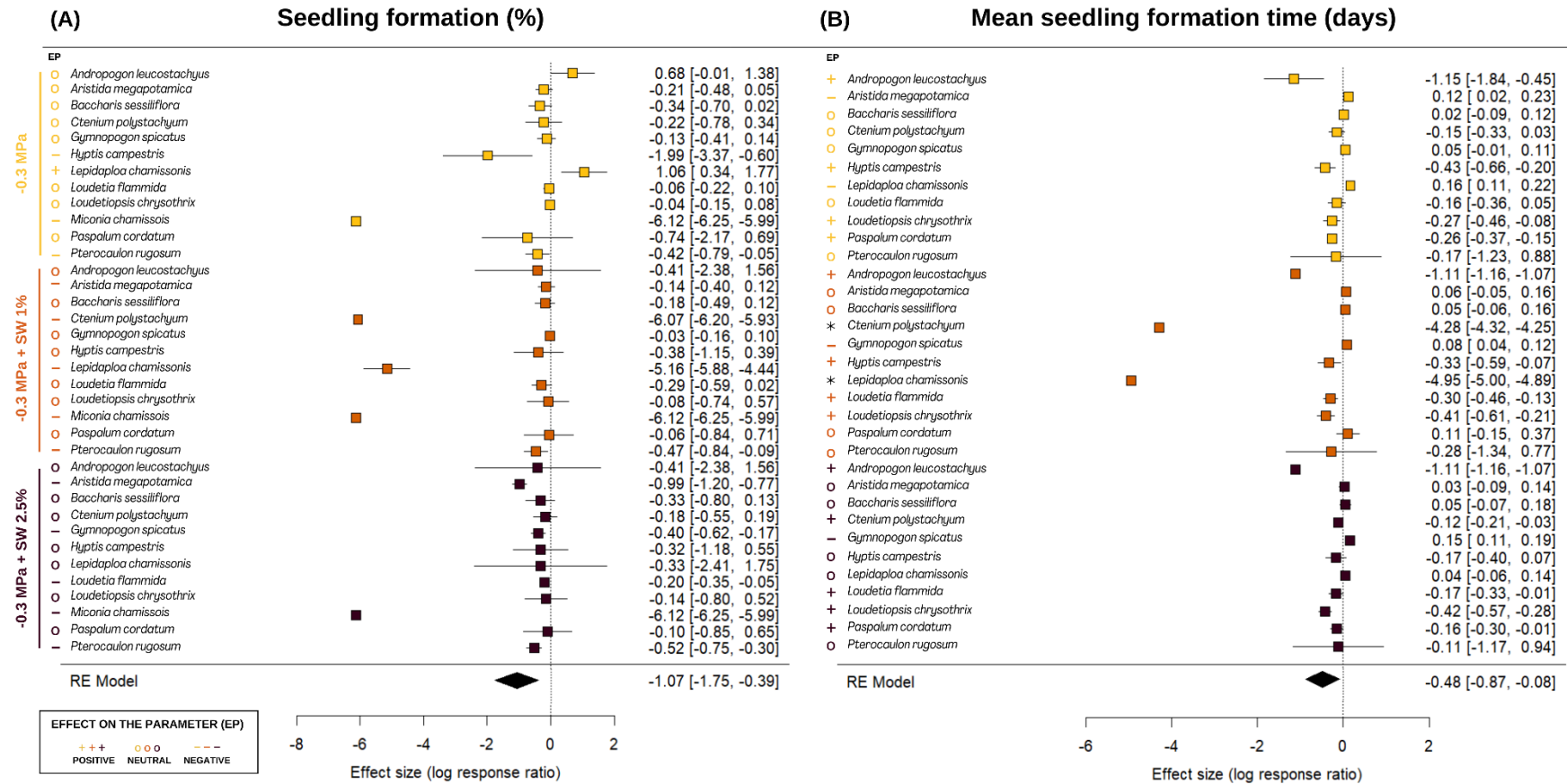


Figure 14. Effect size of osmotic potential and smoke water (SW) on post-germinative responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals. *: with only 1-2 replicates.

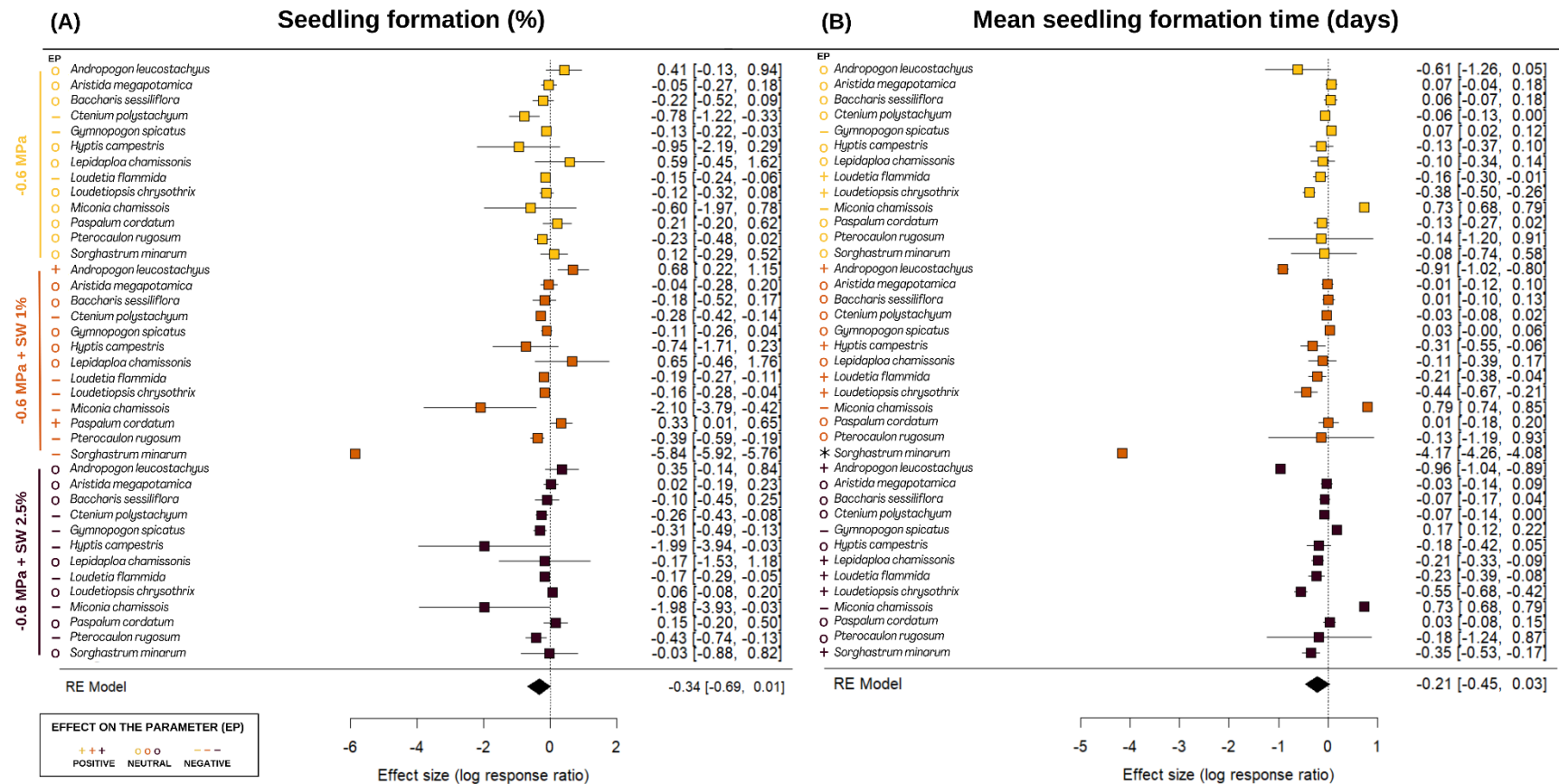


Figure 15. Effect size of osmotic potential and smoke water (SW) on post-germinative responses (A and B) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals.

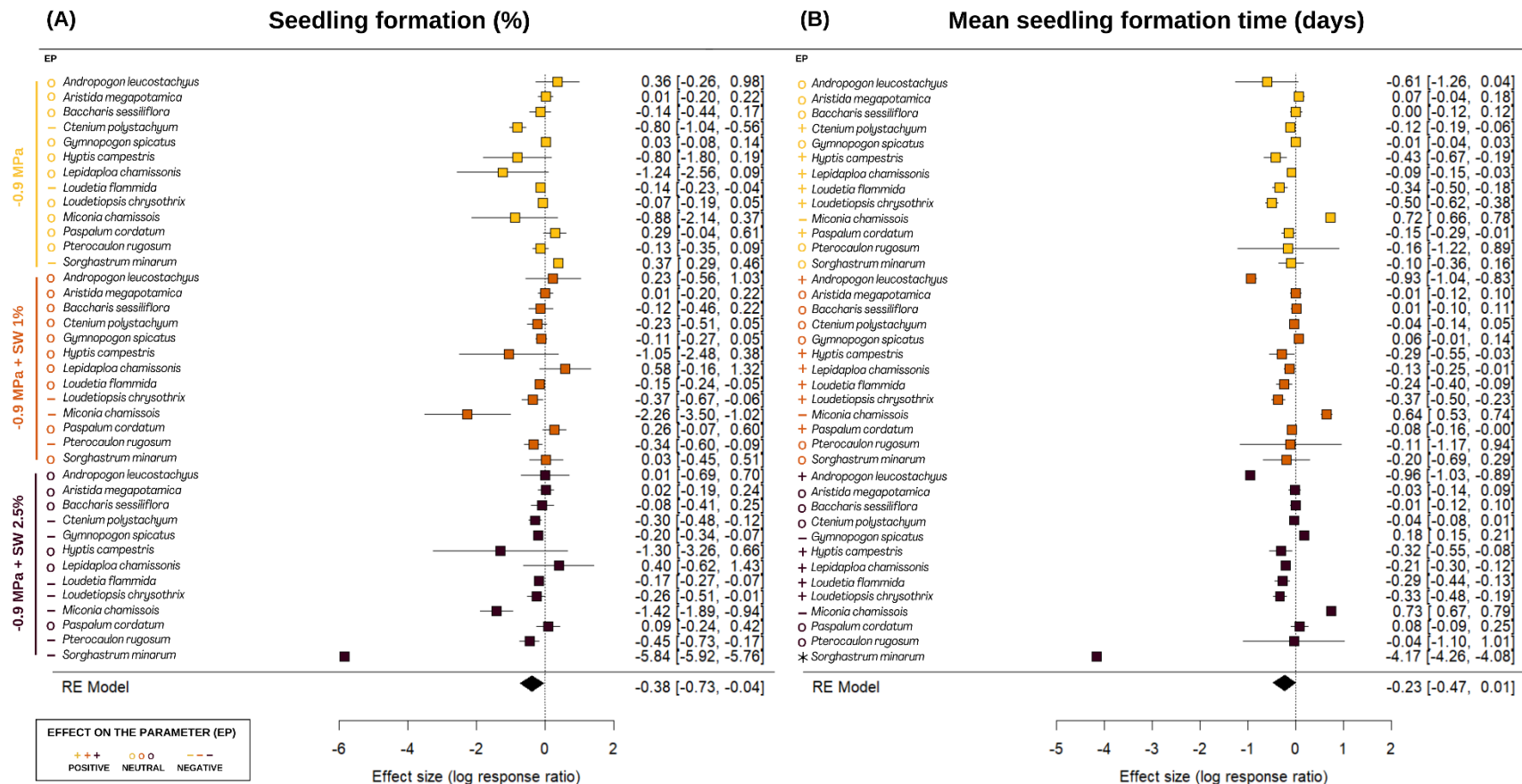


Figure 16. Effect size of osmotic potential and smoke water (SW) on post-germinative responses (**A** and **B**) of Cerrado ground-layer species in recovery treatments. The boxes represent the mean effect sizes and their corresponding 95% confidence intervals for each variable (numerical values for the mean and confidence intervals are displayed on the right). The vertical dashed line denotes the control treatment. Random-effects (RE) model: The diamond represents the pooled effect size and 95% confidence intervals. *: with only 1-2 replicates.

Discussion

In this study, we evaluated the germinative and post-germinative responses of Cerrado ground-layer species under a gradient of water potential reduction, both in isolation and combined with smoke, while also assessing the effects of these factors on post-stress recovery. As expected, water deficit acted as a filter on germination and, consequently, on seedling formation in Cerrado ground-layer species. Smoke, in turn, produced distinct effects on all parameters, being strongly species-specific and varying across developmental stages. Similarly, the recovery results showed that both water deficit levels and smoke exposure influenced different germinative and post-germinative responses.

Water is one of the primary limiting factors for germination (Baskin and Baskin, 2014), as imbibition reactivates seed metabolism (Simon, 1984). Thus, species subjected to water deficit are expected to experience reduced germination, as reported in the literature for other Cerrado species (Silva *et al.* 2001). The results presented here contribute to the limited research on the germinative behavior of Cerrado species under water deficit (Ribeiro and Kolb, 2016; Souza *et al.* 2022) and corroborate the diversity of species-specific tolerance responses to gradients of water potential reduction (Simão *et al.* 2013; Ribeiro and Kolb, 2016).

Germinative capacity, even under more negative osmotic potentials (-0.9 MPa), may indicate a broader germination niche (Braz *et al.* 2016), acting as a plastic trait that ensures germination in heterogeneous and seasonal environments, such as the Cerrado (Vayda *et al.* 2018). However, despite interspecific variability, a pattern of reduced germination percentage and increased mean germination time was observed, inversely proportional to the reduction in water potentials, suggesting that under conditions with less water, the germinative process may be compromised.

Rapid germination allows species to optimize on water availability pulses (Chesson *et al.* 2004). In contrast, prolonged MGT due to water deficit may increase exposure to desiccation, potentially leading to viability loss in recalcitrant seeds (Kijak and Ratajczak, 2020). Seeds with high desiccation tolerance, however, can remain viable in seed banks without damaging metabolic processes (Ribeiro and Borghetti, 2013; Gonçalves *et al.* 2020; Smolikova *et al.* 2021), persisting until favorable microclimatic conditions emerge for radicle protrusion and seedling establishment (Bewley *et al.* 2012; Souza *et al.* 2019; Soares *et al.* 2021). This strategy of staggered

germination events may enhance population survival in unstable environments (Ooi *et al.* 2009), minimizing mortality during unfavorable periods (Ellner, 1987).

Nevertheless, our results demonstrate that if water deficit persists, the few seeds capable of germinating under more negative osmotic potentials will exhibit lower seedling formation capacity. For instance, *Mimosa dolens* var. *rigida*, despite showing high germinability (88 ± 10 SD) under the less negative osmotic potential (-0.3 MPa), displayed severely inhibited seedling formation (2 ± 2 SD). This may occur because water limitation acts as a filter for post-germinative stages (Baskin and Baskin, 2014),

Given that seed germination is heavily influenced by climatic seasonality (Escobar *et al.* 2018) and that prolonged drought periods are predicted in this biome over the coming decades (Ferreira *et al.* 2024), the germinative responses reported here suggest negative impacts on seed bank emergence and species recruitment. While the proportion of seeder species - those regenerating exclusively via seeds - is relatively low in Cerrado (Fidelis *et al.* 2021), reductions in recruitment and genetic variability may severely impact both these species and those employing mixed regeneration strategies.

Additionally, although $\cong 67\%$ of species failed to achieve germination rates $>10\%$ at the most negative osmotic potential (-0.9 MPa), their ability to maintain viability and exhibit rapid germination during recovery suggests strategies for drought stress tolerance (Bhatt *et al.* 2022). Conversely, the considerable number of viable seeds that did not show radicle protrusion during recovery treatments may indicate the onset of secondary dormancy (Garcia *et al.* 2024). This mechanism allows seeds dispersed without dormancy to acquire it under stressful scenarios, thus increasing their chances of establishment under favorable conditions (Zhang *et al.* 2022). During recovery, even without water restriction, seeds of species such as *Ctenium polystachyum*, *Lepidaploa chamissonis*, and *Miconia chamissois*, exposed to the most negative potentials (-0.6 and -0.9 MPa), displayed lower germination rates than controls, indicating that this dormancy mechanism is highly complex.

On the other hand, the recovery capacity of some species, even after 30 days of exposure to water deficit conditions, may be linked to the phenomenon of water memory (Siddique *et al.* 2024). In scenarios with limited water availability, seeds can initiate the imbibition process and, before reaching radicle protrusion, undergo dehydration periods (Dantas *et al.* 2024). While they do not germinate, seeds undergo physiological, biochemical, and genetic changes that can provide advantages under stressful conditions or when conditions become favorable (Tian *et al.*, 2024). However,

passing through these cycles may have ecological implications for field establishment (Dias *et al.* 2024). Thus, in addition to seeds undergoing cycles that enable faster germination and capitalize on water availability pulses (Bicalho *et al.* 2024), seedlings from these seeds also show greater vigor (Lima and Meiado, 2018).

In line with recent studies on Cerrado ground-layer species, smoke exerts various effects on germinative parameters (Sanchez *et al.* 2022; Motta *et al.* 2024). Karrikins (KARs), a group of compounds isolated from smoke, play a significant role in this process, enhancing drought tolerance (Mousavinik *et al.* 2016; Li *et al.* 2017; Pandey *et al.* 2024). Moreover, depending on the stress and its intensity, smoke can induce different alterations in the plant body, regulating metabolic pathways (Aslam *et al.* 2024) or interacting with phytohormones (Li *et al.* 2020). This stimulation and protective effect of smoke and its compounds contribute not only to germination but also to seedling development (Singh *et al.* 2023), a result observed in this study for *Ctenium polystachyum*.

Therefore, this is the first report of the protective effect of smoke water for Cerrado species. However, despite the improvement in germinative or post-germinative parameters in some species, this effect is highly species-specific. Thus, although smoke exposure benefits germination under moderate stress conditions (-0.3 MPa), a contrary effect can be observed in seedling formation, as seen in *Loudetia flammida*, *Loudetiopsis chrysothrix*, and *Miconia chamissois*. It is important to emphasize that the effect on different developmental stages is also linked to smoke concentration. Studies with savanna woody species using aqueous smoke solutions of 1:500 (0.2%) demonstrated beneficial effects for both germination and seedling formation and vigor (Kulkarni *et al.* 2007). Similar results have been observed in studies with commercial species using aqueous solutions at a 1:1000 concentration (Moyo *et al.* 2022). However, as the smoke concentration increases, its beneficial effect on germination tends to decrease (Motta *et al.*, 2024). Therefore, in a climate change scenario, where reduced precipitation is expected (Santos *et al.* 2021), concentrated smoke solutions in the soil may negatively affect post-fire regeneration.

Despite this, the results presented here provide a foundation for further investigation into the germinative and post-germinative responses of the Cerrado ground-layer community. Our findings contribute to understanding the recruitment strategies under water deficit. They suggest that, while species exhibit drought tolerance responses, changes in recruitment dynamics are likely in future scenarios.

Consequently, smoke emerges as a potential research and application tool. Future studies with varying smoke concentrations are needed to evaluate its effects on ground-layer species under combined stresses (e.g., water deficit and elevated temperatures). Additionally, exploring an economically viable seed priming application in restoration projects could help produce more resilient plants to the changing climate.

Conclusion

This study demonstrates that even less negative water potentials negatively affect germinative and post-germinative parameters of Cerrado ground-layer species, with these effects intensifying under more negative potentials. However, while strongly species-specific and dependent on the developmental stage, smoke alleviate the stress induced by water deficit or produce neutral to negative effects. Under non-restrictive conditions, some species successfully resume germination and seedling formation, while others remain in secondary dormancy. Despite these varied responses, Cerrado species seeds maintain viability across all scenarios, demonstrating a complex control of the germination process.

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Supplementary data

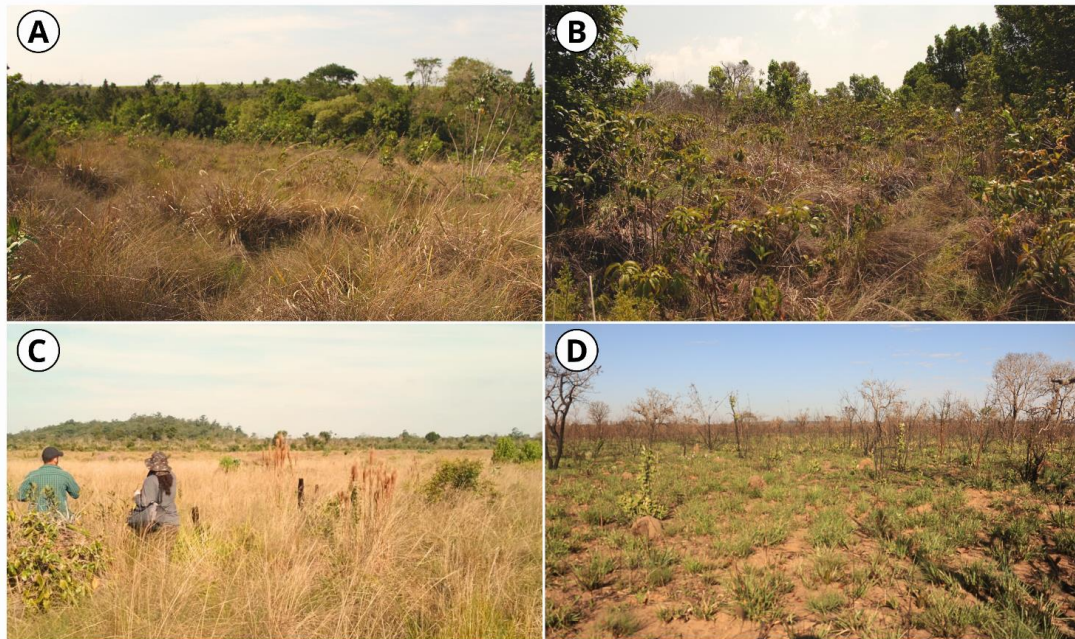


Figure S1. Savanna study areas. A and B: Assis Ecological Station; C and D: Santa Bárbara Ecological Station.

Table S1. Characterization of the environmental and edaphic factors in study areas. AAR: Average Annual Rainfall; AAT: Average Annual Temperature; AES: Assis Ecological Station; SBES: Santa Bárbara Ecological Station.

Factor	AES	SBES
Climate	Cfa ¹	Cwa ¹
AAR (mm)	1,450 ²	1,000 – 1,300 ³
AAT (°C)	≈ 22 ²	≈ 21 ³
Soil type	Oxisols and entisols ⁴	Oxisol ³

¹: Alvares *et al.* 2013; ²: Melo and Durigan, 2011; ³: Assis *et al.* 2011.

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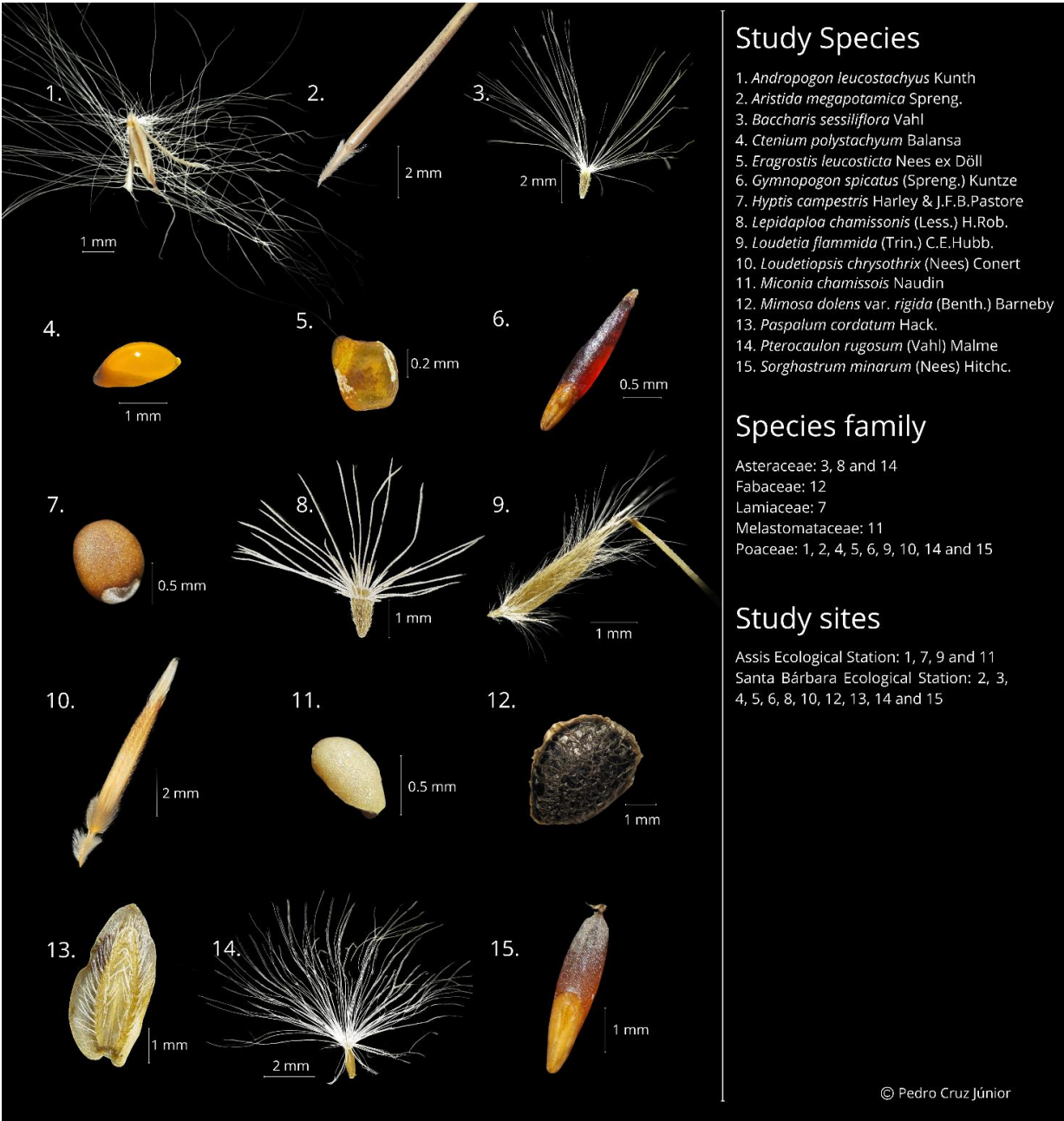


Figure S2. Diaspores of the study species collected under field conditions at Assis Ecological Station and Santa Bárbara Ecological Station, Brazil.

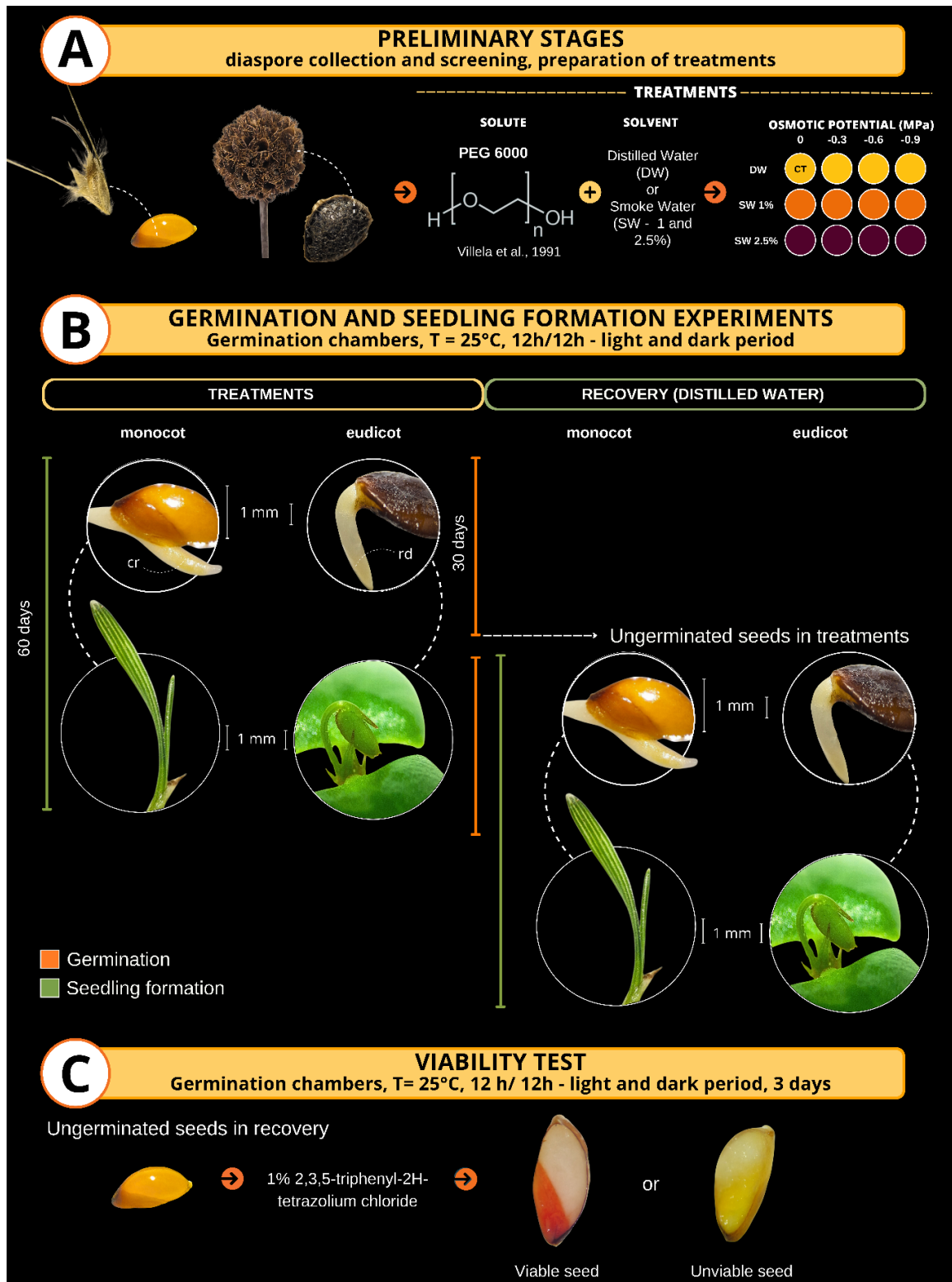


Figure S3. Stages for the preparation of treatments (A), germination experiments and seedling formation (B), and viability (C) of Cerrado ground-layer species under water deficit and smoke water treatments. **cr**:coleorhiza; **rd**: radicle.

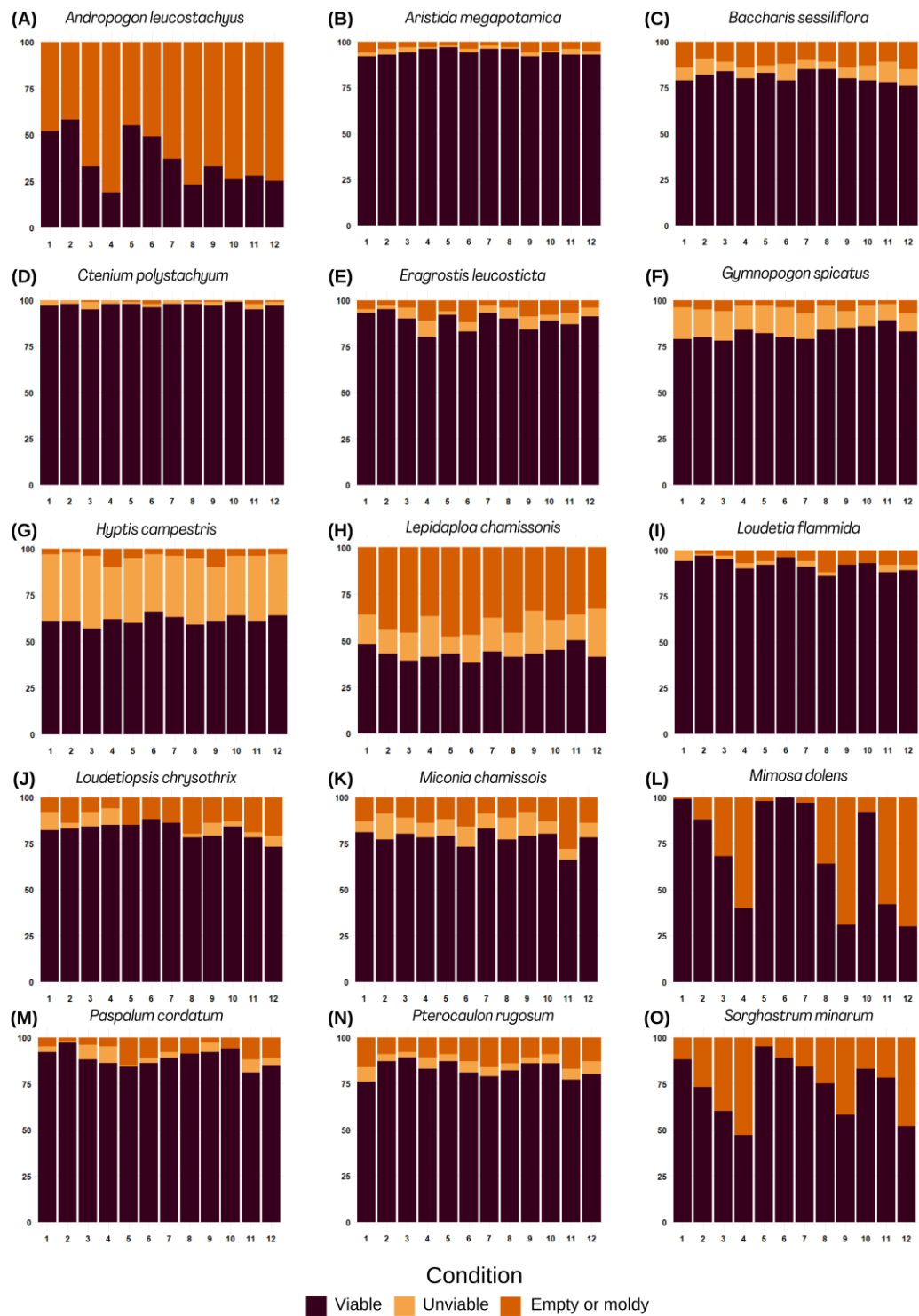


Figure S4. Percentage (y-axis) and condition of seeds from the Cerrado ground-layer under water deficit (MPa) and smoke water (SW) treatments (x-axis). Viability assessed through the tetrazolium test. Treatments: 1: control; 2: -0.3; 3: -0.6; 4: -0.9; 5: SW 1%; 6: SW 2.5%; 7: -0.3 + SW 1%; 8: -0.6 + SW 1%; 9: -0.9 + SW 1%; 10: -0.3 + SW 2.5%; 11: -0.6 + SW 2.5%; 12: -0.9 + SW 2.5%.

CONSIDERAÇÕES FINAIS

Ao avaliar o efeito das alterações ambientais promovidas por distúrbios antrópicos no Cerrado, observamos que a comunidade herbáceo-arbustiva é fortemente impactada. Os resultados evidenciam que essa comunidade está sujeita a filtros ambientais (e.g. sombreamento e déficit hídrico) desde a germinação, passando pela formação de plântulas, até a sobrevivência em condições alteradas. Nossas observações destacam, ainda, a importância de uma abordagem holística no desenvolvimento de políticas ambientais, especialmente no manejo do fogo no Cerrado, considerando que a supressão desse evento natural pode, indiretamente, reduzir a diversidade de espécies não-arbóreas.

Apesar das respostas funcionais das espécies, o sombreamento, mesmo em curto prazo, exerce uma forte filtragem sobre a comunidade, selecionando espécies mais tolerantes e generalistas para ocuparem as áreas alteradas. Além disso, a deficiência hídrica impactou drasticamente os parâmetros germinativos e a formação de plântulas de espécies herbáceo-arbustivas do Cerrado, o que, em condições naturais, pode resultar na redução do estabelecimento e da variabilidade genética dessas populações. No entanto, o efeito protetor da água de fumaça, até onde sabemos, é o primeiro caso relatado para espécies do Cerrado e pode servir como ponto de partida para investigações sobre seu potencial no cultivo de mudas e na semeadura direta para a restauração de campos degradados.

Diante dos processos de invasão lenhosa e do aumento do déficit hídrico em fisionomias campestres, tornam-se indispensáveis estudos de base como o aqui apresentado, que incorporem diferentes delineamentos experimentais, tanto em campo quanto em laboratório, para compreender as dinâmicas e os efeitos das alterações ambientais sobre a comunidade herbáceo-arbustiva do Cerrado. Estudos futuros podem integrar novas variáveis ambientais, possibilitando projetar os potenciais efeitos das mudanças climáticas globais sobre essa comunidade já impactada.