
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

**SELECIONANDO ESTRATÉGIAS ECOFISIOLÓGICAS EM
ESPÉCIES DO CERRADO: UMA ABORDAGEM UTILIZANDO
AUSÊNCIA E PRESENÇA DE DISTÚRBIO**

ARIADNE CRISTINA DE ANTONIO

**Rio Claro – SP
2024**

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ARIADNE CRISTINA DE ANTONIO

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Biologia Vegetal.
Orientador: Dr.Davi Rodrigo Rossatto

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Resumo

O Cerrado, uma das savanas mais biodiversas do mundo, enfrenta frequentemente distúrbios naturais como o fogo, que são cruciais na modelagem da composição e das estratégias ecofisiológicas de sua flora. Esses eventos não apenas influenciam a diversidade de espécies, mas também promovem a seleção de adaptações específicas que permitem às plantas sobreviver e prosperar em condições ambientais adversas. Compreender essas adaptações é essencial para prever como essas comunidades responderão às mudanças climáticas e aos métodos de gestão do fogo. Esta tese investiga as respostas fenológicas, anatômicas e fisiológicas das espécies do Cerrado em face ao regime de fogo anual e à ausência de fogo, explorando como essas respostas afetam a capacidade de recuperação e adaptação das plantas. Os objetivos específicos incluem avaliar as respostas fenológicas das comunidades vegetais à exposição ao fogo frequente, examinar as adaptações na morfologia e anatomia foliar, e investigar as adaptações fisiológicas e nutricionais das espécies em resposta à presença e ausência de fogo. As hipóteses testadas foram que: (1) espécies em áreas frequentemente queimadas apresentariam fenofases antecipadas devido a alterações no microclima e na disponibilidade de recursos após o fogo; (2) a exposição contínua ao fogo levaria ao desenvolvimento de características foliares mesomórficas, refletindo uma estratégia de mais aquisitiva de recursos; (3) espécies em áreas queimadas mostrariam maior eficiência fotossintética e concentração de nutrientes foliares, demonstrando adaptações das plantas para maximizar a captação de luz e nutrientes em ambientes perturbados. No Capítulo I, verificou-se que o fogo não alterou a fenologia das espécies expostas ao fogo frequente. No Capítulo II, constatou-se que, apesar de não haver diferenças significativas na maioria dos atributos foliares examinados entre as parcelas queimadas e não queimadas, a exposição anual ao fogo parece induzir o desenvolvimento de características mesomórficas, como uma maior área foliar específica. No Capítulo III, as áreas queimadas apresentaram maior concentração de fósforo no solo, enquanto as folhas apresentaram maior concentração de potássio. Além disso, houve um aumento significativo das taxas de troca gasosa, refletindo adaptações das plantas para maximizar a absorção de recursos em ambientes com alta disponibilidade de nutrientes pós-distúrbio. Os resultados desta pesquisa destacam a resiliência e a capacidade de adaptação das espécies do Cerrado a distúrbios recorrentes, como o fogo, mostrando poucas mudanças nos atributos foliares em regimes de fogo anuais e pouco intensos.

Palavras – chave: distúrbios de fogo; adaptações ecofisiológicas, fenologia vegetal, anatomia foliar, trocas gasosas.

Abstract

The Cerrado, one of the most biodiverse savannas in the world, frequently experiences natural disturbances such as fire, which play a crucial role in shaping the composition and ecophysiological strategies of its flora. These events not only impact species diversity but also drive the selection of specific adaptations that enable plants to survive and thrive in challenging environmental conditions. Understanding these adaptations is essential for predicting how these communities will respond to climate change and fire management practices. This thesis investigates the phenological, anatomical, and physiological responses of Cerrado species to annual fire regimes and the absence of fire, exploring how these responses influence the plants' recovery and adaptation capabilities. The specific objectives include evaluating the phenological responses of plant communities to frequent fire exposure, examining adaptations in leaf morphology and anatomy, and investigating the physiological and nutritional adaptations of species in response to fire presence or absence. The tested hypotheses were: (1) species in frequently burned areas would exhibit advanced phenophases due to changes in microclimate and resource availability after fire; (2) repeated fire exposure would lead to the development of mesomorphic leaf characteristics, reflecting a resource acquisition strategy; (3) species in burned areas would exhibit higher photosynthetic efficiency and foliar nutrient concentration, demonstrating plant adaptations to maximize light and nutrient capture in disturbed environments. In Chapter I, it was found that fire did not alter the phenology of species exposed to frequent fire. In Chapter II, it was observed that, despite no significant differences in most leaf attributes examined between burned and unburned treatments, annual fire exposure appears to induce the development of mesomorphic characteristics, such as a higher specific leaf area. In Chapter III, burned areas had higher phosphorus concentrations in the soil, while leaves showed higher potassium concentrations. Additionally, there was a significant increase in gas exchange rates, reflecting plant adaptations to maximize resource absorption in environments with high nutrient availability post-disturbance. The results of this research highlight the resilience and adaptability of Cerrado species to recurring disturbances such as fire, showing minimal changes in leaf attributes in annual and low-intensity fire regimes.

Key words: fire disturbance, ecophysiological adaptations, plant phenology, leaf anatomy, gas exchange.

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1.Introdução geral

As comunidades naturais estão sujeitas a fatores ambientais influenciadores que podem moldar não só a composição de espécies, como também selecionar estratégias ecofisiológicas (fenológicas, anatômicas e fisiológicas) ocorrentes em determinado local (Chapin et al., 1987; Lambers et al., 2008). Dentre os fatores influenciadores mais importantes encontram-se os distúrbios. White & Pickett (1985) definem distúrbio como “*qualquer evento relativamente discreto no tempo que modifique a estrutura das comunidades e ecossistemas e a disponibilidade de recursos, substrato ou o ambiente físico*”. Nesta definição, distúrbios podem ser caracterizados pela sua distribuição espacial, sua frequência e seu intervalo de retorno. Nos ecossistemas terrestres, erupções vulcânicas, queimadas, tempestades e alagamentos podem ser considerados distúrbios, pois são capazes de alterar aspectos estruturais dos ecossistemas, e removem a biomassa aérea das plantas, modificando disponibilidade de recursos e aspectos ambientais, como a disponibilidade de luz, de nutrientes e as temperaturas (Turner & Dale 1998; Scalon & Rossatto 2021).

Na América do Sul, sistemas tropicais e subtropicais estão sujeitos a distúrbios frequentes, caso dos ambientes savânicos, que estão representados no Cerrado brasileiro: um conjunto de fisionomias campestres, savânicas e florestais, onde destacam-se as formações savânicas, como o cerrado *sensu stricto* (também chamado de cerrado típico) (Coutinho 1982), um dos tipos fisionômicos do Cerrado mais dominantes e mais comumente presente em toda a extensão territorial do bioma (Ribeiro & Walter 2008). Neste tipo vegetacional pode ser encontrada uma grande diversidade de espécies vegetais (Gottsberger & Silberbauer-Gottsberger 2006; Simon et al., 2009), que compõe desde um estrato herbáceo-arbustivo dominante, até um estrato arbóreo que é mais esparsa (Oliveira-Filho & Ratter 2002). Nestes ambientes savânicos, a presença do fogo promove a estruturação de comunidades em termos de espécies, selecionando determinadas características para persistir sob este evento (Bond et al., 2004; Bond & Keeley 2005; Gottsberger & Silberbauer-Gottsberger 2006; Abreu et al., 2017; Fidelis 2020).

Para as espécies vegetais de cerrado típico, foi demonstrada a existência de características morfofisiológicas envolvendo estratégias de resistência, regeneração e sobrevivência para lidar com a perda da biomassa causada pela frequente passagem do fogo (Coutinho 2002; Hoffmann et al., 2009). Estas estratégias podem variar desde gemas protegidas; casca espessa que atua como isolante térmico dos tecidos vivos; rebrotas vigorosas em curto espaço de tempo; sistemas subterrâneos bem desenvolvidos, e até a presença de folhas escleromórficas,

que possuem características específicas para realizar altas taxas fotossintéticas, com forte controle estomático, em solos de baixa concentração mineral (Hoffmann et al., 2009; Hoffmann et al., 2012; Rossatto et al., 2013; Scalon et al., 2020; Wigley et al., 2020; Chiminazzo et al., 2021). Adicionalmente, ajustes fenológicos são importantes para a persistência sobre eventos de distúrbios, como por exemplo, a rápida produção e expansão de folhas novas durante o período seco para aproveitar as altas disponibilidades de recursos durante a estação chuvosa (Rossatto et al. 2009; Rossatto 2013; Massi et al., 2017).

A presença de uma casca espessa no tronco e nos ramos das espécies arbóreas permite isolar termicamente as gemas de crescimento vegetativo, de modo que as altas temperaturas do fogo não matem os meristemas e as plantas possam brotar rapidamente após a passagem do fogo (Vines 1968; Gignoux et al., 1997; Clarke et al., 2013; Pausas 2015; Rosell 2016; Massi & Franco 2016). O rebrotamento acima do solo também é comum para espécies não arbóreas, e funciona como outra estratégia para crescer rapidamente após a passagem do fogo e recuperar a biomassa aérea perdida, em caso de mortalidade do ramo (Hoffmann et al. 2009; Chiminazzo et al., 2021; 2023;). Isto só é possível devido à presença de sistemas subterrâneos bem desenvolvidos, os quais armazenam carboidratos oriundos das folhas, que possuem alta taxa fotossintética durante a estação chuvosa (Franco et al., 2005; Rossatto et al., 2013; Rossatto & Franco 2017; de Moraes et al., 2016), fornecendo nutrientes e carbono para a rebrota vigorosa (de Moraes et al. 2016, Silva & Rossatto 2019). Assim, estas reservas de carbono, aliadas a disponibilidade de nutrientes no solo, permitem uma rebrota vigorosa (Hoffmann et al., 2009), que possivelmente está associada a produção de folhas com estratégias aquisitivas para repor o carbono e os nutrientes perdidos pela queima da biomassa aérea pré-existente (Silva & Rossatto 2019).

Distúrbios como o fogo podem afetar a disponibilidade de recursos e consequentemente, a formação de novas folhas e de estruturas foliares diferenciadas, já que as folhas são estruturas extremamente plásticas, respondendo a uma série de fatores ambientais, como disponibilidade de água e nutrientes (Junior et al., 2013; Scoffoni et al., 2015). Acredita-se que ambientes expostos a distúrbios como o fogo irão selecionar espécies com estratégias diferentes daquelas que ocorrem em ambientes onde a disponibilidade de recursos e condições ambientais são mais estáveis (Scalon & Rossatto 2021). Deste modo, dependendo das características do ambiente as espécies tendem a possuir estratégias aquisitivas ou conservativas (Díaz et al., 2016). A ideia de colocar as estratégias das folhas dentro de um espectro, entre folhas de espécies com estratégias conservativas e aquisitivas, surgiu a partir de alguns estudos publicados por Ian Wright e colaboradores. Esses estudos propuseram o conceito do espectro

econômico das folhas, que descreve uma gama contínua de estratégias de alocação de recursos foliares (Wright et al., 2001; Wright et al., 2004). Nestes estudos foram analisados dados de taxas fotossintéticas, área foliar específica e teores de N e P foliares para espécies de todo o mundo. Este conceito foi aprimorado por uma série de pesquisadores (ver por exemplo Poorter & Bongers 2006; Wigley et al. 2016; Maracahipes et al., 2018; Pellegrini et al., 2023).

Folhas com atributos aquisitivos são selecionadas em ambientes com alta disponibilidade de recurso, ou seja, plantas com estratégia aquisitiva terão um alto valor de área foliar específica, alto teor de nitrogênio e fósforo na folha e elevadas taxas fotossintéticas, além de possuírem, rápido crescimento e grande utilização de recursos, tudo isso ligado a uma grande produção de folhas de curta duração (Wright et al., 2004; Díaz et al., 2016; Maracahipes et al., 2018). Em contraste, plantas com uma estratégia conservativa terão suas características funcionais selecionadas em situações com baixa capacidade de recursos e baixa capacidade de crescimento, assim apresentarão: baixa produção de folhas, baixa área foliar específica, baixos teores de nitrogênio e fósforo nas folhas e baixa taxa fotossintética, porém com longa longevidade foliar (Reich et al., 1995). Distúrbios podem direcionar a seleção de estratégias funcionais conservativas e aquisitivas em ecossistemas, sendo poucos trabalhos citando os efeitos dos distúrbios, especialmente o fogo, nas estratégias morfoanatômicas e de trocas gasosas foliares (Ackerly 2004; Pakemann et al. 2011; de Bello et al. 2013, Scalón & Rossatto 2021).

Considerando-se o fogo, alguns trabalhos demonstram o aumento substancial de nutrientes no solo quando este evento ocorre com certa frequência, e numa escala de curto prazo (da Silva & Batalha 2008; Miranda et al., 2009; Pivello et al. 2010; de Souza et al., 2016), podendo assim impactar a disponibilidade de recursos para formação de folhas. Em geral, eventos de fogo ao final da estação seca são seguidos das primeiras chuvas, o que disponibiliza grande quantidade de nutrientes para as plantas que tem padrões fenológicos vegetativos (especialmente produção de folhas) associados exatamente a esta época do ano (Rossatto 2013). A fenologia é um evento muito importante no ciclo de vida de uma planta e mudanças nos eventos fenológicos das plantas têm sido apontadas como indicadores de mudanças climáticas (Rodríguez et al., 2014), somado a isso, a frequência e a intensidade do fogo são altamente sensíveis às condições meteorológicas e provavelmente tem uma resposta rápida às mudanças climáticas (Hoffmann et al., 2003).

As respostas de crescimento das árvores (rebrotas e sobrevivência) e seus efeitos nas comunidades frente ao fogo são bem citadas na literatura (Hoffmann et al., 2009; Hoffmann et al. 2012; Charles – Dominique et al., 2015), recentemente alguns trabalhos também vem

avaliando os efeitos do fogo na vegetação herbácea e arbustiva (Durigan et al. 2020; Dairel & Fidelis, 2020; Pilon et al., 2021; Rodrigues et al. 2021,2022) entretanto, os aspectos ecofisiológicos relacionados com trocas gasosas e nutrição mineral e aspectos relacionados a fenologia e anatomia ecológica são raramente abordados para os diversos grupos funcionais de plantas das savanas tropicais considerando-se os efeitos causados pela frequência do fogo (de Souza et al. 2016), apesar de seu grande efeito na captação de recursos e consequentemente no funcionamento dos ecossistemas (estoques e processos de troca de carbono). Desta maneira, termos a compreensão das respostas funcionais foliares em plantas de diferentes formas de crescimento na presença e ausência de distúrbios é crucial num cenário de mudanças climáticas globais (Franco et al. 2014).

2. Objetivos

O presente estudo visa explorar como a ocorrência ou ausência de queimadas anuais influencia a seleção de estratégias morfo-funcionais foliares em diferentes grupos funcionais (arbóreos, arbustivos e subarbustivos) do Cerrado. Focaremos particularmente em aspectos como fenologia foliar, anatomia das folhas, trocas gasosas e nutrição mineral. Assim, o estudo será norteado pelas seguintes questões:

- I. Existem variações significativas nas fenologias vegetativas e reprodutivas das espécies que estão expostas a diferentes frequências de distúrbio: ausência e presença de fogo anual? A sazonalidade das fenofases difere entre diferentes formas de crescimento e na comunidade de plantas de locais queimados em comparação com os não queimados? A ocorrência de distúrbios como o fogo promove a seleção de espécies com maior longevidade foliar? Há um aumento no crescimento dos ramos em ambientes com frequente incidência de fogo?
- II. Qual o impacto de incêndios recorrentes nas características anatômicas e morfológicas das folhas de espécies nativas do Cerrado? Em regiões sujeitas a fogo anual, as espécies tendem a produzir folhas com estratégias morfoanatômicas mais mesomórficas (aquisitivas) em relação a regiões sem presença de fogo, onde as folhas seriam mais escleromórficas?
- III. O distúrbio produz alterações nas taxas de trocas gasosas e na quantidade de macronutrientes foliares em indivíduos sob fogo anual e sob a ausência de

fogo? Como podem estas diferenças estar ligadas às alterações na disponibilidade de nutrientes do solo resultantes do fogo?

3. Hipóteses

Hipótese geral: Ambientes que são expostos a distúrbios frequentes irão selecionar espécies com estratégias foliares aquisitivas (folhas mesomórficas, com curta duração de vida, alta capacidade fotossintética, alto teor de nutrientes foliares, porém com baixa eficiência no uso de nutrientes); por outro lado ambientes sem queima, em que o distúrbio não acontece, as espécies apresentarão folhas com estratégias conservativas (folhas escleromórficas, com alta duração de vida, baixa capacidade fotossintética e baixo teor de nutrientes foliares e alta eficiência no uso de nutrientes).

Assim, especificamente espera-se que:

Hipótese (1): em ambientes com fogo frequente as fenofases serão adiantadas, acelerando o início de atividades fenológicas como brotação, queda foliar (senescência) e floração. Assim, o fogo irá alterar a sazonalidade, rompendo a sincronia dos eventos fenológicos ao adiantar fenofases em espécies expostas ao fogo. Espécies sob regimes frequentes de fogo irão exibir redução na longevidade foliar e desenvolvimento de ramos maiores com crescimento acelerado, como estratégias para otimizar a captação de luz e recuperação pós-fogo.

Hipótese (2): esperamos que as folhas expostas ao fogo frequente (anual) mostrem uma diminuição na espessura geral, favorecendo uma maior proporção de mesofilo, exibindo características mesomórficas mais pronunciadas, resultando em um aumento da área foliar específica.

Hipótese (3): em áreas expostas ao fogo as plantas apresentarão maior eficiência fotossintética aumentando suas taxas fotossintéticas e consequentemente a assimilação de carbono em comparação a ambientes onde não há a presença do fogo, também esperamos que a concentração de nutrientes foliares seja maior, devido ao aumento dos mesmos no solo após a passagem do fogo.

As hipóteses desta tese estão resumidas no seguinte esquema teórico:

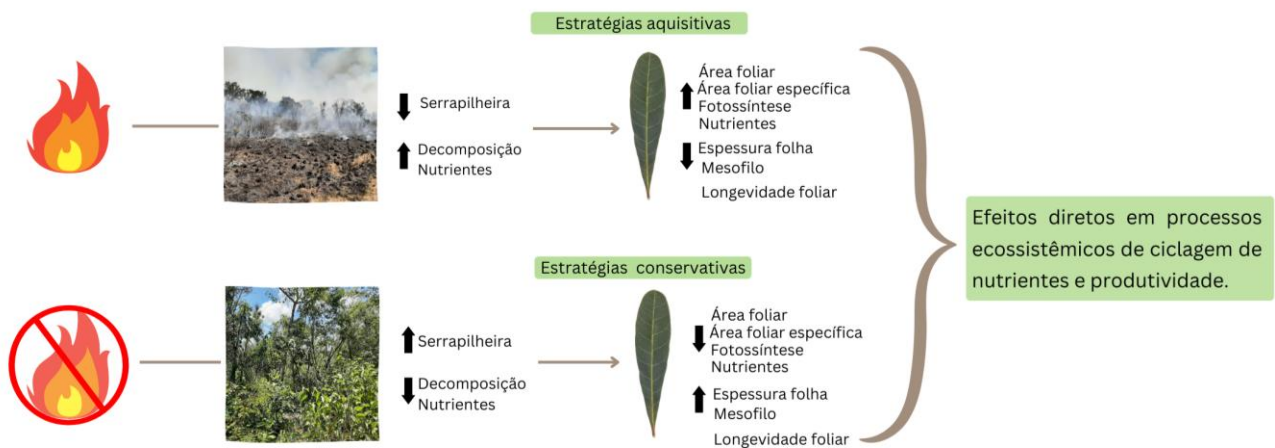


Figura 1. Background teórico resumido e resultados esperados baseados na hipótese proposta para este estudo.

Desenho experimental

Os três capítulos deste estudo foram desenvolvidos na mesma localidade, a Estação Ecológica de Santa Bárbara (EESB), situada no município de Águas de Santa Bárbara, no estado de São Paulo. Desde 2014, esta estação tem sido o local de implementação de práticas de manejo de fogo, nossas coletas foram realizadas entre os anos de 2020 e 2022. As áreas de estudo estão divididas em seis parcelas: três delas são submetidas a queimas prescritas anualmente, enquanto as outras três servem como controle, onde o manejo do fogo não é praticado há mais de 20 anos. Na Figura 2, descrevemos a disposição geográfica das parcelas queimadas e não queimadas para facilitar a compreensão da distribuição espacial e da metodologia empregada.

Na coleta de dados adotada nos três capítulos da tese foram selecionadas 17 espécies distintas, distribuídas equitativamente entre três parcelas submetidas a queima anual e três controle (sem queima), sem manejo de fogo (Figura 3). Cada parcela contém pelo menos um representante de cada espécie selecionada. É importante notar que, em determinados capítulos, algumas espécies podem estar ausentes nos dados coletados devido a danos causados por fogo, geada ou queda de árvores, como ocorreu com a espécie *Tontelea micrantha* no capítulo dedicado à fenologia.

No ano de 2021, entre os meses de junho e julho, foram registrados sete eventos de geada severa na EESB, com temperaturas chegando a -4°C . Essas geadas afetaram as parcelas selecionadas para o estudo, e consequentemente várias espécies que haviam sido selecionadas para coleta dos atributos fotossintéticos e fenológicos sofreram danos (De Antonio et al., 2023). Ao longo desta tese, as parcelas submetidas a queimas anuais serão referidas como

"burned" e as não submetidas como "unburned". Além disso, o termo “tratamento” é alternativamente empregado para especificar cada condição de manejo das parcelas. A organização e o planejamento experimental são ilustrados de maneira esquemática na Figura 3, que detalha a disposição das parcelas dentro da EEsB.

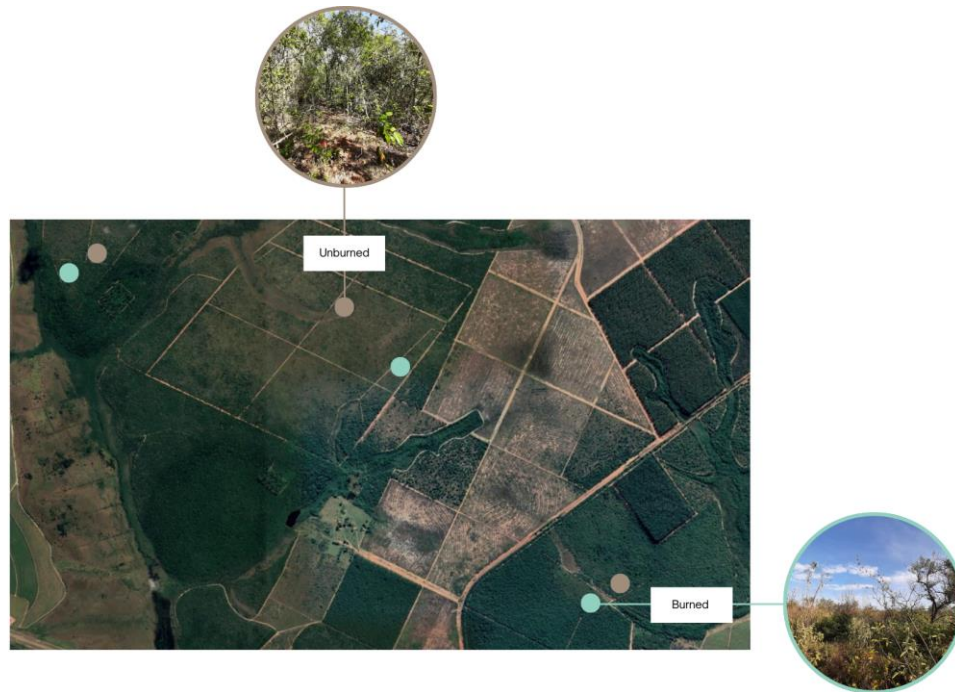


Figura 2. Imagem aérea do local de coleta localizado na Estação Ecológica de Santa Bárbara, em Águas de Santa Bárbara. Na imagem os círculos verdes representam as parcelas queimadas, enquanto os círculos cinzas representam as parcelas sem o tratamento com fogo.



Figura 3. Esquema demonstrativo do desenho experimental das coletas de dados dos 3 capítulos da tese.

Esta tese de doutoramento está organizada em três capítulos:

Chapter I: Este capítulo abordou as respostas fenológicas das comunidades de plantas do Cerrado aos distúrbios causados pelo fogo. Ele explorou como o fogo estimula ou inibe fenômenos como a floração, queda foliar e brotação, e como diferentes formas de crescimento vegetal respondem a esses distúrbios. Os principais objetivos do estudo foram entender como o fogo influenciou a fenologia vegetativa e reprodutiva, examinando as variações nas fenofases vegetativas e reprodutivas entre espécies expostas a diferentes frequências de distúrbio (áreas queimadas e não queimadas). O estudo também investigou se a ocorrência de distúrbios como o fogo promove a seleção de espécies com maior longevidade foliar e se há um aumento no crescimento dos ramos em ambientes com incidência frequente de fogo. Os resultados mostraram que o fogo não alterou significativamente os padrões sazonais de fenofases vegetativas e reprodutivas entre as áreas queimadas e não queimadas, desafiando algumas das hipóteses iniciais.

Chapter II: Este capítulo detalhou as adaptações na morfologia e anatomia foliar em resposta a variações ambientais, influenciadas pelo manejo de fogo na Estação Ecológica de Santa Bárbara. O estudo examinou características como espessura do parênquima paliçádico e esponjoso, epiderme adaxial e abaxial, área total da folha, e área foliar específica, em parcelas submetidas a queimas anuais comparadas com parcelas sem queima. O estudo propôs que a exposição anual ao fogo poderia levar ao desenvolvimento de características foliares mesomórficas, indicativas de uma estratégia de aquisição de recursos, resultando em uma maior área foliar específica. Os resultados mostraram que as plantas em áreas queimadas tendem a exibir características dentro do espectro aquisitivo, como maior área foliar específica, devido ao ressurgimento vigoroso após incêndios.

Chapter III: Este capítulo investigou as adaptações fisiológicas e respostas nutricionais de espécies do Cerrado a ausência e presença de fogo. Focando especificamente nas taxas de trocas gasosas e concentrações de nutrientes foliares, exploramos como os incêndios influenciam a assimilação de carbono e a dinâmica de nutrientes em comunidades vegetais. Os resultados indicaram pouca variação na concentração de nutrientes foliares, e variações significativas nas taxas de fotossíntese e transpiração entre plantas expostas a regimes anuais de fogo em comparação com aquelas em ambientes livres de fogo, refletindo uma adaptação estratégica para melhorar o armazenamento de carboidratos e garantir a persistência individual no caso de futuros incêndios.

4.Referências

- Abreu, R.C., Hoffmann, W.A., Vasconcelos, H.L., Pilon, N.A., Rossatto, D.R., & Durigan, G. (2017). The biodiversity cost of carbon sequestration in tropical savanna. *Science Advances*, 3(8), e1701284. <https://doi.org/10.1126/sciadv.1701284>
- Ackerly, D. (2004). Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs*, 74(1), 25-44. <https://doi.org/10.1890/03-4022>
- Bond, W.J., Woodward, F.I., & Midgley, G.F. (2004). The global distribution of ecosystems in a world without fire. *New Phytologist*, 165(2), 525–538. <https://doi.org/10.1111/j.1469-8137.2004.01252.x>
- Bond, W.J., & Keeley, J.E. (2005). Fire as a global “herbivore”: The ecology and evolution of flammable ecosystems. *Trends in Ecology and Evolution*, 20(7), 387–394. <https://doi.org/10.1016/j.tree.2005.04.025>
- Charles-Dominique, T., Beckett, H., Midgley, G.F., & Bond, W.J. (2015). Bud protection: A key trait for species sorting in a forest-savanna mosaic. *New Phytologist*, 207(4), 1052–1060. <https://doi.org/10.1111/nph.13406>
- Chapin, F.S., Bloom, A.J., Field, C.B., & Waring, R.H. (1987). Plant responses to multiple environmental factors. *Bioscience*, 37(1), 49-57. <https://doi.org/10.2307/1310177>
- Chiminazzo, M.A., Bombo, A.B., Charles-Dominique, T., & Fidelis, A. (2021). Your best buds are worth protecting: Variation in bud protection in a fire-prone cerrado system. *Functional Ecology*, 35, 2424–2434. <https://doi.org/10.1111/1365-2435.13907>
- Chiminazzo, M.A., Bombo, A.B., Charles-Dominique, T., & Fidelis, A. (2023). To protect or to hide: Why not both? An investigation of fire-related strategies in Cerrado woody species. *Flora*, 306, 152350. <https://doi.org/10.1016/j.flora.2023.152350>
- Clarke, P.J., Lawes, M.J., Midgley, J.J., Lamont, B.B., Ojeda, F., Burrows, G.E., Enright, N.J., & Knox, K.J.E. (2013). Resprouting as a key functional trait: How buds, protection and resources drive persistence after fire. *New Phytologist*, 197(1), 19–35. <https://doi.org/10.1111/nph.12001>
- Coutinho, L.M. (1982). Ecological effects of fire in Brazilian cerrado. In: Ecology of tropical savannas. Berlin, Heidelberg: *Springer*, pp. 273–291.
- Coutinho, L.M. (2002). O Bioma Cerrado. In A.L. Klein (Org.), Eugen Warming e o cerrado brasileiro: Um século depois (pp. 273-291). São Paulo: *UNESP, Imprensa Oficial do Estado*.
- Dairel, M., & Fidelis, A. (2020). How does fire affect germination of grasses in the Cerrado? *Seed Science Research*, 30(4), 275–283. <https://doi.org/10.1017/S0960258520000094>

- da Silva, D.M., & Batalha, M.A. (2008). Soil–vegetation relationships in cerrados under different fire frequencies. *Plant and Soil*, 311(1-2), 87-96. <https://doi.org/10.1007/s11104-008-9651-5>
- de Antonio, A.C., Scalon, M.C., & Rossatto, D.R. (2023). Leaf size and thickness are related to frost damage in ground layer species of Neotropical savannas. *Flora*, 299, 152208. <https://doi.org/10.1016/j.flora.2022.152208>
- de Bello, F., Vandewalle, M., Reitalu, T., Lepš, J., Prentice, H.C., Lavorel, S., & Sykes, M.T. (2013). Evidence for scale-and disturbance-dependent trait assembly patterns in dry semi-natural grasslands. *Journal of Ecology*, 101(5), 1237-1244. <https://doi.org/10.1111/1365-2745.12133>
- de Moraes, M.G., de Carvalho, M.A.M., Franco, A.C., Pollock, C.J., & Figueiredo-Ribeiro, R.D.C.L. (2016). Fire and drought: Soluble carbohydrate storage and survival mechanisms in herbaceous plants from the Cerrado. *BioScience*, 66, 107–117. <https://doi.org/10.1093/biosci/biv178>
- de Souza, M.C., Rossatto, D.R., Cook, G.D., Fujinuma, R., Menzies, N.W., Morellato, L.P.C., & Habermann, G. (2016). Mineral nutrition and specific leaf area of plants under contrasting long-term fire frequencies: A case study in a mesic savanna in Australia. *Trees*, 30(1), 329-335. <https://doi.org/10.1007/s00468-015-1285-1>
- Díaz, S., Kattge, J., Cornelissen, J.H., Wright, I.J., Lavorel, S., Dray, S., & Garnier, E. (2016). The global spectrum of plant form and function. *Nature*, 529(7585), 167. <https://doi.org/10.1038/nature16489>
- Durigan, G., Pilon, N., Abreu, R., Hoffmann, W., Martins, M., Fiorillo, B., Antunes, A., Carmignotto, A., Maravalhas, J., Vieira, J., & Vasconcelos, H. (2020). No net loss of species diversity after prescribed fires in the Brazilian savanna. *Frontiers in Forests and Global Change*, 3, 1-15. <https://doi.org/10.3389/ffgc.2020.00013>
- Fidelis, A. (2020). Is fire always the “bad guy”? *Flora: Morphology, Distribution, Functional Ecology of Plants*, 268(May), 151611. <https://doi.org/10.1016/j.flora.2020.151611>
- Franco, A.C., Bustamante, M., Caldas, L.S., Goldstein, G., Meinzer, F.C., Kozovits, A.R., Rundel, P., & Coradin, V.T.R. (2005). Leaf functional traits of Neotropical savanna trees in relation to seasonal water deficit. *Trees*, 19, 326-335. <https://doi.org/10.1007/s00468-004-0394-z>
- Franco, A.C., Rossatto, D.R., Silva, L.C.R., & Ferreira, C.S. (2014). Cerrado vegetation and global change: The role of functional types, resource availability and disturbance in regulating plant community responses to rising CO² levels and climate warming. *Theoretical and Experimental Plant Physiology*, 26(1), 19-38. doi 10.1007/s40626-014-0002-6
- Gignoux, J., Clobert, J., & Menaut, J. (1997). Alternative fire resistance strategies in savanna trees. *Oecologia*, 110, 576-583. <https://doi.org/10.1007/s004420050198>

Gottsberger, G., & Silberbauer-Gottsberger, I. (2006). Life in the cerrado: A South American tropical seasonal ecosystem. Vol I *Origin, Structure, Dynamics and Plant use*. Ulm: Reta Verlag.

Hoffmann, W.A., & Solbrig, O.T. (2003). The role of topkill in the differential response of savanna woody species to fire. *Forest Ecology and Management*, 180(1-3), 273–286. [https://doi.org/10.1016/S0378-1127\(02\)00566-2](https://doi.org/10.1016/S0378-1127(02)00566-2)

Hoffmann, W.A., da Silva, E.R., Machado, G.C., Bucci, S.J., Scholz, F.G., Goldstein, G., & Meinzer, F.C. (2005). Seasonal leaf dynamics across a tree density gradient in a Brazilian savanna. *Oecologia*, 145(2), 306-315. <https://doi.org/10.1007/s00442-005-0110-8>

Hoffmann, W.A., Adasme, R., Haridasan, M., Carvalho, M.T., Geiger, E.L., Pereira, M.A., & Franco, A.C. (2009). Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. *Ecology*, 90(5), 1326-1337. <https://doi.org/10.1890/08-0741.1>

Hoffmann, W.A., Geiger, E.L., Gotsch, S.G., Rossatto, D.R., Silva, L.C., Lau, O.L., Haridasan, M., & Franco, A.C. (2012). Ecological thresholds at the savanna-forest boundary: How plant traits, resources and fire govern the distribution of tropical biomes. *Ecology Letters*, 15(7), 759-768. <https://doi.org/10.1111/j.1461-0248.2012.01789.x>

Junior, J.J.D.M.S., Rossatto, D.R., Kolb, R.M., & Bruno, O.M. (2013). A computer vision approach to quantify leaf anatomical plasticity: A case study on *Gochnatia polymorpha* (Less.) Cabrera. *Ecological Informatics*, 15, 34-43. <https://doi.org/10.1016/j.ecoinf.2013.02.007>

Lambers, H., Chapin, F.S., & Pons, T.L. (2008). Plant physiological ecology. *Springer*, New York, NY. <https://doi.org/10.1007/978-0-387-78341-3>

Maracahipes, L., Carlucci, M.B., Lenza, E., Marimon, B.S., Marimon, B.H., Guimarães, F.A.G., & Cianciaruso, M.V. (2018). How to live in contrasting habitats? Acquisitive and conservative strategies emerge at inter- and intraspecific levels in savanna and forest woody plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 34, 10-21. <https://doi.org/10.1016/j.ppees.2018.07.006>

Massi, K.G. & Franco, A.C. (2016). How does resprouting response differ among three species of savanna trees and in relation to plant size? *Acta Botanica Brasilica* - 30(4): 693-699. doi: 10.1590/0102-33062016abb0264

Massi, K.G., Eugênio, C.U.O., and Franco, A.C. (2017). Post-fire reproduction of herbs at a savanna-gallery forest boundary in Distrito Federal, Brazil. *Braz. J. Biol.* 77 (4), 876-886. <https://doi.org/10.1590/1519-6984.06416>

Miranda, H.S., Sato, M.N., Neto, W.N., & Aires, F.S. (2009). Fires in the cerrado, the Brazilian savanna. In: Tropical Fire Ecology (pp. 427-450). Berlin, Heidelberg: *Springer*. https://doi.org/10.1007/978-3-540-77381-8_14

Oliveira-Filho, A.T., & Ratter, J.A. (2002). Vegetation physiognomies and woody flora of the Cerrado Biome. In P.S. Oliveira & R.J. Marquis (Eds.), *The Cerrados of Brazil: Ecology and natural history of a neotropical savanna* (pp. 91-120). *New York: Columbia University Press.*

Pakemann, R.J., Lennon, J.J., & Brooker, R.W. (2011). Trait assembly in plant assemblages and its modulation by productivity and disturbance. *Oecologia*, 167(1), 209-218. <https://doi.org/10.1007/s00442-011-1980-6>

Pausas, J. (2015). Bark thickness and fire regime. *Functional Ecology*, 29(3), 315-327. <https://doi.org/10.1111/1365-2435.12372>

Pellegrini, A.F.A., Anderegg, L., Pinto-Ledezma, J.N., Cavender-Bares, J., Hobbie, S.E., & Reich, P.B. (2023). Consistent physiological, ecological and evolutionary effects of fire regime on conservative leaf economics strategies in plant communities. *Ecology Letters*, 26(3), 597–608. <https://doi.org/10.1111/ele.14182>

Pilon, N.A.L., Durigan, G., Rickenback, J., Pennington, R.T., Dexter, K.G., Hoffmann, W.A., Abreu, R.C.R., & Lehmann, C.E.R. (2021). Shade alters savanna grass layer structure and function along a gradient of canopy cover. *Journal of Vegetation Science*, 32(1). <https://doi.org/10.1111/jvs.12959>

Pivello, V.R., Oliveras, I., Miranda, H.S., Haridasan, M., Sato, M.N., & Meirelles, S.T. (2010). Effect of fires on soil nutrient availability in an open savanna in Central Brazil. *Plant and Soil*, 337(1-2), 111-123. <https://doi.org/10.1007/s11104-010-0508-x>

Poorter, L., & Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, 87(7), 1733-1743. [https://doi.org/10.1890/0012-9658\(2006\)87\[1733:LTAGPO\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1733:LTAGPO]2.0.CO;2)

Reich, P. B. (1995). Phenology of tropical forests: patterns, causes and consequences. *Canadian Journal of Botany*, 73, 164-174. <https://doi.org/10.1139/b95-020>

Ribeiro, J.F. & Walter, B.M.T. (2008). As principais fisionomias do bioma Cerrado. In: S.M. Sano, S.P. Almeida and J.F. Ribeiro, eds. *Cerrado: ecologia e flora*. Brasília: *Embrapa Informação Tecnológica*, pp. 151-199.

Rodrigues, C.A., Zironi, H.L., & Fidelis, A. (2021). Fire frequency affects fire behavior in open savannas of the Cerrado. *Forest Ecology and Management*, 482. <https://doi.org/10.1016/j.foreco.2020.118850>

Rodrigues, C.A., & Fidelis, A. (2022). Should we burn the Cerrado? Effects of fire frequency on open savanna plant communities. *Journal of Vegetation Science*, 33(6). <https://doi.org/10.1111/jvs.13159>

Rodriguez, H.G., Maiti, R., & Sarkar, N.C. (2014). Phenology of woody species: A review. *International Journal of Bio-Resource and Stress Management*, 5(3), 436. <https://doi.org/10.5958/0976-4038.2014.00595.8>

Rosell, J.A. (2016). Bark thickness across the angiosperms: More than just fire. *New Phytologist*, 211(1), 90–102. <https://doi.org/10.1111/nph.13889>

- Rossatto, D.R., Hoffmann, W.A., & Franco, A.C. (2009). Differences in growth patterns between co-occurring forest and savanna trees affect the forest–savanna boundary. *Functional Ecology*, 23, 689–698. <https://doi.org/10.1111/j.1365-2435.2009.01568.x>
- Rossatto, D.R., Hoffmann, W.A., Silva, L.C.R., Haridasan, M., Sternberg, L.S., & Franco, A.C. (2013). Seasonal variation in leaf traits between congeneric savanna and forest trees in Central Brazil: Implications for forest expansion into savanna. *Trees*, 27(4), 1139–1150. <https://doi.org/10.1007/s00468-013-0864-2>
- Rossatto, D.R., & Franco, A.C. (2017). Expanding our understanding of leaf functional syndromes in savanna systems: The role of plant growth form. *Oecologia*, 183(4), 953–962. doi: 10.1007/s00442-017-3815-6
- Scalon, M.C., Domingos, F.M.C.B., Cruz, W.J.A.da, Júnior, B.H.M., Marimon, B.S., Oliveras, I., (2020). Diversity of functional trade-offs enhances survival after fire in Neotropical savanna species. *J. Veg. Sci.* 31, 139–150. <https://doi.org/10.1111/jvs.12823>
- Scalon, M. C., and Rossatto, D. R. (2021). Ground layer Cerrado plants sustain higher maximum photosynthetic rates after medium-term fire events. *Flora: Morphology, Distribution, Functional Ecology of Plants*, 285. <https://doi.org/10.1016/j.flora.2021.151962>
- Scoffoni C., Kunkle J., Pasquet-Kok J., Vuong C., Patel A.J., Montgomery R.A., Givnish T.J., and Sack L. (2015). Light-induced plasticity in leaf hydraulics, venation, anatomy, and gas exchange in ecologically diverse Hawaiian lobeliads. *New Phytologist*. 207(1), 43–58. doi: 10.1111/nph.13346.
- Silva, B.H.P., & Rossatto, D.R. (2019). Are underground organs able to store water and nutrients? A study case in non-arboreal species from the Brazilian Cerrado. *Theoretical and Experimental Plant Physiology*, 31(3), 413–421. <https://doi.org/10.1007/s40626-019-00155-9>
- Simon, M.F., Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T., Hughes, C.E., & Queiroz, L.P. (2009). Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Sciences*, 106, 20359–20364. <https://doi.org/10.1073/pnas.0903410106>
- Turner, M.G., & Dale, V.G. (1998). Comparing large, infrequent disturbances: What have we learned? *Ecosystems*, 1, 493–496. <https://doi.org/10.1007/s100219900045>
- Vines, R.G. (1968). Heat transfer through bark and the resistance of trees to fire. *Australian Journal of Botany*, 16, 499–514. <https://doi.org/10.1071/BT9680499>
- White, P.S., & Pickett, S.T.A. (1985). Natural disturbance and patch dynamics: An introduction. In S.T.A. Pickett & P.S. White (Eds.), *The ecology of natural disturbance and patch dynamics* (pp. 3–13). New York: Academic.
- Wigley, B.J., Slingsby, J.A., Díaz, S., Bond, W.J., Fritz, H., & Coetsee, C. (2016). Leaf traits of African woody savanna species across climate and soil fertility gradients: Evidence for conservative versus acquisitive resource-use strategies. *Journal of Ecology*, 104(5), 1357–1369. <https://doi.org/10.1111/1365-2745.12598>

Wigley, B.J., Charles-Dominique, T., Hempson, G.P., Stevens, N., Tebeest, M., Archibald, S., Bond, W.J., Bunney, K., Coetsee, C., Donaldson, J., Fidelis, A., Gao, X., Gignoux, J., Lehmann, C., Massad, T.J., Midgley, J.J., Millan, M., Schwilk, D., Siebert, F., Solofondranohatra, C., Staver, A.C., Zhou, Y., & Kruger, L.M. (2020). A handbook for the standardised sampling of plant functional traits in disturbance-prone ecosystems, with a focus on open ecosystems. *Australian Journal of Botany*, 68(8), 473-531. <https://doi.org/10.1071/BT20048>

Wright, I.J., Reich, P.B., & Westoby, M. (2001). Strategy shifts in leaf physiology, structure and nutrient content between species of high-and low-rainfall and high-and low-nutrient habitats. *Functional Ecology*, 15(4), 423-434. <https://doi.org/10.1046/j.0269-8463.2001.00542.x>

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, F.S., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J., & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428, 821–827. <https://doi.org/10.1038/nature02403>

Chapter I

Time for a Change? Phenological Responses of plants subjected or not to annual fire in the Cerrado



5. Introduction

Fire is a significant environmental factor in savannas (Pausas and Bond, 2019). It has the ability to impact species composition, plant growth, filtering the phylogenetic and functional structure of these vegetations (Silva and Batalha, 2010; Cianciaruso et al., 2012; Dantas et al., 2013, Dantas et al., 2016). Natural fires are of common occurrence in savanna regions during the transition from the dry to the wet season, often ignited by lightning strikes, resulting in surface burns that primarily consume the fine biomass of the herbaceous layer, especially grasses (Miranda et al., 2009; Oliveiras et al., 2013; Oyedeji et al., 2016). The regular occurrence of these fires over geological time has played a crucial role in the evolution of savannas, significantly influencing their structure, dynamics, and biodiversity significantly (Andersen, 2003; Durigan and Ratter, 2006; Durigan, 2020). This natural selection process favors species with morphological and physiological adaptations that enable survival and regeneration in environments subject to this type of disturbance (Lamont and He, 2017; Keeley and Pausas, 2022).

The Cerrado, among savannas, harbors the world's richest flora (Klink and Machado, 2005), known for its vast biodiversity and adaptability to seasonal climatic conditions. It is the second largest vegetative formation in the country and a biodiversity hotspot (Ratter et al., 1997; Myers et al., 2000). The Central Brazilian Cerrado stands out for its rich diversity of grasses, herbs, and woody plants, with noticeable variation in vegetative structure across the landscape, particularly along topographic gradients in watersheds (Rossatto et al., 2023). This diversity of life forms is essential for the adaptation of plant communities to the challenging environmental conditions typical of the Cerrado, such as marked rainfall seasonality, nutrient-poor soils, and recurrent fires (Franco et al., 2014). One observed adaptive strategy includes the development of leaves with short life longevity (Keeley et al., 2011), allowing for faster replacement and facilitating plant adaptation to environmental fluctuations, maximizing growth during the wet season (Vico et al., 2015).

In addition to fire, savannas are also affected by other disturbances, such as frost, which play a crucial role in the dynamics and maintenance of this vegetation (Childes and Walker 1987; Brando and Durigan 2005; Holdo 2006; Chafota and Owen-Smith 2009; Finckh 2016; Muller et al., 2016; Hoffmann et al., 2019). Frost is a natural and recurring disturbance in the southern savannas of Africa and South America (Davies et al., 2016; Finckh, et al., 2016; Hoffmann et al., 2019; Whitecross et al., 2012), impacting vegetation, especially during winter, with varying intensities and frequencies (Frost et al. 1985). Research in South Africa

has demonstrated that, despite its brief duration, frost events have a significant impact on the flora of savanna regions, causing herbaceous species to burn and succumb to the effects of frost (Finckh 2016).

Frost, like fire or herbivory, can cause a demographic bottleneck that hinders the growth of woody species to maturity when these disturbances occur frequently (Whitecross et al., 2012; Hoffmann et al., 2019). This can create a "freezing trap" similar to the "fire trap" (repeated cycles of biomass loss and regeneration) (Bond and Midgley 2001; Grady and Hoffmann 2012; Hoffmann et al. 2019), where frost keeps species in a suppressed state through continuous cycles of biomass loss and regeneration. Frost shares key characteristics with damage from fire and herbivory, potentially worsening the impacts of these disturbances (Hoffmann et al., 2019).

Studies have shown that fire has distinct effects on plant phenology, which vary depending on the plant community involved (Bison et al., 2022). Fire can stimulate flowering and fruiting, accelerating these phenological events, but it can also hinder them by damaging floral buds and fruits, particularly in species that bloom or bear fruit during the fire season (D'Antonio, 2000; Bison et al., 2022). The extent of these impacts is influenced by factors such as fire intensity, frequency, and specific species traits (Rodrigues et al., 2021; Rodrigues and Fidelis, 2022). However, certain plants, particularly herbs, and shrubs, may benefit from fire as it promotes their vegetative propagation (Hoffmann, 1999; Hoffmann et al., 2009; Rodrigues et al., 2021; Pilon et al., 2021; Zanzarini et al., 2022). Understanding how fire interacts with plant phenology is essential for comprehending its effects on fire prone ecosystems.

Research indicates that fire damage immediately affects vegetative and reproductive phenophases (Miranda et al., 2002; Silvério et al., 2015; Dodonov et al., 2018). The high temperatures from flames lead to great leaf abscission, causing immediate damage to the canopy of woody species (Miranda et al., 2002; Hoffmann and Solbrig, 2003; Dodonov et al., 2018; Bison et al., 2022). While these studies have shed light on the responses of savanna woody species to fire damage, there is still a limited understanding of how fire affects different growth forms within the Cerrado ecosystem (subshrubs, shrubs, and trees) and its impact on the timing of vegetative phenological events in woody and shrub plants. Recognizing the effects of fire on the seasonal patterns of plant phenophases is crucial for the management and conservation of these ecosystems, particularly in fire-prone areas. Therefore, the changes in plant phenology induced by fire are a critical consideration for the management and conservation of vegetation (Morellato et al., 2016).

Phenology is an integrative science that allows us to monitor, understand, and predict recurring biological events in the life cycle and their correlation with abiotic factors. This helps us link these events to climate and the evolutionary history of species (Rosenzweig et al., 2008; Morellato et al., 2016). This field of study not only enables the identification of changing patterns in the life cycles of plants and animals but also allows for predictions on how these changes can affect entire ecosystems. Therefore, phenology can serve to describe plant community responses to fire (Carvalho and Batalha, 2013) and predict vegetation recovery dynamics (Andersen et al., 2005). Within this context, our study focuses on vegetative and reproductive phenology in ecosystems regularly affected by fires, which are also susceptible to severe and rare frost events (Brando and Durigan 2005; Hoffmann et al., 2019; De Antonio et al., 2021), leading to similar consequences as fire, such as topkill in woody plants (Whitecross et al., 2012; Hoffmann et al., 2019).

In this study, we analyzed the phenological responses of selected species under the influence and absence of fires, using the same spatio-temporal scale for a direct comparison. We investigated how fire influences the timing and seasonality of vegetative (e.g., leaf flushing, leaf fall, and resprouting) and reproductive (flowering) phenophases in a savanna ecosystem. The aim is to understand how a frequent disturbance like fire influences plant phenology, addressing questions such as: Do species exposed to burned and unburned treatments significantly differ in their vegetative and reproductive phenologies? Does the seasonality of phenophases differ among different growth forms and in the plant community of burned sites compared to unburned ones? Does the occurrence of disturbances like fire promote the selection of species with lower leaf longevity? Is there an increase in shoot growth in environments with frequent fire incidence?

Based on the guiding questions, we tested the following hypotheses: (1) Fire triggers plant phenophases, accelerating the onset of phenological activities such as sprouting, leaf fall, and flowering, due to induced changes in microclimate and resource availability; (2) Fire alters the seasonality of phenophases, disrupting the synchrony of phenological events among species by advancing phenophases in individuals exposed to fire; and (3) Species under frequent fire regimes exhibit specific morphological and physiological adaptations, such as reduced leaf longevity and the development of larger shoots with accelerated growth, as strategies to optimize light capture and post-fire recovery.

6. Materials and Methods

6.1 Study site and selected species

The study was conducted at the Santa Bárbara Ecological Station (SBES), located in the municipality of Águas de Santa Bárbara, in the state of São Paulo, southeastern Brazil. The Köppen climate classification shows that the region where SBES is located falls under climate type Cwa - hot and humid climate with a dry winter. The annual rainfall is between 1000 and 1300 mm, and the average monthly temperatures vary between 16° and 24°C (Fig.1) (Melo and Durigan, 2011, data from: <http://www.ciiagro.org.br/cpmensal/pmensal>). The region is characterized by preserving remnants of grassland and savanna physiognomies of the Cerrado, which are very rare in the state of São Paulo (Melo and Durigan, 2011). The SBES encompasses a gradient of vegetation types ranging from open grassland (campo and cerrado típico) to closed forest (cerradão) (Abreu et al., 2017, Flake et al., 2021). In this study, data collection was carried out in treatments located in the “cerrado típico” or “*sensu strictu*”. Until the 1980s, when the area was designated as an ecological station, fire was a recurrent feature in the study areas and was therefore subject to a protection policy aimed at suppressing fires, as was common in Brazil at the time (Pilon et al., 2021). Currently, the reserve has a variety of gradients representing different stages of development of cerrado vegetation (Flake et al., 2021).

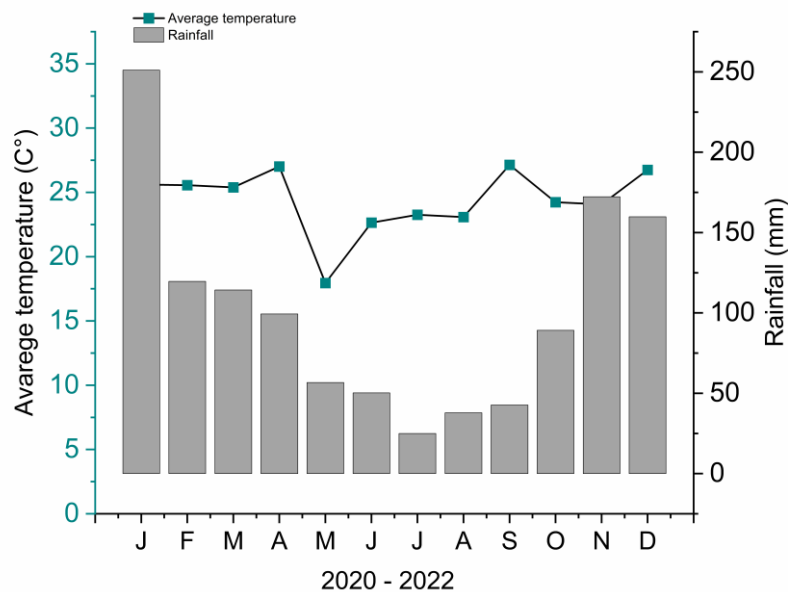


Figure 1. Average monthly rainfall (mm) and temperature (°C) at the study site between January 2020 and December 2022. Information obtained from CIIAGRO.

For this chapter, we used the list of tree species in the area provided by Abreu et al. (2017) and conducted field surveys to confirm the presence of individuals in the treatment. We selected 16 species that were present in both, annually burned and unburned treatment (Table 1). These species came from different families and habits (sub-shrubs, shrubs and trees). The species were classified according to their growth form based on Souza et al. (2018) and Durigan et al. (2004), as well as our field observations. We only measured the species that were present in both treatments (burned and unburned). At the beginning of the growing season, we marked one individual of each species per treatment (16 species), distinguishing between the burned and unburned treatment three months before the fire.

Table 1. List of species selected for this chapter.

Family	Species	Abbreviation	Growth form
Anacardiaceae	<i>Anacardium humile</i> A.St.-Hil	ANA	Subshrub
Annonaceae	<i>Duguetia furfuracea</i> (A.St.-Hil.) Saff.	DF	Shrub
Araliaceae	<i>Schefflera vinosa</i> (Cham. and Schltdl.) Frodin and Fiaschi	SV	Tree
Asteraceae	<i>Moquiniastrum barrosoae</i> (Cabrera) G. Sancho	MB	Shrub
Celestraceae	<i>Peritassa campestris</i> (Cambess.) A.C.Sm.	PC	Shrub
Chrysobalanaceae	<i>Licania humilis</i> Cham. and Schltdl.	LH	Subshrub
Ebenaceae	<i>Diospyros hispida</i> A.DC.	DH	Shrub
Fabaceae	<i>Stryphnodendron rotundifolium</i> Mart.	SR	Tree
	<i>Leptolobium elegans</i> Vogel	LE	Tree
	<i>Andira humilis</i> Mart. Ex. Benth	AH	Shrub
Lamiaceae	<i>Aegiphila verticillata</i> Vell.	AV	Shrub
	<i>Miconia albicans</i> (Sw.) Triana	MA	Shrub
Melastomataceae	<i>Miconia ligustroides</i> (DC.) Naudin	ML	Tree
Myrtaceae	<i>Campomanesia adamantium</i> (Cambess.) O.Berg	CA	Shrub
Ochnaceae	<i>Ouratea spectabilis</i> (Mart.) Engl.	OS	Tree
Sapotaceae	<i>Pradosia brevipes</i> (Pierre) T.D.Penn	PB	Subshrub

Selected species is based on Abreu et al. (2017) and field observations. Growth form classification is based on Durigan et al. (2004); Souza et al. (2018) and observation in the field.

6.2 Data collection

The six plots used in this study, of which three were burned annually and three remained unburned (controls), are part of a broader fire management experiment conducted across the biomass gradient of savanna vegetation within the reserve. The landscape mosaic ranges from open savanna to forest-type vegetation (Abreu et al., 2017). In this area, Prof. Davi Rodrigo Rossatto collaborates with Dr. William Hoffmann and Dr. Giselda Durigan,

where a controlled fire experiment has been conducted since 2014. The experiment consists of 20 x 50 m plots established in grassland (6 plots) and savanna (9 plots), with different fire frequencies (Abreu et al. 2017), including the absence of fire (half of the plots) and regular burning (the other half). Our plots are burned annually, with frequent fires occurring every year since 2014 at the end of the dry season, between August and September. From April 2021 to May 2022 (a total of 387 days), we conducted monthly monitoring of all selected individuals in each plot (both burned and unburned) and recorded the following measurements.

6.3 Phenology, Shoot Growth and Leaf Longevity

From April 2021 to May 2022, we observed all individuals and assessed leaf flushing, leaf fall, resprouting and flowering monthly observed. Leaf flushing was identified as the period when small, shiny, light green, yellowish or reddish leaves first appeared. We considered leaf flushing complete when these leaves had reached three quarters the size of a mature leaf, according to the approach proposed by Morellato et al. (1989). Leaf fall was determined by the senescent appearance of the tree crowns, whereby leaves were considered senescent if they were completely yellowed and/or could be easily detached when touched by hand (Wright and Westoby 2003). A plant was considered to be flowering when it showed flower buds and/or open flowers (Valentin et al., 2021). Resprouting was defined as the process in which a plant generated new branches and leaves with non-expanded leaf blades, including both basal and aerial resprouting. We will define this phenophase as resprouting rather than sprouting. Sprouting typically refers to the initial growth from buds and is not specifically related to a response to disturbances. Resprouting, on the other hand, is used to describe the resprouting or regeneration of plants in response to disturbances (Clarke et al., 2013).

We initiated data collection at the beginning of the dry season, 8 months after the 2020 burn, and completed data collection 8 months after the 2021 burn. Leaf flushing was defined as the emergence of new leaf shoots, while resprouting referred to the development of new growth structures post-burning. To evaluate resprouting, we employed the Fournier intensity index (1974) in the same manner as it was applied to the other phenophases.

We assessed resprouting in 2021 during our data collection in July at the Santa Bárbara Ecological Station following a severe frost event. The frost occurred on 7 nights out of 30, with temperatures dropping to -5°C (see De Antonio et al., 2023). This frost significantly impacted a large number of species. Subsequently, annual burning was carried

out the following month, resulting in complete or partial burning of individuals in the burned plot. We continued collecting data and included resprouting for species affected by both the burned and unburned treatments. The effects of frost and fire are indistinguishable in the analysis because the damage caused by frost, particularly in woody species, is similar to that caused by fire. (see Fig.2 and Fig.3) (Hoffmann et al., 2019; De Antonio et al., 2021).

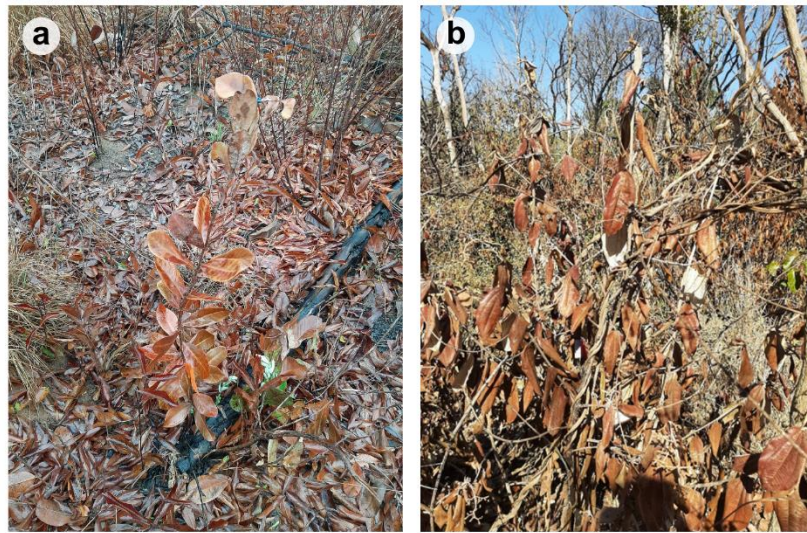


Figure 2. Example of a plant damaged by fire and another damaged by frost. (a) *Licania humilis* after a fire in July 2021; (b) *Miconia albicans* after frost in July 2021.

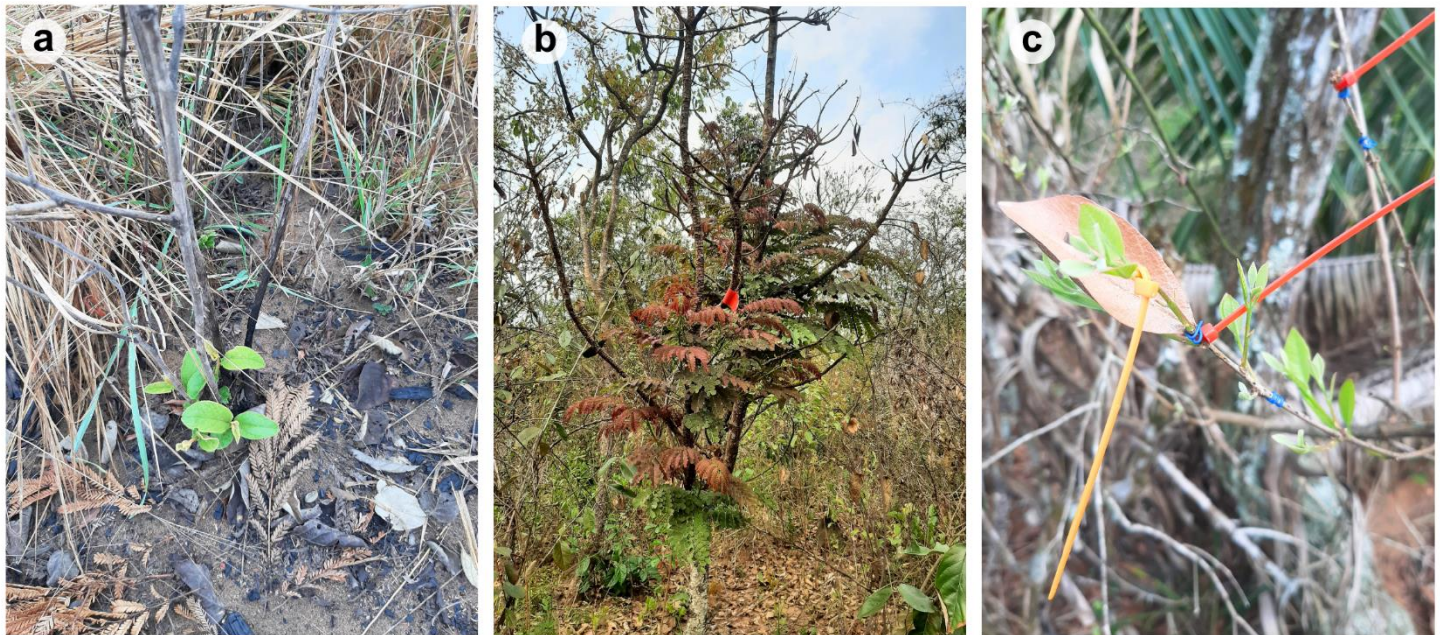


Figure 3. Example of a resprouting after fire and frost, and an example of leaf flushing (a) *Moquiniastrum barrosae* after a fire in September 2021; (b) *Stryhnodendron rotundifolium* after frost in October 2021; (c) *Campomanesia adamantium* leaf flushing.

Phenological patterns were recorded monthly according to the Fournier Intensity Index (1974), in which scores are estimated using a semi-quantitative interval scale with five categories (0 to 4) and a 25% interval between each category as follows: 0 - no observation of the phenomenon; 1 - 1 to 25%; 2 - 26 to 50%; 3 - 51 to 75%; 4 - 76 to 100%, recorded on the basis of a visual estimate of the percentage of observed phenomena in each individual (Rossato 2013). We calculated the monthly average percentage values for each species by summing the intensity values per month for all individuals and dividing by the maximum possible value (number of individuals multiplied by four), resulting in a proportion multiplied by 100, which was converted into a percentage (Lacerda et al., 2018).

To monitor and compare shoot growth in burned and unburned areas, we marked one to three terminal shoots in each individual of each previously selected species. We selected the shoots according to the definition of Maia (1999), which considers the part between the meristematic region and the first branching of the branch. We marked all selected branches with colored nylon cable ties to monitor their growth according to the methodology proposed by Rossatto and Franco (2008). The determination of the branch used was similar for all species, regardless of their phyllotaxis and growth form.

We assessed shoot growth and leaf longevity on these shoots once a month for one year between April 2021 and May 2022. We measured the initial size of the shoots by attaching colored wires at their initial position (near the branching point) and at the terminal piece in the first month (Fig. 4). The initial size of the selected shoots varied among the studied species. In the months in which growth occurred, we marked the shoots with a color (nylon cable tie) corresponding to each month (Fig. 4) and then measured the elongation of the shoots that had grown using a digital caliper (Mitutoyo, resolution of 0.03 mm).

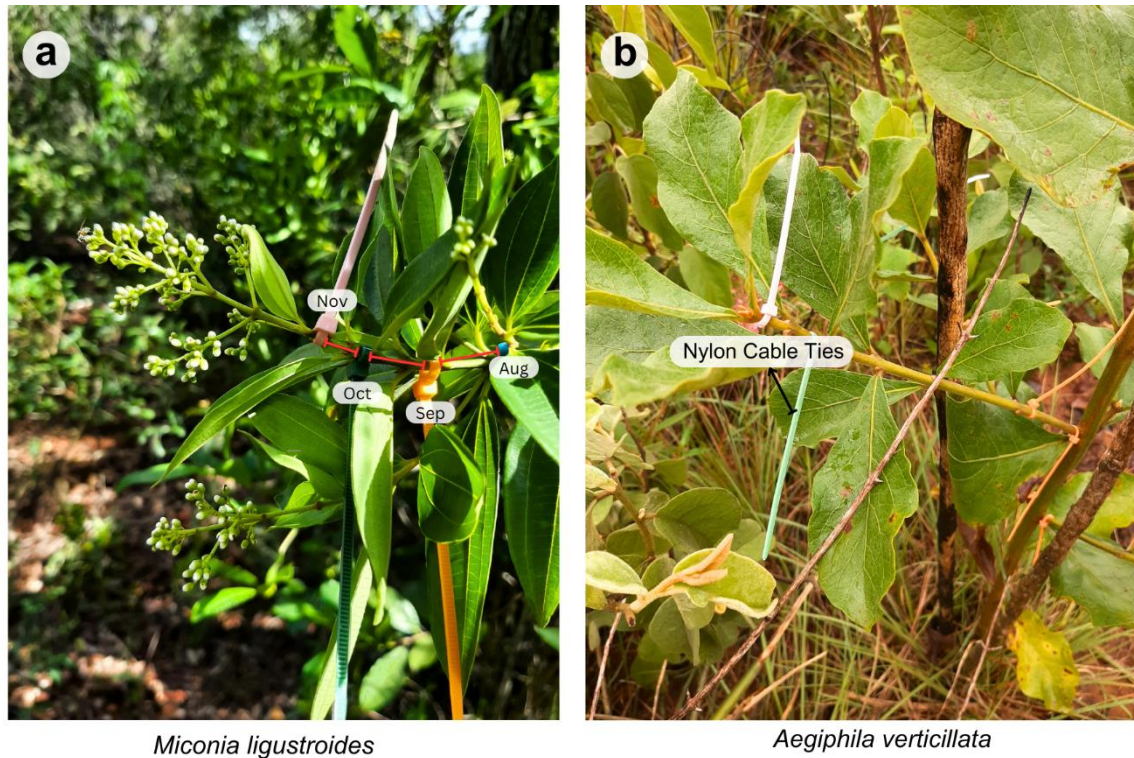


Figure 4. Branch marking method for measuring shoot growth and leaf longevity assessment. Branches were marked with colored nylon cable ties for identification during monitoring. (a) *Miconia ligustroides* - Demonstrating the use of monthly markings and the location for measuring shoot growth (b) *Aegiphila verticillata* – nylon cable ties used as a monthly marker.

To estimate the average leaf life (LL), we observed all leaves produced on the selected shoots (Rossatto 2013). The number of leaves observed varied among species due to intrinsic patterns of leaf production. We estimated leaf longevity using the method described by Craine et al. (1999), which incorporates the average date of leaf production and the average date of leaf death. We conducted the first observation in 2021, which was considered day zero, and subsequent observations were conducted every 30 days, increasing each day by 30, and so on. This methodology was successfully applied by Rossatto (2013) for Cerrado plants. To estimate average leaf longevity (LL), we observed all leaves produced on the identified shoots to monitor shoot growth and leaf longevity. We estimated leaf longevity using the following formula:

Leaf longevity was calculated by dividing the total number of days lived by all leaves (both born and senescent) during the census period by the total number of leaves present during that time (Craine et al., 1999). Following a fire, new branches started to regrow from the burnt structure. These emerging branches were identified and monitored for growth and leaf longevity. If resprouting originated from an apical bud of the existing structure, the branch was marked from that point onwards to track shoot growth and leaf longevity.

6.4 Statistical analysis

Circular statistics were utilized to evaluate the consistency of phenophases throughout the year in both burned and unburned treatments and to identify seasonal trends and peak dates for each species or group (subshrub, shrub, and tree). The null hypothesis (H_0) tested whether the data were uniformly distributed around the circle; otherwise, the peak occurrence would indicate the time of year when the growth form groups exhibited the most pronounced phenological patterns. The mean month in which each phenological event reached its peak intensity was determined (Morellato et al., 2010). Months were converted into angles at 30° intervals for analysis. Circular parameters such as mean angle, peak date, and mean vector length (r) were estimated for the community and groups. The Rayleigh z-test (Zar, 2010) was utilized for this analysis. A high probability value indicates a strong concentration intensity around the mean angle, represented by the vector r , reflecting the degree of seasonality. The value ranges from 0 to 1, where 0 indicates complete uniformity of the data, and 1 indicates total aggregation of the data around the mean angle (Morellato et al., 2010). Significance was set at $p < 0.05$. The length and direction of the arrow in the circular histogram indicate data concentration throughout the year, reflecting seasonality rather than a random distribution. The black circle in the center of the diagram represents the Rayleigh confidence interval for the arrow length (Morellato et al., 2010). The Watson-Williams two-sample (F) test was conducted when the mean angle was significant to determine whether the community and groups exhibited similar or distinct seasonal patterns between burned and unburned areas. All analyses were performed using Oriana 4.1 software (Kovach Computing Services, Anglesey, UK).

ANOVA was performed to the cumulative leaf longevity (LL) and cumulative shoot growth (SG) between burned and unburned treatments for all species (Community) and growth form (subshrub, shrub and tree), with separate analyses for each group. Normality tests (Shapiro-Wilk) and tests for homogeneity of variances (Levene) were performed to test the assumptions of ANOVA. Treatment differences were then assessed for each variable (LL and SG), with treatment (burned and unburned) as a fixed factor. The Tukey test was used as a post hoc test for multiple comparisons between burned and unburned treatment. Statistical analyses were conducted using R software, version 4.1.1, with a significance level of 0.05 for all test.

7. Results

Seasonal patterns in plant community and growth forms were observed in most phenophases, as confirmed by the Rayleigh test ($p < 0.05$) for both treatments. However, the data indicate that fire did not significantly alter these seasonal patterns or accelerate the occurrence of phenophases in the burned treatment as anticipated. This suggests a consistent occurrence of each phenophase throughout the year, regardless of the treatment or growth form considered.

Community leaf flushing was observed nearly every month, with intensity peaks at the beginning of dry season particularly between April and May. The analysis revealed the minimal impact of fire on the flushing patterns of the studied species (Watson-Williams F test = 0.218, $p > 0.05$), with flushing peaks in May (mean angle of 139.955° , unburned) and June (mean angle of 176.396° , burned). Notably, trees exhibited a significant difference in flushing, with slight variations post-fire (Rayleigh test $p < 0.05$, Watson-Williams F test = 50.914, $p < 0.05$), peaking in May (mean angle of 104.069° , burned) and April (mean angle of 129.8° , unburned). Shrubs maintained a similar trend, showing peaks at the onset of the dry season, with consistent patterns in April and May (mean angle of 103.752° , unburned; mean angle of 126.696° , burned; Watson-Williams F test = 0.004, $p > 0.05$). Subshrubs exhibited the highest seasonality, with no significant difference between treatments, peaking in dry season in the unburned treatment, specifically in August (mean angle = 233.826° , unburned; mean angle = 217.405° , burned) (see Table 2, Fig.5). Among the phenophases, leaf flushing displayed the lowest r values, indicating less data aggregation around the mean angle, making it the phenophase with the least seasonality.

Leaf fall and resprouting were observed during the dry season, in early dry season mainly in May, across the entire plant community and various growth form groups in both treatments. Notably, there was a variation in subshrubs alone, with the average occurrence date shifting to June in burned treatment (mean 150.155° for burned) (Fig.5). The high circular variance (Community: circular variance 0.87 unburned, 0.929 burned) indicates a dispersion in the occurrence of leaf fall without a fixed directional pattern. However, the Rayleigh test ($p < 0.05$) and high r values suggest a well-aggregated seasonal trend in leaf fall for both the evaluated community (Community $r = 0.751$ unburned, $r = 0.718$ burned) and the three analyzed growth forms, although there is no significant difference between treatments in the occurrence of this phenophase (Community: $F = 0.196$, $p > 0.05$; tree: $F = 1.732$, $p > 0.05$; subshrub: $F = 0.284$, $p > 0.05$; shrub: $F = 1.479$, $p > 0.05$).

The flowering phenophase showed distinct seasonality, with different periods of occurrence in both the community and the various growth forms under both treatments. For unburned treatment, flowering was more common in July (Community: mean angle = 191.483°), while for burned treatment, it was more frequent in August (Community mean angle = 237.194°) (Fig.5). Trees, in both burned and unburned treatment, peaked in flowering in dry season, typically in April (mean angle = 118.665° or 105°), showing a significant direction (Rayleigh test $p < 0.05$). Subshrubs in unburned conditions had flowering more common in September, with a mean angle of 262.141° (Rayleigh test $p < 0.05$). Shrubs flowered at the end of the dry season (Table 2), with September being the predominant month in both treatment conditions (mean angle = 241.613° , Rayleigh test $p < 0.05$). Among subshrubs, a distinct seasonality was observed between treatments, with flowering occurring at the end of the dry season for treatment without fire (mean angle = 262.141° or September 2021) and at the beginning of the dry season in the frequent fire treatment (mean angle = 105° or April 2022) (Fig.5).

Resprouting showed seasonal patterns in treatment subjected to frequent fire and those without fire but affected by frost. No significant differences were observed between growth forms regarding the period of occurrence of the phenophases among treatments, with a general peak of occurrence in spring, in September (2021) (see Table 2, Fig.5). For the community, post-frost resprouting (unburned) was more frequent in October (mean angle = 271.635°), while in burned treatment, the trend was similar among growth forms, in September (mean angle = 263.15° , Rayleigh test $p < 0.05$). The mean direction indicates a concentration peak in regrowing plants (Rayleigh test $p < 0.05$), and the high r values indicate aggregated phenological activity (Community $r = 0.933$ unburned; $r = 0.933$ burned, see Table 2), but without significant differences between treatments (Community: $F = 0.738$, $p > 0.05$; tree: $F = 0.812$, $p > 0.05$; subshrub: $F = 1.607$, $p > 0.05$; shrub: $F = 0.135$, $p > 0.05$). The Watson-Williams test did not reveal significant differences in the mean directions of the phenophases for burned and unburned conditions, indicating a moderate effect of fire on the observed phenological patterns (Table 4).

Table 2. Summary of circular statistics for the various phenophases of the subshrub, shrub, and tree groups, as well as the community, in both burned and unburned areas within a cerrado *stricto sensu*.

Phenophases	Results	Community Unburned	Community Burned	Tree Unburned	Tree Burned	Shrub Unburned	Shrub Burned	Subshrub Unburned	Subshrub Burned
Leaf flushing	Mean Vector (μ)	139.955°	176.396°	129.8°	104.069°	103.752°	126.696°	233.826°	217.405°
	Mean Group	may	june	may	april	april	may	august	august
	Length of Mean Vector (r)	0.13	0.071	0.207	0.127	0.312	0.183	0.285	0
	Circular Variance	0.87	0.929	0.793	0.873	0.688	0.688	0.715	*****
	Rayleigh Test (Z)	47.762**	12.185**	50.914**	18.589**	138.923**	42.579**	16.789**	*****
Leaf fall	Mean Vector (μ)	141.932°	147.54°	142.211°	128.652°	149.196°	156.916°	121.222°	150.155°
	Mean Group	may	may	may	may	may	may	may	june
	Length of Mean Vector (r)	0.751	0.718	0.787	0.81	0.853	0.695	0.711	0.786
	Circular Variance	0.221	0.286	0.213	0.19	0.147	0.305	0.289	0.214
	Rayleigh Test (Z)	549.559**	348.441**	248.208**	147.703**	247.968**	191.725**	117.395**	76.068**
Flowering	Mean Vector (μ)	191.483°	237.194°	118.665°	105°	241.613°	241.613°	262.141°	105°
	Mean Group	july	august	april	april	september	september	september	april
	Length of Mean Vector (r)	0.288	0.521	0.633	1	0.594	0.849	0.986	1
	Circular Variance	0.712	0.479	0.367	*****	0.406	0.151	0.014	*****
	Rayleigh Test (Z)	32.544**	58.555**	56.932**	50**	76.545**	95.819**	32.103**	*****
Resprouting	Mean Vector (μ)	271.635°	263.15°	268.574°	267.168°	282.574°	259.654°	260.279°	257.795°

Mean Group	october	september	september	september	september	september	september	september
Length of Mean Vector (r)	0.933	0.963	0.899	0.899	0.918	0.965	0.991	1
Circular Variance	0.067	0.037	0.101	0.041	0.082	0.035	0.009	3.57E-4
Rayleigh Test (Z)	549.53**	846.671**	160.75**	412.918**	210.03**	354.986**	179.823**	82.941**

** $p < 0.05$; *****Non-significant values

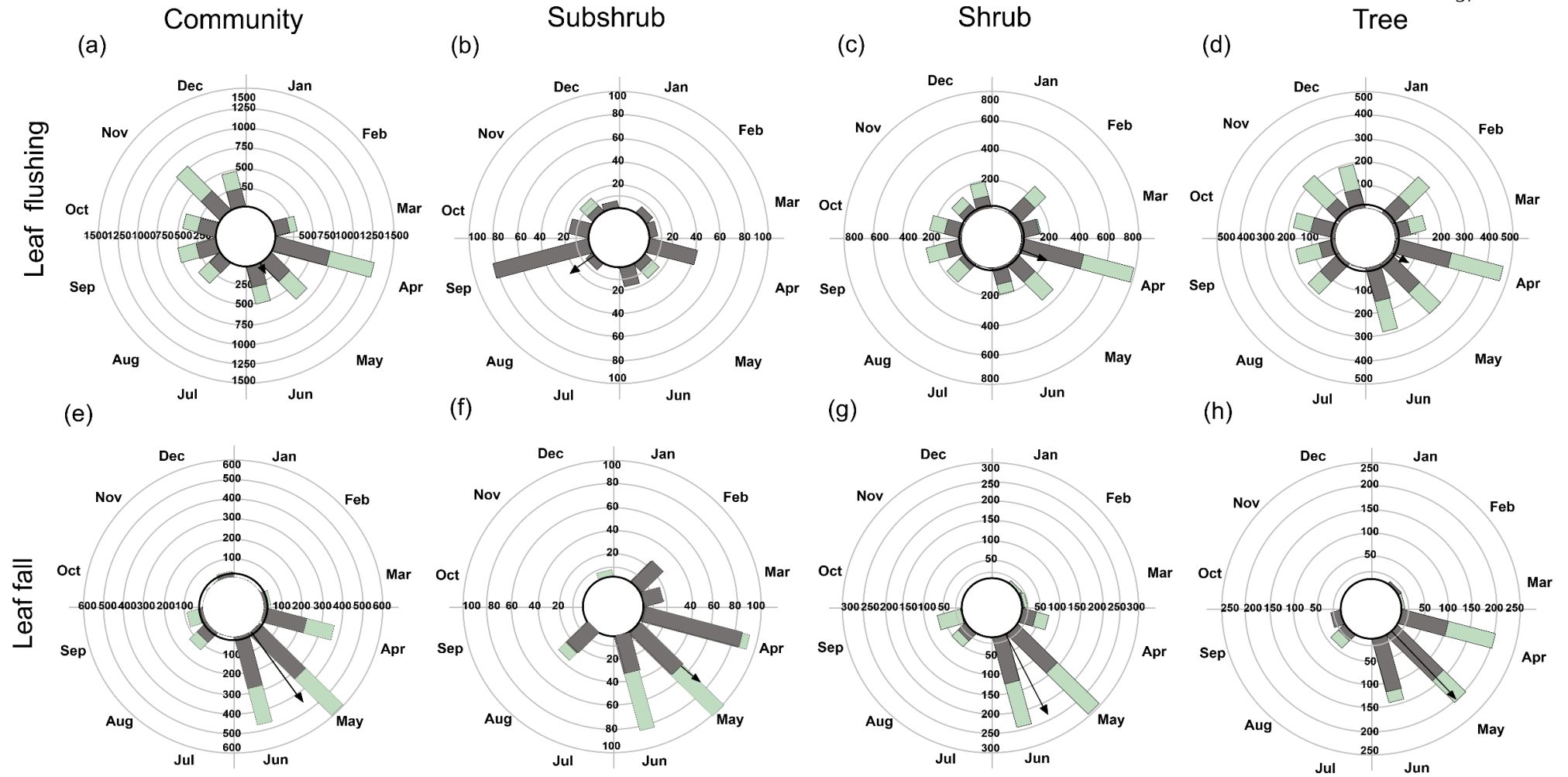


Figure 5. Circular histogram of plant phenology in a cerrado *stricto sensu* area at the Santa Bárbara Ecological Station, showing the average intensity of phenophases among evaluated individuals: leaf flushing (a – d), leaf fall (e – h), flowering (i – l), and resprouting (m – p). Unburned (gray) and burned (light aqua green) treatments. The length and direction of the arrow indicate the concentration of data throughout the year, revealing a seasonal pattern rather than random distribution. The black circle at the center of the histogram represents the Rayleigh confidence interval for the arrow's length.

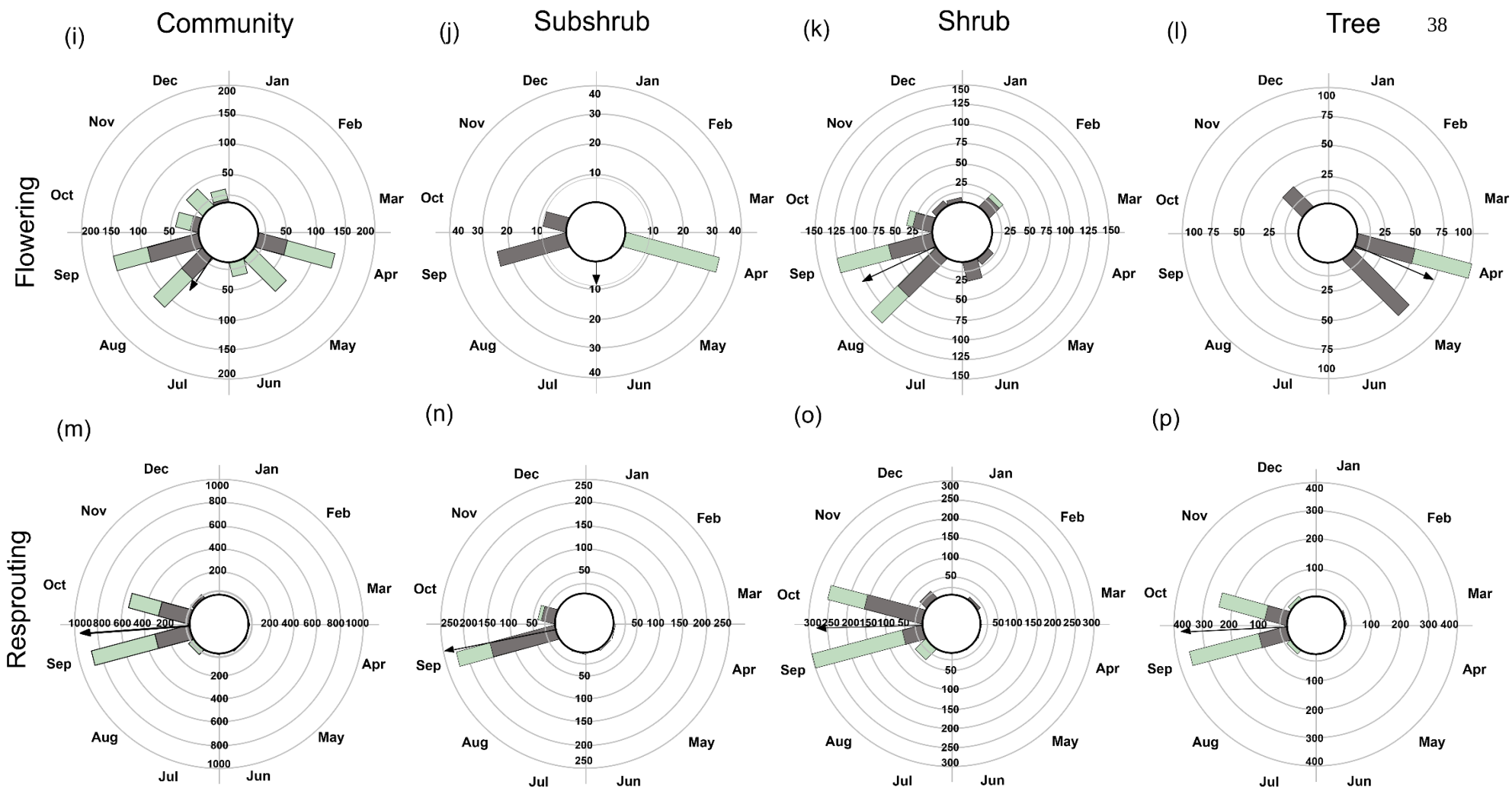


Figure 5. Circular histogram of plant phenology in a cerrado *stricto sensu* area at the Santa Bárbara Ecological Station, showing the average intensity of phenophases among evaluated individuals: leaf flushing (a – d), leaf fall (e – h), flowering (i – l), and resprouting (m – p). Unburned (gray) and burned (light aqua green) treatments. The length and direction of the arrow indicate the concentration of data throughout the year, revealing a seasonal pattern rather than random distribution. The black circle at the center of the histogram represents the Rayleigh confidence interval for the arrow's length.

The cumulative monthly average shoot growth did not show a significant difference in the studied species community (ANOVA $F = 0.142$, $p = 0.709$). When considering growth forms, trees (10.82 ± 0.48 cm unburned; 19.17 ± 0.35 cm burned), subshrubs (2.22 ± 0.41 cm unburned; 6.60 ± 0.59 cm burned) and shrubs (19.81 ± 0.53 cm unburned; 14.45 ± 0.22 cm burned) (Fig. 7; Table 3), were not statistically significant (Tree: ANOVA $F = 1.982$, $p > 0.05$; Subshrub: ANOVA $F = 0.197$, $p > 0.05$; Shrub: ANOVA $F = 5.760$, $p > 0.05$) (see Fig. 9). Among tree species, growth appeared more consistent compared to the other groups (Fig.7).

Table 3. Cumulative shoot growth and leaf longevity of burned and unburned treatments in the species studied. Mean followed by standard error ($n = 3$ for each species).

Growth form	Species	Shoot Growth (cm) Unburned	Shoot Growth (cm) Burned	Leaf longevity (days) Unburned	Leaf longevity (days) Burned
Tree	<i>S. rotundifolium</i>	11.16 (0.87)	20.58 (1.28)	164.84 (20)	241.2 (17.8)
	<i>O. spectabilis</i>	5.14 (0.70)	21.19 (1.52)	189.9 (9.8)	234.74 (7.46)
	<i>M. ligustroides</i>	20.48 (1.6)	13.71 (1.22)	154.54 (49)	182.6 (5.48)
	<i>L. elegans</i>	12.42 (0.8)	15.27 (1.34)	151.8(19.6)	152.(16.45)
	<i>S. vinosa</i>	4.90 (0.71)	25.6 (1.02)	164.5 (33)	217.8 (82)
Shrub	<i>D. furfuracea</i>	5.03 (0.22)	17.06 (1.40)	173.6 (29.3)	205.6 (11.6)
	<i>M. albicans</i>	20.44 (1.1)	22.04 (1.61)	178.1(14.5)	176.7 (38.7)
	<i>M. barrosoae</i>	21.24 (1.29)	27.12 (0.99)	217.7 (15)	189 (33)
	<i>D. hispida</i>	5.80 (0.67)	16.21 (1.15)	140.7 (37.4)	237.8 (1.75)
	<i>C. adamantium</i>	5.15 (0.42)	7.28 (0.59)	161.63 (19)	236.6 (31)
	<i>A. verticillata</i>	18.57 (1.56)	47.21 (2.43)	145 (70)	177.7 (72)
Subshrub	<i>P. campestris</i>	12.42 (1.08)	3.79 (0.59)	181.6 (14.6)	184.12 (38)
	<i>A. humilis</i>	1.18 (1.67)	2.45 (0.60)	78.6 (33)	127.7 (24.6)
	<i>L. humilis</i>	6.93 (0.37)	7.58 (0.70)	224.21 (5)	151 (28)
	<i>P. brevipes</i>	0	0	222.39 (22)	201.76 (28)
	<i>A. humile</i>	0.77 (0.30)	0.55 (0.24)	222 (9.36)	172.68 (25)

Table 4. Table displaying the outcomes of a Watson-Williams test for phenophases and the interaction among treatments, with F and p-values for various categories (community, tree, shrub, and subshrub).

	Community (F, p – value)	Tree (F, p – value)	Shrub (F, p – value)	Subshrub (F, p – value)
Leaf flushing	0.22, 0.64	7.26, 0.008	<0.05, 0.94	0.27, 0.59
Leaf fall	0.19, 0.66	1.73, 0.19	1.47, 0.22	0.28, 0.59
Flowering	0.55, 0.46	<0.05, 0.98	0.66, 0.41	<0.05, 0.98
Resprouting	0.74, 0.39	0.81, 0.37	1.74, 0.18	1.60, 0.20

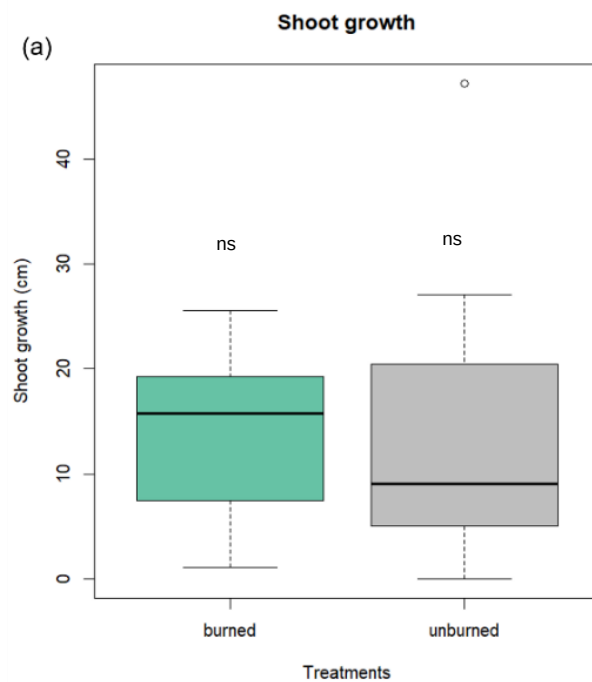


Figure 6. Average shoot growth (cm). Box plot showing the average shoot growth between burned and unburned treatments. Error bars represent standard errors. Unburned (gray) and burned (green).

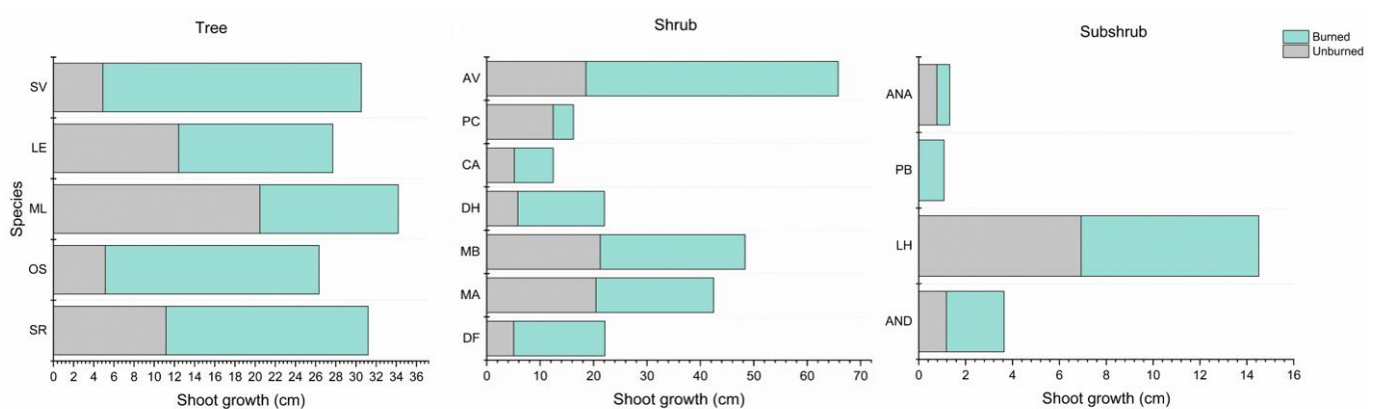


Figure 7. Stacked bar of the average shoot growth by growth form (tree; shrub and subshrub). In gray – unburned and in green – burned.

Leaf longevity did not differ significantly between treatments in the studied community (ANOVA $F = 2.92$, $p = 0.0976$) (Fig. 8). Evaluating the cumulative means, contrary to expectations, the average leaf longevity did not differ between the treatments (172.14 ± 0.38 unburned; 194.73 ± 0.37 burned (Table 3). In all three groups assessed, there were consistently no differences in leaf longevity between the treatments (see Table 5), (ANOVA $F = 3.027$, $p > 0.05$) (Fig. 9). A consistent trend of leaf longevity around 5 to 7 months was observed (Table 5).

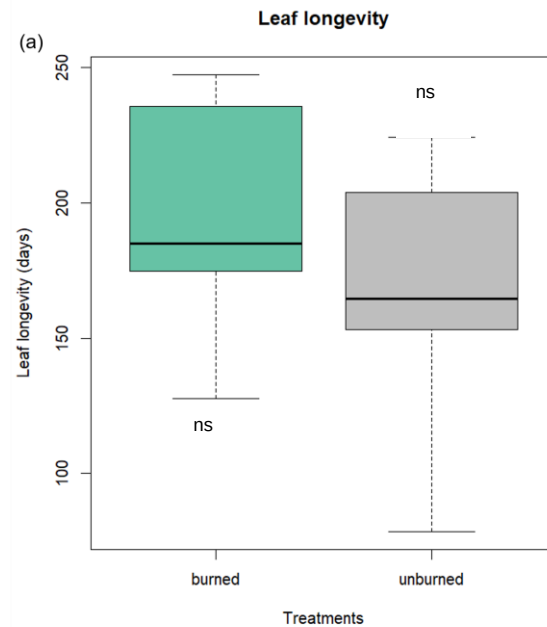


Figure 8. Average leaf longevity (cm). Box plot showing the average leaf longevity between burned and unburned treatment. Error bars represent standard errors. Unburned (gray) and burned (green).

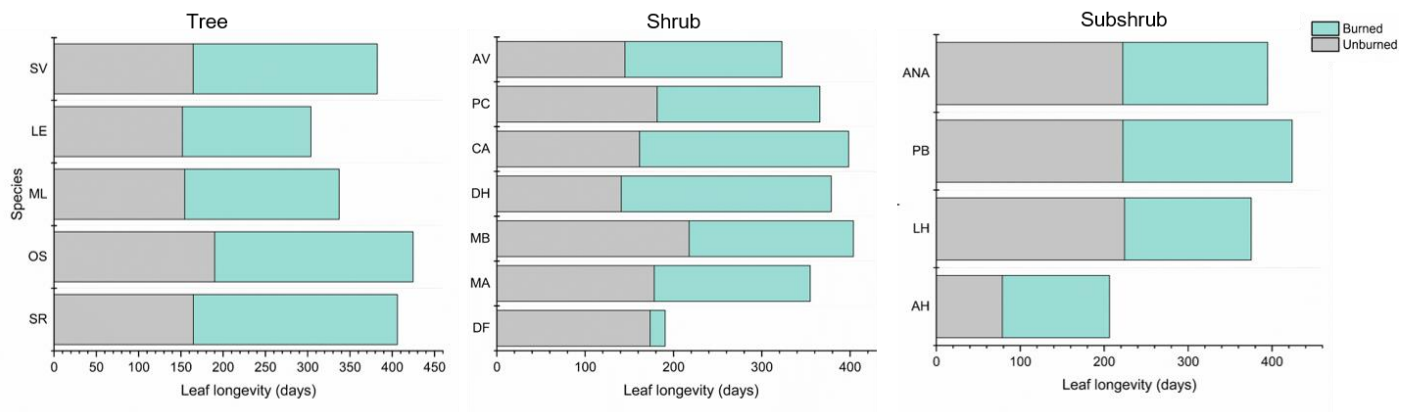


Figure 9. Stacked bar of the average leaf longevity by growth form (tree; shrub and subshrub). In gray – unburned and in green – burned

Table 5. Cumulative shoot growth and leaf longevity of burned and unburned treatment in growth forms studied.

	Community Unburned	Community Burned	Tree Unburned	Tree Burned	Shrub Unburned	Shrub Burned	Subshrub Unburned	Subshrub Burned
Shoot growth (annual cm)	9.49 ± 0.17	15.52 ± 0.23	10.82 ± 0.48	19.17 ± 0.35	19.81 ± 0.53	14.45 ± 0.22	2.22 ± 0.41	6.60 ± 0.59
Leaf longevity (days)	172.14 ± 0.38	194.73 ± 0.3	165.15 ± 0.73	211.58 ± 1.23	168.75 ± 0.71	200.66 ± 0.71	186.82 ± 1.98	163.29 ± 1.30

Mean followed by standard error

8. Discussion

The study revealed that fire did not hasten the vegetative and reproductive phenophases in the burned areas compared to the unburned areas, contradicting the initial hypothesis. Moreover, the seasonality of these phenophases remained relatively stable across treatments, and there were no significant differences in phenophases between the treatments as anticipated (hypothesis 2). Additionally, the fire did not enhance branch growth or reduce leaf longevity as hypothesized (hypothesis 3).

The fire did not alter the seasonality between burned and unburned environments, with leaf flushing showing the least seasonality, occurring almost year-round for both the community and trees and shrubs, with peaks of intensity at the beginning of the dry season between April and May. In environments with seasonal climates like the cerrado, leaf flushing typically occurs at the end of the dry season, when most cerrado trees produce new leaves and flowers, just before the onset of the rainy season, suggesting that individuals have access to water at greater depths to sustain themselves even during low precipitation periods (Batalha and Mantovani 2000; Batalha and Martins 2004; Pirani et al. 2009; Pilon et al., 2015; Bosio et al., 2016). While cerrado woody species are known to concentrate leaf production at the end of the dry season (August and September) (Felfili et al. 1999; Lenza and Klink 2006), the highest concentration was observed in April and May in both treatments. Various species exhibit adaptive mechanisms to cope with water stress, such as xylopodia or deeply developed roots (Eiten 1972; Franco et al., 2005), enabling them to maintain leaf production during the dry season and retain foliage throughout the year (Sarmiento et al. 1985, Franco et al. 2005, Lenza and Klink 2006).

Subshrubs exhibited a peak in leaf production at the beginning of the rainy season in September, following the typical leaf flushing pattern with a phase of marked intensity throughout the year (Pilon et al., 2015; Bosio et al., 2016). Recent community-level studies have shown that fire does not alter the average timing of sprouting or flowering in Cerrado plants (Valentin et al., 2021). The similarity in leaf flushing phases between burned and unburned areas highlights the plants' adaptation to stable environmental cycles rather than specific disturbances (Miranda et al., 2002). This observation suggests that fire-adaptive mechanisms may also confer resilience against frosts (Brando and Durigan, 2005; Hoffmann et al., 2019; De Antonio et al., 2021), indicating a multifunctional evolutionary strategy against various environmental stressors. Given the diverse adaptations to fire displayed by

plants (Coutinho, 1976, 1977; Keeley et al., 2011; Simon and Pennington, 2012), these adaptations may also enhance survival during drought, frost, or herbivory (Durigan, 2020).

Leaf fall exhibited high seasonality but did not differ between burned and unburned treatment. Previous studies have shown this phenophase occurring throughout the year, with greater intensity at the end of the dry season (Lenza and Klink, 2006; Mantovani and Martins, 1988; Batalha et al., 1997; Pirani et al., 2009; Bosio et al., 2016). The slightly higher frequency of species shedding leaves during the driest months from April to September represents a strategic response to cope with water scarcity by enhancing water use efficiency (Franco et al., 2005). Our study also observed a peak in leaf fall during the dry season, mainly in May, indicating strong seasonality rather than a year-round phenophase. The consistency in leaf fall patterns across growth forms and treatments throughout a phenological cycle is closely tied to seasonal weather conditions, particularly the dry season, underscoring the ecological synchrony of Cerrado species with their environment (Fenner, 1998; Borchert, 2000; Silvério and Lenza, 2010). This suggests that water restriction plays a crucial role in vegetative phenological events (Pirani et al. 2009), surpassing the influence of fire.

Flowering shows high seasonality, which was not initially affected by fire in any of the studied groups, with high r values and remaining consistent across all groups evaluated. This suggests that the reproductive phenology of species may be more influenced by biotic factors, such as pollinators, or abiotic factors, like water availability, rather than disturbances such as fire (Morellato et al., 2000; Batalha and Martins, 2004; Tannus et al., 2006). In contrast to findings in other studies (Batalha and Martins, 2004; Pilon et al., 2015; Fidalgo 2019), where reproductive events were reported to be concentrated in the warmer and wetter months of the year, the community flowered during the dry season. Different growth forms varied in their seasonal periods, with trees flowering in April regardless of treatment, while shrubs flowered in September, at the end of the dry season. In the cerrado, the flowering pattern can be quite diverse, especially among trees, which may exhibit varied reproductive phenological behavior. Some species can flower at different times of the year, demonstrating a reproductive phenological flexibility that is not confined to a specific period (Mantovani and Martins 1988, Batalha and Mantovani 2000, Lenza and Klink 2006).

Consistent with Valentin et al. (2021), subshrubs exhibited distinct seasonality between treatments, with the peak occurring in September in the burned treatment similar to shrub species, while in the unburned plot, it occurred in April similar to woody species. The apparent seasonal variations observed among subshrubs under varied treatments imply that certain species may alter their reproductive timing in response to disturbances (Gottsberger

and Silberbauer-Gottsberger, 2006). However, not all subshrub species in this study flowered during the collection period, which could explain the difference in the occurrence period of seasonality between treatments. The phenological cycles observed in the cerrado reflect the adaptation of its species to the environmental specifics, demonstrating an impressive capacity to withstand disturbances like fires (Fidalgo, 2019). Richardson and Wagenius (2022) emphasize that the alignment of flowering cycles is determined by individual phenological characteristics of plants, such as the start and end times of flowering, as well as their duration. Changes in these phenological elements, caused by events like fires, can have significant consequences. Understanding these dynamics and their relation to natural processes is crucial for the conservation and management of savannas, ecosystems of remarkable global value known for their biodiversity and ecological services (Fidalgo 2019).

In a study conducted by Valentin et al. (2021), an increase in resprouting was observed in herbaceous-subshrub species in the first months after burning. This finding supports the high resurgence of subshrubs in September, two months post-burn, both within the community and across different growth forms, with a peak in resprouting shortly after the fire. Rapid recovery through resprouting is an effective process for restoring lost biomass (Bond & Midgley, 2001; Massi and Franco, 2016). Smaller plants with resilient root systems show a remarkable ability to regenerate repeatedly after fire exposure (Coutinho, 1982; Pausas and Keeley, 2009). On the other hand, trees benefit from the protection of their vegetative buds (Silva and Batalha, 2011; Charles-Dominique et al., 2017), which is crucial for effective and vigorous resprouting after fires (Clarke et al., 2013).

This may explain why there was a peak in resprouting shortly after the disturbance (2 months), regardless of growth form. Additionally, in unburned areas, resprouting occurred in a similar pattern to burned treatment, with peaks in the same month. This suggests that Cerrado plants may respond similarly to the effects of fire and frost due to adaptations for enduring high temperatures (Hoffmann et al., 2019; De Antonio et al., 2021). The ability to regenerate after fires and frosts, maintaining consistent seasonal patterns regardless of treatments, highlights the robust adaptive strategies of Cerrado species against different disturbances. This resilience is supported by the presence of protected buds and reserves that enable rapid and efficient resprouting, characteristic of ecosystems accustomed to recurrent fires (Bond and Midgley, 2001; Paula and Pausas, 2008; Keeley et al., 2011; Clarke et al., 2013).

9. Conclusion

The study did not find a clear correlation between environmental disturbances like fire and the phenological patterns of the cerrado *sensu stricto* community. This indicates a strong phenological synchrony within the community that can withstand disturbances. Previous research has shown the cerrado's resilience to natural disturbances, emphasizing its ability to adapt to fire regimes.

The study revealed that leaf flushing in cerrado species occurs consistently throughout the year, especially at the beginning of the dry season. This highlights the species' capability to access deep water sources, ensuring leaf maintenance and new leaf production even during dry periods. This adaptation is crucial for survival in an environment with limited nutrients and seasonal rainfall. The research also showed variations in plant responses to fire, with some species resprouting vigorously while others exhibit changes in leaf longevity. This complexity demonstrates the cerrado's resilience and adaptive strategies to fire disturbances, essential for understanding survival mechanisms in resource-limited environments where fire influences biodiversity and ecosystem dynamics.

10. References

- Abreu, R.C., Hoffmann, W.A., Vasconcelos, H.L., Pilon, N.A., Rossatto, D.R., and Durigan, G. (2017). The biodiversity cost of carbon sequestration in tropical savanna. *Science Advances*, 3(8), e1701284. <https://doi.org/10.1126/sciadv.1701284>
- Andersen, A. N. (2003). Burning issues in savanna ecology and management. In A. N. Andersen, G. D. Cook, and R. J. Williams (Eds.), *Fire in Tropical Savannas: the Kapalga Experiment* (pp. 1–14). *Springer*.
- Andersen, A., Cook, G., and Williams, D. (2012). Savanna burning: The ecology and economy of fire in tropical savannas. *Austral Ecology*, 37, 633–633. <https://doi.org/10.1111/J.1442-9993.2012.02410.X>
- Batalha, M. A., and Mantovani, W. (2000). Reproductive phenological patterns of Cerrado plant species at the Pé-de-Gigante Reserve (Santa Rita do Passa Quatro, SP, Brazil): comparison between the herbaceous and woody floras. *Revista Brasileira de Biologia*, 60, 129–145. <https://doi.org/10.1590/S0034-71082000000100016>
- Batalha, M. A., and Martins, F. R. (2004). Reproductive phenology of the cerrado plant community in Emas National Park (central Brazil). *Australian Journal of Botany*, 52, 149–161. doi:10.1071/BT03098
- Bison, M., Gatto, L. C., Pilon, N. A., and Fidelis, A. (2022). Trait phenology and fire seasonality co-drive seasonal variation in fire effects on tree. *New Phytologist* (2022) 234: 1654–1663. <https://doi.org/10.1111/nph.18047>
- Bond, W. J., and Midgley, J. J. (2001). Ecology of sprouting in woody plants: The persistence niche. *Trends in Ecology and Evolution*, 16(1), 45–51. [https://doi.org/10.1016/S0169-5347\(00\)02033-4](https://doi.org/10.1016/S0169-5347(00)02033-4)
- Bosio, F. (2016). Periodicity and environmental drivers of apical and lateral growth in a Cerrado woody species. *Trees*, 30(5), 1495–1505. <https://doi.org/10.1007/s00468-016-1383-8>
- Brando, P. M., and Durigan, G. (2004). Changes in cerrado vegetation after disturbance by frost (São Paulo State, Brazil). *Plant Ecology*, 175(2), 205–215. <https://doi.org/10.1007/s11258-005-0014-z>
- Carvalho, G. H., and Batalha, M. A. (2013). The drivers of woody species richness and density in a Neotropical savannah. *Biology Letters*, 9, 20130412. <https://doi.org/10.1098/rsbl.2013.0412>
- Chafota, J. and Owen – Smith, N. (2009). Episodic Severe Damage to Canopy Trees by Elephants: Interactions with Fire, Frost and Rain. *Journal of Tropical Ecology*. 25(3), 341–345. <https://www.jstor.org/stable/25562624>
- Charles-Dominique, T., Midgley, G. F., and Bond, W. J. (2017). Fire frequency filters species by bark traits in a savanna–forest mosaic. *Journal of Vegetation Science*, 28(4), 728–735. <https://doi.org/10.1111/jvs.12528>
- Childes, S.L., Walker, B.H., 1987. Ecology and dynamics of the woody vegetation on the Kalahari sands in Hwange National Park, Zimbabwe. *Vegetation* 72, 111–128. <https://doi.org/10.1007/BF00044841>

- Cianciaruso, M. V., Silva, I. A., Manica, L. T., and Souza, J. P. (2013). Leaf habit does not predict leaf functional traits in cerrado woody species. *Basic and Applied Ecology*, 14(5), 404–412. <https://doi.org/10.1016/j.baae.2013.05.002>
- Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., Enright, N. J., and Knox, K. J. E. (2013). Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist*, 197, 19–35. <https://doi.org/10.1111/nph.12001>
- Coutinho, L. M. (1976). Contribuição ao conhecimento do papel ecológico das queimadas na floração de espécies do Cerrado. <https://doi.org/10.11606/T.41.2014.tde-04092014-095647>
- Coutinho, L. M. (1982). Ecological effects of fire in Brazilian cerrado. In *Ecology of Tropical Savannas* (pp. 273–291). *Springer*.
- Craine, J. M., Berin, D. M., Reich, P. B., Tilman, D. G., and Knops, J. M. H. (1999). Measurement of leaf longevity of 14 species of grasses and forbs using a novel approach. *New Phytologist*, 142(3), 475–481. <https://doi.org/10.1046/j.1469-8137.1999.00411.x>
- Damascos, M. A., Prado, C. H. B. A., and Ronquim, C. C. (2005). Bud composition, branching patterns and leaf phenology in Cerrado woody species. *Annals of Botany*, 96(6), 1075–1084. <https://doi.org/10.1093/aob/mci258>
- D'Antonio, C. M. (2000). Fire, plant invasions, and global changes. In H. A. Mooney and R. J. Hobbs (Eds.), *Invasive Species in a Changing World* (pp. 65–93). *Island Press*.
- Dantas, V. L., Pausas, J. G., Batalha, M. A., Loiola, P. P., and Cianciaruso, M. V. (2013). The role of fire in structuring trait variability in Neotropical savannas. *Oecologia*, 171(2), 487–494. <https://doi.org/10.1007/s00442-012-2431-8>
- Dantas, V. L., Hirota, M., Oliveira, R. S., and Pausas, J. G. (2016). Disturbance maintains alternative biome states. *Ecology Letters*, 19(1), 12–19. <https://doi.org/10.1111/ele.12537>
- Davies AB, van Rensburg BJ, Robertson MP, Levick SR, Asner GP, Parr CL (2016) Seasonal variation in the relative dominance of herbivore guilds in an African savanna. *Ecology* 97, 1618–1624. doi:10.1890/15-1905.1
- De Antonio, A. C., Hoffmann, W. A., and Rossatto, D. R. (2021). The role of morphophysiological traits in frost tolerance of neotropical savanna trees. *Trees - Structure and Function*, 35(5), 1687–1696. <https://doi.org/10.1007/s00468-021-02150-7>
- De Antonio, A. C., Scalon, M. C., and Rossatto, D. R. (2023). Leaf size and thickness are related to frost damage in ground layer species of Neotropical savannas. *Flora*, 299. <https://doi.org/10.1016/j.flora.2022.152208>
- Dodonov, P., Zanelli, C. B., and Silva-Matos, D. M. (2017). Effects of an accidental dry-season fire on the reproductive phenology of two Neotropical savanna shrubs. *Brazilian Journal of Biology*, 78(3), 564–573. <https://doi.org/10.1590/1519-6984.174660>

- Durigan, G., Baitello, J. B., Corrêa Franco, G. A. D., and de Siqueira, M. F. (2004). Plantas do Cerrado paulista: Imagens de uma paisagem ameaçada. *Páginas & Letras*.
- Durigan, G., and Ratter, J. A. (2016). The need for a consistent fire policy for Cerrado conservation. *Journal of Applied Ecology*, 53, 11–15. <https://doi.org/10.1111/1365-2664.12559>
- Durigan, G. (2020). Zero-fire: Not possible nor desirable in the Cerrado of Brazil. *Flora*, 268, 151612. <https://doi.org/10.1016/j.flora.2020.151612>
- Eiten, G. (1972). The cerrado vegetation of Brazil. *The Botanical Review*, 38, 201–341.
- Fenner, M. (1998). The phenology of growth and reproduction in plants. *Perspectives in Plant Ecology*, 1, 78–91. <https://doi.org/10.1078/1433-8319-00053>
- Felfili, J. M., Silva-Junior, M. C., Dias, B. J., and Rezende, A. V. (1999). Estudo fenológico de *Stryphnodendron adstringens* (Mart.) Coville no cerrado *sensu stricto* da Fazenda Água Limpa no Distrito Federal, Brasil. *Brazilian Journal of Botany*, 22(1), 83-90. <https://doi.org/10.1590/S0100-84041999000100011>
- Fidalgo, A. D. O. (2019). Reproductive phenology of shrubs and trees in a Cerrado area of Mogi Guaçu, SP, Brazil. 32(3), 1–9. <https://doi.org/10.5007/2175-7925.2019v32n3p1>
- Finckh, M. (2016) Letters Climate refugees going underground— a response to Maurin et al. (2014). *New Phytologist*. 209, 904–909. <https://doi.org/10.1111/nph.13567>
- Flake, S. W., Abreu, R. C. R., Durigan, G., and Hoffmann, W. A. (2021). Savannas are not old fields: Functional trajectories of forest expansion in a fire-suppressed Brazilian savanna are driven by habitat generalists. *Functional Ecology*, 35(8), 1797–1809. <https://doi.org/10.1111/1365-2435.13818>
- Fournier, L. A. (1974). Un método cuantitativo para la medición de características fenológicas en árboles. *Turrialba*, 24, 422-423.
- Franco, A. C. (1998). Seasonal patterns of gas exchange, water relations and growth of *Roupala montana*, an evergreen savanna species. *Plant Ecology*, 136, 69-76. <https://doi.org/10.1023/A:1009763328808>
- Franco, A. C., Bustamante, M., Caldas, L. S., Goldstein, G., Meinzer, F. C., Kozovits, A. R., Rundel, P., and Coradin, V. T. R. (2005). Leaf functional traits of Neotropical savanna trees in relation to seasonal water deficit. *Trees*, 19, 326-335. <https://doi.org/10.1007/s00468-004-0394-z>
- Franco, A. C., Rossatto, D. R., de Carvalho Ramos Silva, L., and da Silva Ferreira, C. (2014). Cerrado vegetation and global change: the role of functional types, resource availability and disturbance in regulating plant community responses to rising CO2 levels and climate warming. *Theoretical and Experimental Plant Physiology*, 26(1), 19–38. <https://doi.org/10.1007/s40626-014-0002-6>
- Frost, P. G. H., Medina, E., Menaut, J.-C., Solbrig, O., Swift, M., and Walker, B. H. (1985). Responses of savannas to stress and disturbance. A proposal for a collaborative programme of research. *Biology International*, 10, 63.

Grady, J.M. and Hoffmann, W. (2012). Caught in a fire trap: Recurring fire creates stable size equilibria in woody resprouters. *Ecology*, 93, 2052–2060. <https://doi.org/10.1890/12-0354.1>

Gottsberger, G., and Silberbauer-Gottsberger, I. (2006). Life in the Cerrado: a South American Tropical Seasonal Vegetation (Vol. 2). *Reta Verlag*.

Hoffmann, W. A. (1999). Fire and population dynamics of woody plants in a neotropical savanna: matrix model projections. *Ecology*, 80(4), 1354–1369. [https://doi.org/10.1890/0012-9658\(1999\)080\[1354:FAPDOW\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1354:FAPDOW]2.0.CO;2)

Hoffmann, W. A., and Solbrig, O. T. (2003). The role of topkill in the differential response of savanna woody species to fire. *Forest Ecology and Management*, 180(1–3), 273–286. [https://doi.org/10.1016/S0378-1127\(02\)00566-2](https://doi.org/10.1016/S0378-1127(02)00566-2)

Hoffmann, W. A., Dasme, R. A., Haridasan, M., Carvalho, M. T., Geiger, E. L., Pereira, M. A. B., Gotsch, S. G., and Franco, A. C. (2009). Tree topkill, not mortality, governs the dynamics of savanna–forest boundaries under frequent fire in central Brazil. *Ecology*, 90, 1326–1337. doi: 10.1890/08-0741.1

Hoffmann, W. A., Flake, S. W., Abreu, R. C. R., Pilon, N. A. L., Rossatto, D. R., and Durigan, G. (2019). Rare frost events reinforce tropical savanna–forest boundaries. *Journal of Ecology*, 107(1), 468–477. <https://doi.org/10.1111/1365-2745.13047>

Keeley, J. E., Pausas, J. G., Rundel, P. W., Bond, W. J., and Bradstock, R. A. (2011). Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science*, 16(8), 406–441. <https://doi.org/10.1016/j.tplants.2011.04.002>

Keeley, J. E., and Pausas, J. G. (2022). Evolutionary ecology of fire. *Annual Review of Ecology, Evolution, and Systematics*, 53, 203–225. <https://doi.org/10.1146/annurev-ecolsys-102320-095612>

Kikuzawa, K., and Ackerly, D. (1999). Significance of leaf longevity in plants. *Plant Species Biology*, 14(1), 39–45. <https://doi.org/10.1046/j.1442-1984.1999.00005.x>

Kikuzawa, K., and Lechowicz, M. J. (2011). Ecology of Leaf Longevity. *Springer*. <https://doi.org/10.1007/978-4-431-53918-6>

Klink, C. A., and Machado, R. B. (2005). Conservation of the Brazilian Cerrado. *Conservation Biology*, 19, 707–713. <https://doi.org/10.1111/j.1523-1739.2005.00702.x>

Lacerda, D. M. A., Rossatto, D. R., Ribeiro-Novais, É. K. M. D., and Almeida, E. B. (2018). Reproductive phenology differs between evergreen and deciduous species in a Northeast Brazilian savanna. *Acta Botanica Brasilica*, 32(3), 367–375. <https://doi.org/10.1590/0102-33062017abb0343>

Lamont, B. B., and He, T. (2017). Fire-proneness as a prerequisite for the evolution of fire-adapted traits. *Trends in Plant Science*, 22(4), 278–288. <https://doi.org/10.1016/j.tplants.2016.11.004>

Lenza, E., and Klink, C. A. (2006). Comportamento fenológico de espécies lenhosas em um cerrado sentido restrito de Brasília, DF. *Revista Brasileira de Botânica*, 29, 627–638. <https://doi.org/10.1590/S0100-84042006000400013>

- Maia, J. M. F. (1999). Variações sazonais das relações fotossintéticas, hídricas e crescimento de *Caryocar brasiliense* e *Rapanea guianensis* em um cerrado *sensu stricto*. Tesis de Maestria, Universidade de Brasília, Brasília.
- Mantovani, W., and Martins, F. R. (1988). Variações fenológicas das espécies do cerrado da Reserva Biológica de Moji Guaçu, Estado de São Paulo. *Revista Brasileira de Botânica*, 11, 101–112.
- Melo A.C.G. and Durigan G. (2011). Encarte 3: Análise da Estação Ecológica de Santa Bárbara. In: ACG Melo, G Durigan (coordinators.) Estação Ecológica de Santa Bárbara: Plano de Manejo, Águas de Santa Bárbara, Instituto Florestal, SP. 66-100
- Miranda, H. S., Bustamante, M. M., and Miranda, A. C. (2002). The fire factor. In P. Oliveira and R. Marquis (Eds.), *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (pp. 51–61). *Columbia University Press*.
- Miranda, H., Sato, M., Neto, W., and Aires, F. (2009). Fires in the cerrado, the Brazilian savanna. 427–450. https://doi.org/10.1007/978-3-540-77381-8_15.
- Muller, K., O'Connor, T.G., Henschel, J.R. (2016). Impact of a severe frost event in 2014 on woody vegetation within the Nama-Karoo and semi-arid savanna biomes of South Africa. *Journal of Arid Environments*. 133, 112-121. <http://dx.doi.org/10.1016/j.jaridenv.2016.06.010>
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858. <https://doi.org/10.1038/35002501>
- Morellato, L. P. C., Rodrigues, R. R., Leitão-Filho, H. F., et al. (1989). Estudo comparativo da fenologia de espécies arbóreas de floresta de altitude e floresta mesófila semidecídua na Serra do Japi, Jundiaí, São Paulo. *Revista Brasileira de Botânica*, 12, 85–98.
- Morellato, L. P. C., Talora, D. C., Takahasi, A., et al. (2000). Phenology of Atlantic rain forest trees: a comparative study. *Biotropica*, 32, 811–823.
- Morellato, L. P. C., Alberti, L. F., and Hudson, I. L. (2010). Applications of circular statistics in plant phenology: A case studies approach. In *Phenological Research: Methods for Environmental and Climate Change Analysis* (pp. 339–359). *Springer*. https://doi.org/10.1007/978-90-481-3335-2_16
- Morellato, L. P. C., Alberton, B., Alvarado, S. T., Borges, B., Buisson, E., Camargo, M. G. G., Cancian, L. F., Carstensen, D. W., Escobar, D. F. E., Leite, P. T. P., Mendoza, I., Rocha, N. M. W. B., Soares, N. C., Silva, T. S. F., Staggemeier, V. G., Streher, A. S., Vargas, B. C., and Peres, C. A. (2016). Linking plant phenology to conservation biology. *Biological Conservation*, 195, 60–72. <https://doi.org/10.1016/j.biocon.2015.12.033>
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858. <https://doi.org/10.1038/35002501>

- Paula, S., and Pausas, J. G. (2008). Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology*, 96, 543–552. <https://doi.org/10.1111/j.1365-2745.2008.01359.x>
- Pausas, J. G., and Keeley, J. E. (2009). A burning story: The role of fire in the history of life. *BioScience*, 59(7), 593–601. <https://doi.org/10.1525/bio.2009.59.7.10>
- Pausas, J. G., and Bond, W. J. (2019). Humboldt and the reinvention of nature. *Journal of Ecology*, 107, 1031–1037. <https://doi.org/10.1111/1365-2745.13109>
- Pilon, N. A. L., Udulutsch, R. G., and Durigan, G. (2015). Padrões fenológicos de 111 espécies de Cerrado em condições de cultivo. *Hoehnea*, 42(3), 425–443. <https://doi.org/10.1590/2236-8906-07/2015>
- Pilon, N. A. L., Durigan, G., Rickenback, J., Pennington, R. T., Dexter, K. G., Hoffmann, W. A., Abreu, R. C. R., and Lehmann, C. E. R. (2021). Shade alters savanna grass layer structure and function along a gradient of canopy cover. *Journal of Vegetation Science*, 32(1), e12959. <https://doi.org/10.1111/jvs.12959>
- Pirani, F. R., Sanchez, M., and Pedroni, F. (2009). Fenologia de uma comunidade arbórea em cerrado sentido restrito, Barra do Garças, MT, Brasil. *Acta Botanica Brasilica*, 23, 1096–1109. <https://doi.org/10.1590/S0102-33062009000400019>
- Ratter, J. A., Ribeiro, J. F., and Bridgewater, S. (1997). The Brazilian Cerrado vegetation and threats to its biodiversity. *Annals of Botany*, 80, 223–230. <https://doi.org/10.1006/anbo.1997.0469>
- Reich, P. B. (1995). Phenology of tropical forests: patterns, causes and consequences. *Canadian Journal of Botany*, 73, 164–174.
- Richardson, L. K., and Wagenius, S. (2022). Fire influences reproductive outcomes by modifying flowering phenology and mate-availability. *New Phytologist*, 233, 2083–2093. <https://doi.org/10.1111/nph.17923>
- R Core Team. (2020). R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing. Retrieved from R Project
- Rodriguez, H. G., Maiti, R., and Sarkar, N. C. (2014). Phenology of woody species: A review. *International Journal of Bio-Resource and Stress Management*, 5(3), 436. <https://doi.org/10.5958/0976-4038.2014.00595.8>
- Rodrigues, C. A., Zironi, H. L., and Fidelis, A. (2021). Fire frequency affects fire behavior in open savannas of the Cerrado. *Forest Ecology and Management*, 482, 118850. <https://doi.org/10.1016/J.FORECO.2020.118850>
- Rodrigues, C. A., and Fidelis, A. (2022). Should we burn the Cerrado? Effects of fire frequency on open savanna plant communities. *Journal of Vegetation Science*, 33. <https://doi.org/10.1111/jvs.13159>
- Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., Menzel, A., Root, T. L., Estrella, N., Seguin, B., Tryjanowski, P., Liu, C., Rawlins, S., and Imeson, A. (2008). Attributing physical and biological impacts to anthropogenic climate change. *Nature*, 453, 353–357.

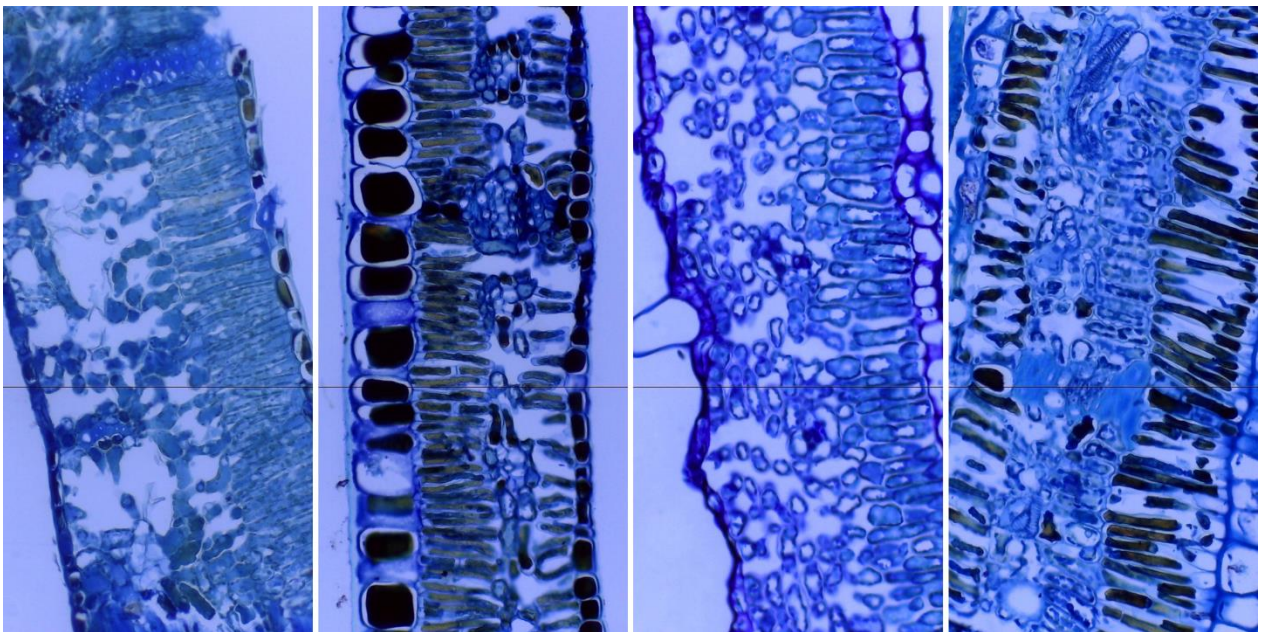
- Rossatto, D. R., and Franco, A. C. (2008). Expansão e mortalidade de ramos em espécies arbóreas do cerrado sensu stricto. *Revista Brasileira de Botânica*, 31(4), 715–719. <https://doi.org/10.1590/s0100-84042008000400016>
- Rossatto, D. R., and Franco, A. C. (2023). Plant competition across topographic gradients in neotropical cerrado savannas: An isotopic approach. In *Neotropical Gradients and Their Analysis* (pp. 65-82). *Springer*. https://doi.org/10.1007/978-3-031-22848-3_4
- Oliveras, I., Meirelles, S., Hirakuri, V., Freitas, C., Miranda, H., and Pivello, V. (2013). Effects of fire regimes on herbaceous biomass and nutrient dynamics in the Brazilian savanna. *International Journal of Wildland Fire*, 22 (3), 368-380. doi:10.1071/wf10136
- Oyedepi, S., Onuche, F., Animasaun, D., Ogunkunle, C., Agboola, O., and Isichei, A. (2016). Short-term effects of early-season fire on herbaceous composition, dry matter production and soil fertility in Guinea savanna, Nigeria. *Archives of Biological Sciences*, 68, 7-16. doi:10.2298/ABS150526002O
- Schmidt, I. B., and Eloy, L. (2020). Fire regime in the Brazilian Savanna: Recent changes, policy and management. *Flora*, 268, 151613. <https://doi.org/10.1016/j.flora.2020.151613>
- Silvério, D., and Lenza, E. (2010). Fenologia de espécies lenhosas em um cerrado típico no Parque Municipal do Bacaba, Nova Xavantina, Mato Grosso, Brasil. 10(3), 451-464. <https://doi.org/10.1590/S1676-06032010000300024>
- Silva, I. A., and Batalha, M. A. (2010). Woody plant species co-occurrence in Brazilian savannas under different fire frequencies. *Acta Oecologica*, 36(1), 85-91. <https://doi.org/10.1016/j.actao.2009.10.004>
- Silva, D. M., and Batalha, M. A. (2011). Defense syndromes against herbivory in a cerrado plant community. *Plant Ecology*, 212, 181-193. <https://doi.org/10.1007/s11258-010-9813-y>
- Simon, M. F., and Pennington, R. T. (2012). Evidence for adaptation to fire regimes in the tropical savannas of the Brazilian Cerrado. *International Journal of Plant Sciences*, 173(6), 711–723. <https://doi.org/10.1086/665973>
- Souza, V. C., Flores, T. B., Colletta, G. D., and Coelho, R. L. G. (2018). Guia das Plantas do Cerrado. *Taxon Brasil*.
- Tannus, J. L. S., Assis, M. A., and Morellato, L. P. C. (2006). Fenologia reprodutiva em campo sujo e campo úmido numa área de cerrado no sudeste do Brasil, Itirapina – SP. *Biota Neotropica*, 6(3), 1–27. <https://doi.org/10.1590/S1676-06032006000300008>
- Valentin-Silva, A., Alves, V. N., Tunes, P., and Guimarães, E. (2021). Fire does not change resprouting nor flowering, but affects fruiting phenology in a Neotropical savanna community. *Flora*, 283, 151901. <https://doi.org/10.1016/j.flora.2021.151901>
- Vico, G., Thompson, S. E., Manzoni, S., Molini, A., Albertson, J. D., Almeida-Cortez, J. S., Fay, P. A., Feng, X., Guswa, A. J., Liu, H., Wilson, T. G., and Porporato, A. (2015). Climatic, ecophysiological, and phenological controls on plant ecohydrological strategies in seasonally dry ecosystems. *Ecohydrology*, 8(4), 660–681. <https://doi.org/10.1002/eco.1533>

Zanzarini, V., Andersen, A. N., and Fidelis, A. (2022). Flammability in tropical savannas: variation among growth forms and seasons in Cerrado. *Biotropica*, 54, 979–987. <https://doi.org/10.1111/btp.13121>

Whitecross MA, Archibald S, Witkowski ETF (2012) Do freeze events create a demographic bottleneck for *Colophospermum mopane*. *South Africa Journal Botany*, 83:9–18. <https://doi.org/10.1016/j.sajb.2012.07.008>

Chapter II

Leaf Anatomical Responses to Recurrent Fires in a Cerrado vegetation



11. Introduction

The morpho-anatomical traits of plants, especially on leaves, are significantly influenced by environmental variations. As such organs are primarily exposed to diverse biotic and abiotic conditions, leaves can adapt to these changes (Fahn, 1986). This adaptive capacity often becomes the subject of detailed studies to understand how plants respond to their environment (Cuter et al., 2007). Research on the morpho-anatomy of plants, particularly in the Cerrado, demonstrates a wide variation in leaf structure among woody, shrubby, and herbaceous species, indicating an adaptation to variations in light, soil nutrients, and seasonality (Rossatto et al., 2015; Pinheiro et al., 2017). An important characteristic of the leaf in this regard is the specific leaf area (SLA), which is a crucial trait that measures the efficiency of light capture and CO₂ assimilation, with species in resource-rich environments generally having higher SLAs than those in nutrient-poor environments (Reich et al., 1997; Pérez-Harguindeguy et al., 2013). The phenotypic plasticity of SLA is vital for the adaptability of plants to fluctuating environmental conditions, directly influencing species survival (Scheepens et al., 2010; Stotz et al., 2022). Thus, SLA has become increasingly important for characterizing vegetation responses to environmental changes (Hoffmann et al., 2005; Scalon et al., 2017; Flake et al., 2021; Pellegrini et al., 2023).

Specific Leaf Area is an important factor that can affect the ability of plants to photosynthesize, as demonstrated by Dijkstra and Lambers (1989) and Xiong et al., (2016). Variations in SLA result from changes in leaf density and thickness, which depend on factors such as leaf shape, the number of layers in the palisade parenchyma, the length of its cells, mesophyll thickness, as well as the characteristics of the epidermis (Witkowski and Lamont, 1991). Leaf anatomy plays a significant role in determining photosynthetic efficiency; the composition and complex structure of leaf cells and the intercellular space are crucial for gas exchange and photosynthesis (Evans et al., 2009; Crang et al., 2018). Leaves typically consist of an epidermis, a parenchymal tissue called mesophyll, and a vascular system (Oguchi et al., 2006). The spongy parenchyma, with larger intercellular spaces, facilitates gas transport, while the palisade parenchyma is denser and contains more chloroplasts, maximizing light absorption (Crang et al., 2018). Studies suggest that both palisade and spongy tissues are essential limiting factors for optimizing photosynthesis, as these processes primarily occur in the chloroplasts of these tissues (Terashima et al., 2011; Crang et al., 2018). Changes in

mesophyll structure directly influence light capture, gas movement, and water distribution within the leaf, influencing the plant's ability to tolerate environmental and biotic stresses (Oguchi et al., 2006). Zhu et al., (2015) discuss that one strategy for plants to adapt to changing environmental conditions is to alter leaf morphology (Niinemets 2001; Wright et al., 2004). How would leaf traits of species exposed to frequent disturbance respond to environmental changes? While some authors suggest that savanna species typically adopt conservative strategies due to drought and low soil fertility (Marachipes et al., 2018), research indicates that different fire incidence patterns may promote the emergence of trees with distinct functional characteristics, particularly those associated with survival and post-fire regeneration capacity (Scalon et al., 2019; Pellegrini et al., 2023).

Fire, although a disturbance (White and Pickett, 1985), is a vital ecological agent in the Cerrado (Hardesty et al., 2005), shaping its vegetation. It occurs naturally during the rainy season, often ignited by lightning, and in the dry season, induced by human activities (Miranda et al., 1993; Miranda et al., 2002). Fire not only stimulates flowering and seed production, particularly in shrubby and herbaceous species but can also alter the balance between sexual and vegetative reproduction, favoring the latter (Coutinho, 1982; Hoffmann, 1999; Zironi et al., 2021). Controlled burning, deliberately used as part of landscape management, is globally recognized as an environmental management practice (Penman et al., 2011). Researchers such as Pivello and Coutinho (1996) were pioneers in advocating for fire management in the Cerrado (Durigan 2020), and since 2014, integrated fire management programs have been implemented in protected areas of the biome (Abreu et al., 2017; Durigan, 2020). Despite the novelty of these initiatives, they face uncertainties due to the still incomplete understanding of the impact of fire behavior on various ecological processes and fire regimes (Gomes et al., 2018).

The Cerrado, an ancient biome endemic to Brazil (Durigan et al., 2004), is known for its rich biodiversity and ability to adapt to seasonal climatic conditions. It is the second largest vegetation formation in the country and is considered a biodiversity hotspot (Ratter et al., 1997; Myers et al., 2000). The vegetation in this biome features an open tree layer with twisted branches and trunks, along with a lower layer of grasses and small shrubs (Rizzini, 1976; Coutinho, 1990; Miranda et al., 2009). The functional traits of Cerrado vegetation provide resilience to plant communities in the face of fire events, supporting ecosystem regeneration and persistence (Keeley et al., 2011; Clarke

et al., 2013; Pausas, 2015; Archibald et al., 2018). These traits reflect adaptations to environmental disturbances that affect resource availability (Pellegrini et al., 2023). Plants in the Cerrado employ ecological strategies that balance conservation and resource acquisition, with some prioritizing survival under harsh conditions and others optimizing growth in favorable conditions (Díaz et al., 2004; Wright et al., 2004; Hoffmann et al., 2012; Reich, 2014; Pellegrini, 2016; Wigley et al., 2016). Understanding species traits at the community level is essential for identifying factors that support savanna resilience to fire and guiding conservation and restoration efforts (Pellegrini et al., 2023).

To investigate the impact of recurrent fires on the anatomical and morphological features of native Cerrado species leaves, this study examined the effects of annual burning on leaf traits. The analysis focused on the thickness of palisade parenchyma (PP), spongy parenchyma (SP), cuticle (Cu), adaxial (Ade), and abaxial (Abe) face epidermis, as well as total leaf thickness, leaf area, and specific leaf area (SLA). The study aims to determine whether annual fire exposure leads to the development of leaves with mesomorphic morphoanatomical traits indicative of a resource acquisition strategy. The study questions whether leaves in burned areas will exhibit higher SLA, reflecting mesomorphic characteristics such as reduced parenchymal and leaf thickness compared to leaves from unburned areas which would be more scleromorphic.

Based on these questions, the hypothesis is that the vigorous resprouting of leaves observed after fire events, coupled with the high nutrient availability in the topsoil layer, will lead to the development of leaves that optimize resource acquisition. Therefore, it is hypothesized that these leaves will show a decrease in overall thickness, favoring a higher proportion of mesophyll and displaying more pronounced mesomorphic characteristics, resulting in an increased SLA. This hypothesis is in line with the expectation that plant adaptive strategies to post-fire conditions involve optimization for light capture and utilization, as well as for CO₂ assimilation, enhancing photosynthetic efficiency in an environment with seasonal abundant resources.

12. Materials and Methods

12.1 Study site

The study was conducted at the Santa Bárbara Ecological Station (EESB) in the municipality of Águas de Santa Bárbara, São Paulo, located between the coordinates 22°46'33" and 22°50'33"S, and 49°10'27" and 49°15'36"W. This area is situated at the southeastern edge of the Cerrado biome (Abreu et al., 2017). According to the Köppen climate classification, the climate of the EESB area is classified as Cwa, characterized by warm and humid conditions with dry winters. The average temperatures range between 16 and 24°C throughout the year, with an annual rainfall variation between 1000 and 1300 mm (Melo and Durigan, 2011). The EESB encompasses a diverse range of vegetation types, from open grasslands to dense forests, including the 'cerradão' forest type (Melo and Durigan, 2011; Pilon et al., 2018). Currently, the reserve is a mosaic of grasslands, savannas, and forests (Abreu et al., 2017). The areas studied in this research primarily fall within the cerrado *sensu stricto* or cerrado típico.

This study included six experimental plots, evenly split between burned (annually burned) and unburned areas, as part of a larger investigation into fire management and its effects on Cerrado biomass at EESB, covering campo cerrado to cerradão (Abreu et al., 2017). The research on burning impacts has been ongoing since 2014. The study utilized 20 x 50 m plots in both grassland formations (6 plots) and savanna formations (9 plots), with varying burning regimes: half without burning and the other half with annual burns. The burned treatment were always treated at the end of the dry season, between August and September. This chapter evaluates the impact of fire on three completely burned treatment, while three other treatment were designated as controls without burning for comparison (unburned). The treatment that are burned annually will be referred to as "burned," and those that are not burned will be referred to as "unburned."

12.2 Data Collection and Species Selection

To characterize the morpho-anatomical leaf traits of species in both burned and unburned treatments, a specimen from each species in the cerrado *sensu stricto* areas was selected. Seventeen species from various families, all present in both treatment conditions, were chosen based on field observations and data from previous studies conducted at the ecological station (Abreu et al., 2017). Each species was found in both

burned and unburned locations, as listed in Table 1. For each species, three healthy, fully developed leaves were harvested from the third or fourth node for evaluation. The collection took place in August 2020, before the annual burning regime was implemented in the designated treatment areas. The leaves were stored in paper bags after collection and processed immediately thereafter.

Table 1. List of species selected for the leaf anatomical analyses.

Family	Species	Abbreviation	Growth form
Anacardiaceae	<i>Anacardium humile</i> A.St.-Hil	ANA	Subshrub
Annonaceae	<i>Duguetia furfuracea</i> (A.St.-Hil.) Saff.	DF	Shrub
Araliaceae	<i>Schefflera vinosa</i> (Cham. and Schltdl.) Frodin and Fiaschi	SV	Tree
Asteraceae	<i>Moquiniastrum barrosoae</i> (Cabrera) G. Sancho	MB	Shrub
Celestraceae	<i>Peritassa campestris</i> (Cambess.) A.C.Sm.	PC	Shrub
	<i>Tontelea micrantha</i> (Mart.) A.C.Sm	TM	Shrub
Chrysobalanaceae	<i>Licania humilis</i> Cham. and Schltdl.	LH	Subshrub
Ebenaceae	<i>Diospyros hispida</i> A.DC.	DH	Shrub
Fabaceae	<i>Stryphnodendron rotundifolium</i> Mart.	SR	Tree
	<i>Leptolobium elegans</i> Vogel	LE	Tree
	<i>Andira humilis</i> Mart. ex Benth.	AH	Subshrub
Lamiaceae	<i>Aegiphila verticillata</i> Vell.	AV	Shrub
Melastomataceae	<i>Miconia albicans</i> (Sw.) Triana	MA	Shrub
	<i>Miconia ligustroides</i> (DC.) Naudin	ML	Tree
Myrtaceae	<i>Campomanesia adamantium</i> (Cambess.) O.Berg	CA	Shrub
Ochnaceae	<i>Ouratea spectabilis</i> (Mart.) Engl.	OS	Tree
Sapotaceae	<i>Pradosia brevipes</i> (Pierre) T.D.Penn	PB	Subshrub

Selected species is based on Abreu et al. (2017) and field observations. Growth form classification is based on Durigan et al. (2004); Souza et al. (2018) and observation in the field.

12.3 Morpho-Anatomical traits

To address our questions, the following leaf morphological traits were determined:

Leaf thickness, leaf area, and leaf dimensions: Leaf thickness (mm) was measured using a digital caliper (Vonder, 150 mm, resolution of 0.01 mm). Measurements were taken on three leaves per individual in the morning. The same leaves were used to determine the total leaf area (cm²). Total leaf area and leaf dimensions, such as width and length (cm), were determined using a high-resolution desktop scanner (4800 x 9600 dpi) and the free software Image J.

Specific leaf area: The specific leaf area (cm²g⁻¹) was calculated by dividing the leaf area by the dry mass obtained after drying the leaves in an oven at 70°C for three days, followed by weighing on a precision scale (± 0.0001 g).

Leaf Anatomy: To analyze and quantify leaf anatomical traits, transverse sections were taken from the median regions of the leaves, including the central vein, in the selected species (Table 2). This method was used to compare the differences between the presence and absence of fire conditions. Therefore, three fully expanded and disease-free leaves were collected from each species (Table 1). The middle thirds of the collected leaves were fixed in FAA 70 for 48 hours, subsequently dehydrated in an ethanol series, and embedded in paraffin or historesin (Johansen 1940). The thickness of the transverse sections was 8 μ m. Staining was performed with Toluidine Blue and mounting with Entellan. Thickness measurements of leaf structures were obtained using 20x or 40x objective lenses on a Zeiss Primostar optical microscope with an integrated image capture system (AxioCam ERc5s). Typically, three slides per species were prepared, and in some cases, additional slides were created due to the delicate nature of certain tissues to aid in photography. A photo was taken per slide, and for each of the 17 species in both conditions (with and without fire), the leaf structures were measured three times on each anatomical section per slide, yielding approximately nine measurements per leaf structure for each species, which were then averaged to obtain a mean value per individual. All measurements were conducted using Image Pro 4.0 software (Rossato et al., 2015). The analyzed leaf structures included palisade and

spongy parenchyma, abaxial and adaxial epidermis, and the cuticle, when present (Table 2).

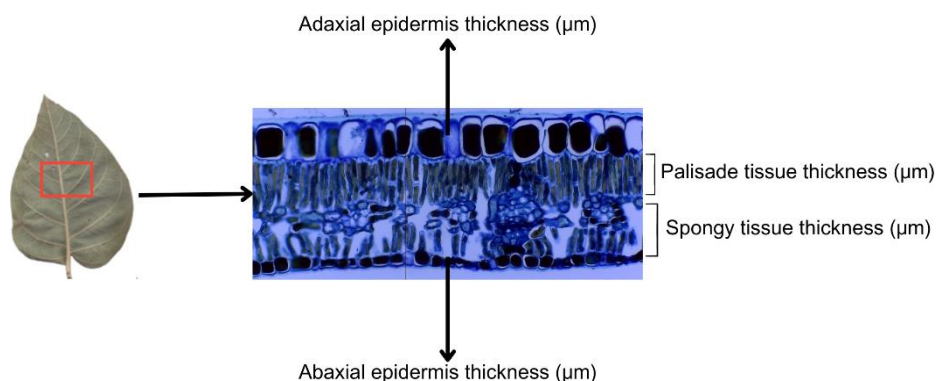


Figure 1. Exemple of an optical microscopy image of a leaf cross-section with measurements of anatomical traits that were evaluated.

Table 2. List of measured leaf traits and their respective abbreviations

Abreviattion	Leaf traits
Abe	Abaxial face of epidermis (μm)
Ade	Adaxial face of epidermis (μm)
Cu	Cuticle (μm)
PP	Palisade parenchyma (μm)
SP	Spongy parenchyma (μm)
LA	Leaf area (cm^2)
SLA	Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)
Thickness	Leaf thickness (mm)

12. 4 Statistical Analysis

The data collected underwent various statistical analyses to investigate variations in leaf traits resulting from different burning treatments in the study years 2020 and 2021. Initially, a descriptive analysis was conducted, utilizing histograms and boxplots to illustrate the distribution of leaf data based on treatment (burned vs. unburned) and scatter plots to explore correlations between variables. To ensure the data's suitability for analysis of variance (ANOVA), Shapiro-Wilk (normality) and Levene (homogeneity of variances) tests were performed.

Multivariate Analysis of Variance (MANOVA) was utilized to evaluate the influence of treatment on aggregated leaf traits. This analysis also incorporated the

species variable to comprehend the combined effects on different species. To assess the significance of multivariate differences in the MANOVA models, statistics such as Wilks' Lambda, Pillai's Trace, Hotelling's Trace, and Roy's Largest Root were employed to test the hypothesis of equality of multivariate means (Gotelli and Ellison 2011).

We conducted a univariate ANOVA by species to compare leaf traits between burned and unburned areas. This analysis was extended to include the interaction between species and treatments to examine how different species responded to fire. Significant differences were further investigated using Tukey's post hoc test with a significance level set at $p < 0.05$. Principal Component Analysis (PCA) was conducted on eight anatomical leaf traits: PP, SP, Ade, Abe, Cu, SLA, leaf thickness, and LA, considering the treatment. PCA was employed to simplify the data and identify significant patterns. To ensure equal weighting of all variables in the analysis, the data were standardized using the z-score method.

Furthermore, we calculated correlations between leaf traits and treatments separately for burned and unburned areas. The Pearson correlation coefficient (r) and corresponding p-value were calculated for each pair of variables to identify significant relationships. All analyses were performed using R software (version 4.1.1).

13. Results

When evaluating the treatment (burned and unburned) in relation to all dependent variables collectively, it was determined that there is no statistically significant difference. This indicates that, overall, the treatments do not have a significant impact on the measured leaf traits (MANOVA: $F = 0.9521$, $p > 0.05$).

The results, taking into account both species and treatments for the measured leaf traits, revealed no significant interaction (see more in Table 4 in the supplementary materials). This is evident in the graphs, where the values of the traits exhibit minimal variation between treatments (see Figs. 2, and 3).

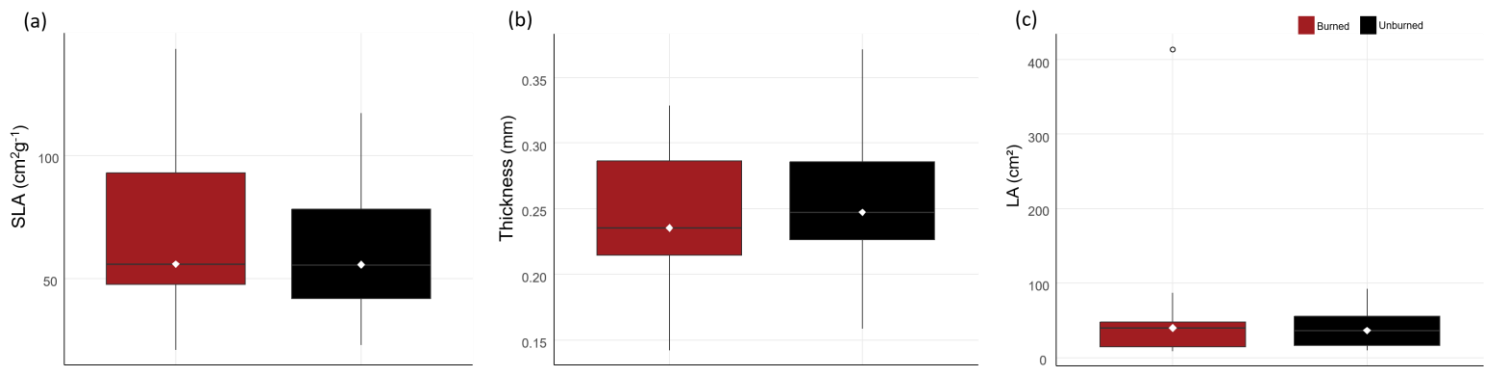


Figure 2. Boxplot showing the average differences in leaf traits between different treatments (burned and unburned) in a cerrado *sensu stricto*. (a) SLA (specific leaf area); (b) Thickness; (c) LA (leaf area). Burned treatments: red; Unburned treatments: black. Vertical bars indicate standard error ($n = 9$).

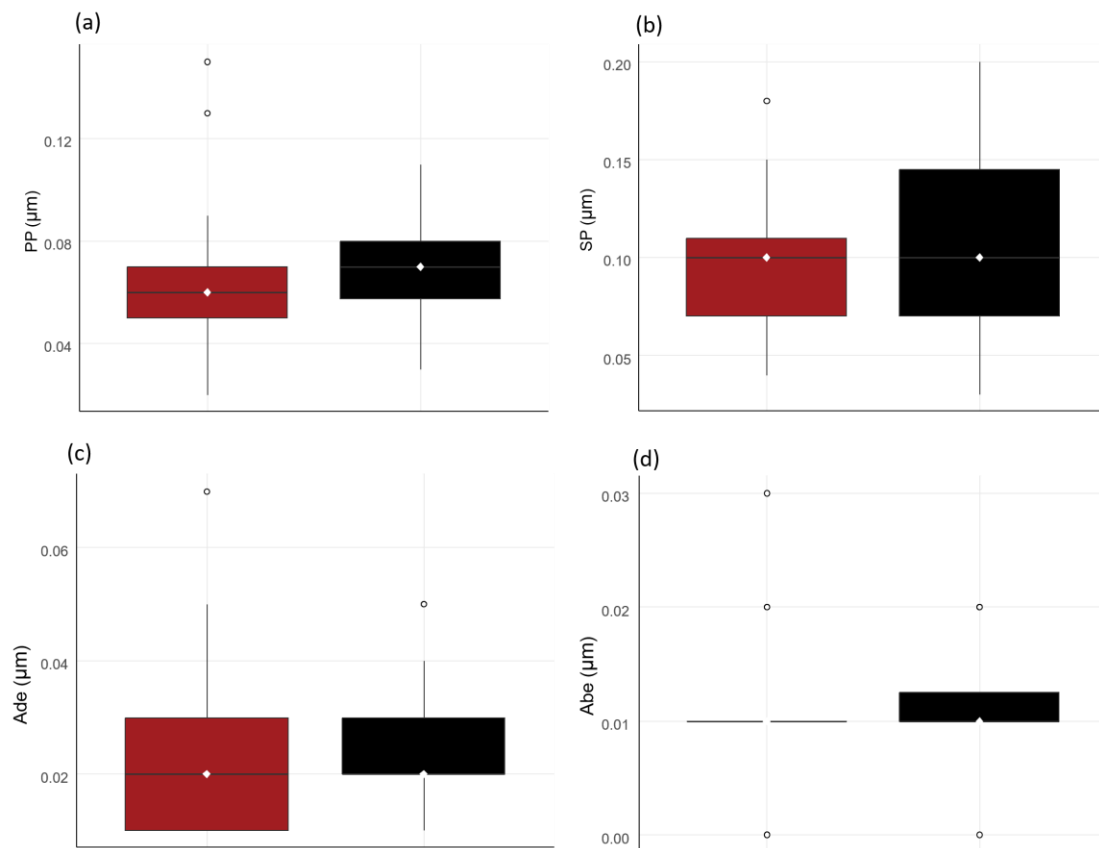


Figure 3. Boxplot showing the average differences in leaf traits between different treatments (burned and unburned) in a cerrado *sensu stricto*. (a) Palisade parenchyma (PP); (b) Spongy parenchyma (SP); (c) Adaxial face of epidermis (Ade); (d) Abaxial face of epidermis (Abe). Burned treatments: red; Unburned treatments: black. Vertical bars indicate standard error ($n = 9$).

In the analysis of the leaf anatomy of the studied species, the qualitative analysis did not reveal any distinguishing characteristics between the studied conditions (Fig.4). Most of the studied species had a single-layered epidermis, except for *L. humilis* and *S. vinosa*, which had a bilayered epidermis. The mesophyll of the species was predominantly dorsiventral, with the exception of *Andira humilis* and *Anacardium humile*, which had homogeneous mesophyll. *Duguetia furfuracea* and *Diospyros hispida* exhibited isobilateral mesophyll. The palisade and spongy parenchymas were differentiated, with the palisade parenchyma consisting of rectangular cells organized into one or two layers. The spongy parenchyma exhibited variability in compaction and the number of cell layers among the species. These observations are depicted in Figure 4.

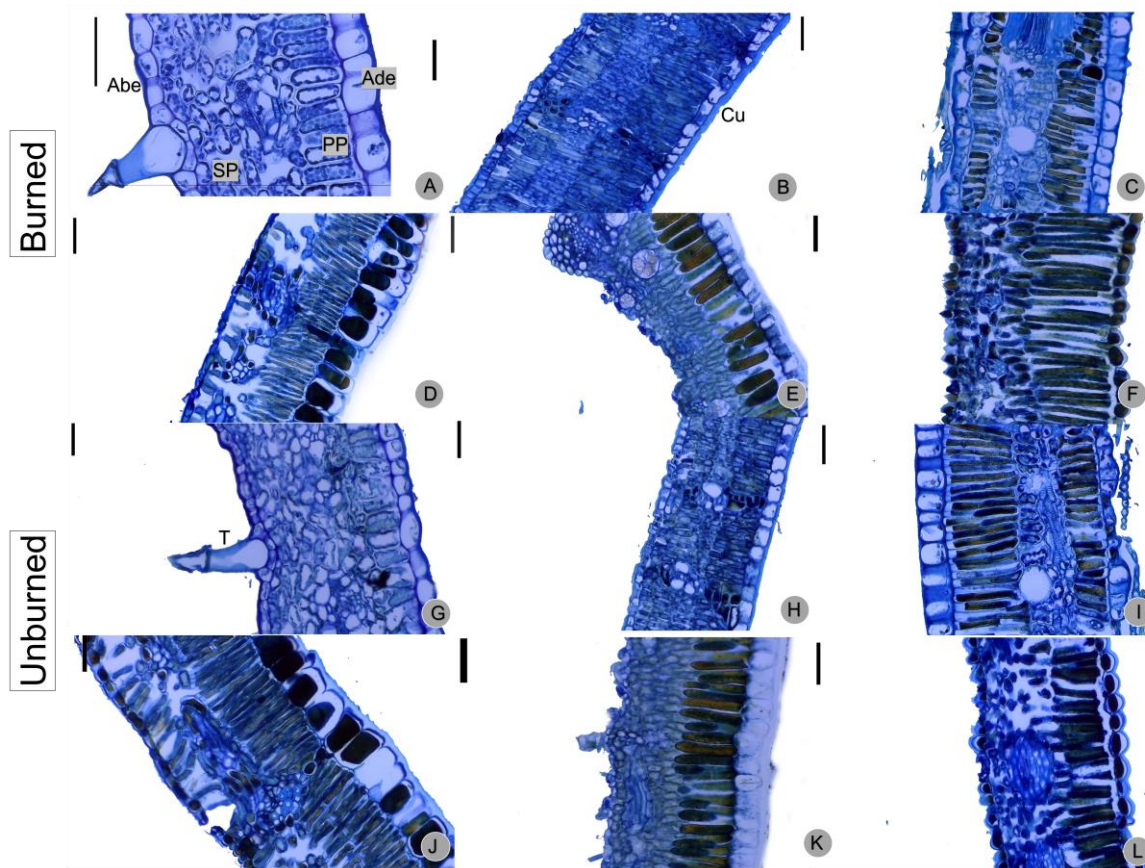


Figure 4. Comparative structural diversity of leaf cross sections of the studied in burned and unburned areas at cerrado stricto sensu: Burned - (A) *Aegiphila verticillata* ; (B) *Andira humilis* ; (C) *Duguetia furfuracea*; (D) *Diospyros hispida*; (E) *Miconia albicans* (F) *Stryphnodendron rotundifolium*; Unburned - (G) *Aegiphila verticillata*; (H) *Andira humilis*; (I) *Duguetia furfuracea*; (J) *Diospyros hispida*; (K) *Miconia albicans*; (L) *Stryphnodendron rotundifolium*; Abe, abaxial face of epidermis; Ade, adaxial face of epidermis; Cu, cuticle; Hy, hypodermis; PP, palisade parenchyma; SP, spongy parenchyma; T, trichome; Scale bars = 40µm. Scale bars in (B) = 20 µm.

All tests indicate a statistically significant difference between species in relation to the set of dependent variables analyzed (leaf traits) (MANOVA: $F = 6.05$, $p < 0.05$) (Figs. 5 and 6). The results of statistical analyses to determine if species under different treatments exhibit considerable variation in responses to the measured leaf traits are as follows: *A. verticillata* showed the highest leaf area in unburned areas (ANOVA: $F = 18.49$, $p < 0.05$) (Fig. 5c), and *S. rotundifolium* had lower SLA values in the unburned treatment (Fig. 5a) (ANOVA: $F = 10.88$, $p < 0.05$). *C. adamantinum* exhibited higher Cu values in burned environments (Fig. 6e) (ANOVA: $F = 24.88$, $p < 0.05$), while for *M. ligustroides*, PP and SP values were higher in unburned areas (ANOVA: PP – $F = 26.12$, $p < 0.05$; SP – $F = 35.92$, $p < 0.05$) (Figs. 6a, b). *L. elegans* also showed higher PP values in the unburned treatment (ANOVA: $F = 108.8$, $p < 0.05$). Regarding the epidermis, only *L. humilis* showed significant differences, with higher Ade in locations with frequent fire and lower Abe (ANOVA: $F = 90.64$, $p < 0.05$) under frequent fire (ANOVA; $F = 35.6$, $p < 0.05$) (Fig 6c). In summary, the responses of different species to treatments varied considerably. Other species showed little or no significant difference between treatments.

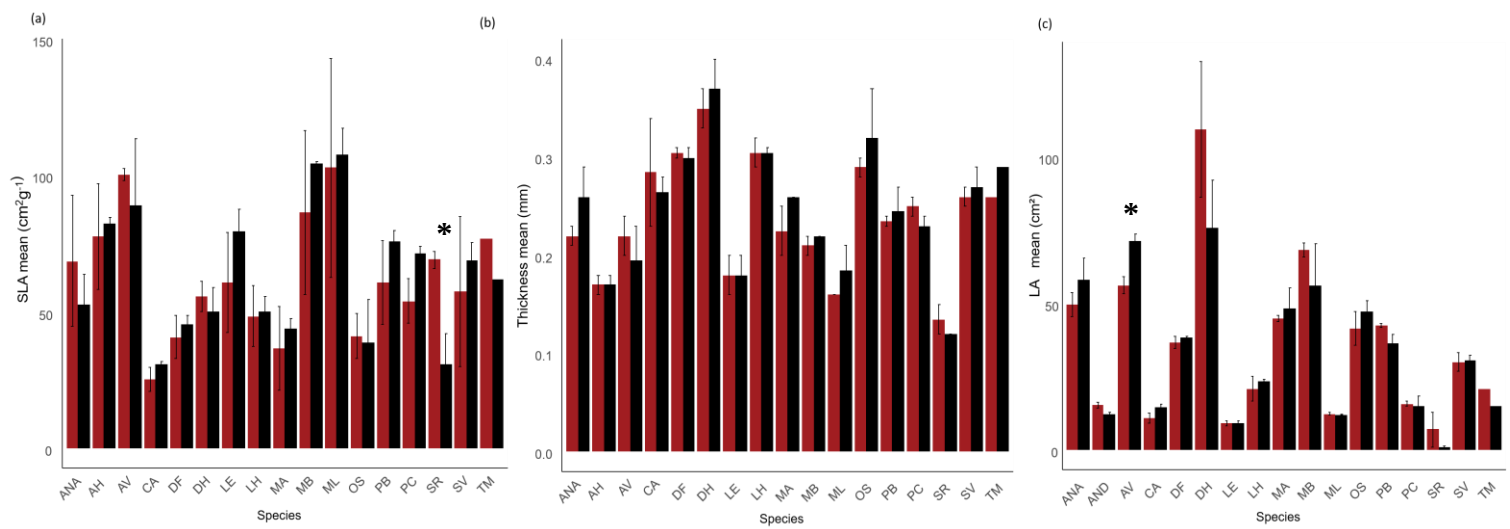


Figure 5. Average values of leaf traits by species collected in the cerrado *sensu stricto* under different treatments (burned and unburned): (a) SLA (specific leaf area) (b) Thickness; (c) LA (leaf area). Burned treatments: red; Unburned treatments: black. The symbol * indicates species that had significant differences between treatments. Vertical bars indicate standard error (n = 9).

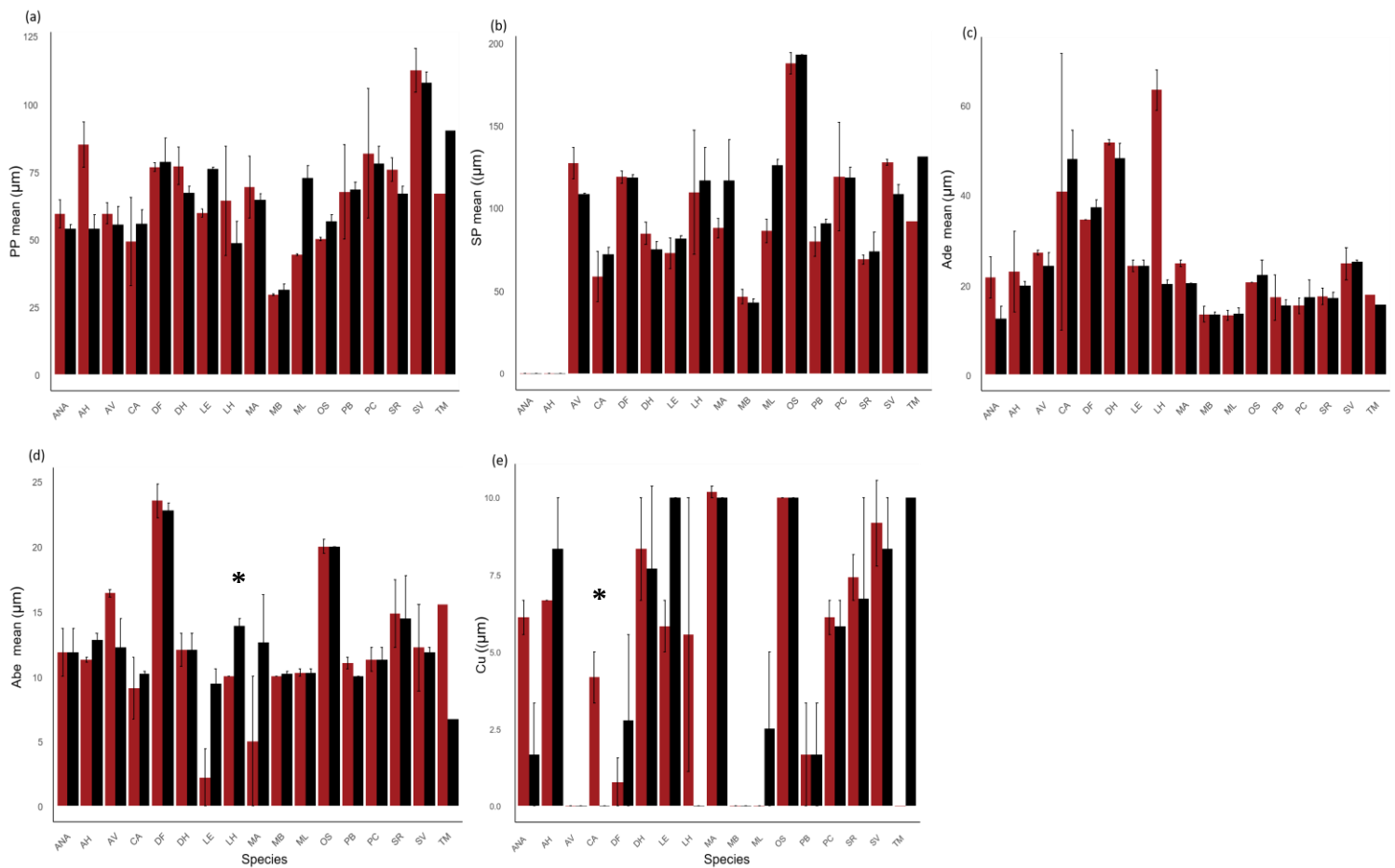


Figure 6. Average values of leaf traits by species collected in the cerrado *sensu stricto* under different treatments (burned and unburned): (a) Palisade parenchyma (PP); (b) Spongy parenchyma (SP); (c) Adaxial face of epidermis (Abe); (d) Abaxial face of epidermis (Abe); (e) Cuticle (Cu). Burned treatments: red; Unburned treatments: black. Th* indicates species that had significant differences between treatments. Vertical bars indicate standard error (n = 9).

The PCA results indicate that Dimension 1 and Dimension 2 collectively account for a significant portion of the total variance in the data (Fig. 7). In Dimension 1, Thickness had the highest contribution (27.56%), followed by Ade (23.29%) and SLA (25.69%). SP contributed 7.72%, PP 3.77%, and Abe, Cu, and LA contributed 10.35%, 1.57%, and 0.05%, respectively (Fig. 7).

Although LA had a minimal contribution to Dimension 1, it was the highest contributor in Dimension 2 (24.86%), followed by Cu (22.63%) and PP (17.88%). The graph illustrates an overlap between the treatments across Dimensions 1 and 2. Dimension 1 primarily separates the groups based on Thickness and SLA, while Dimension 2 distinguishes them based on LA and PP (Fig. 7).

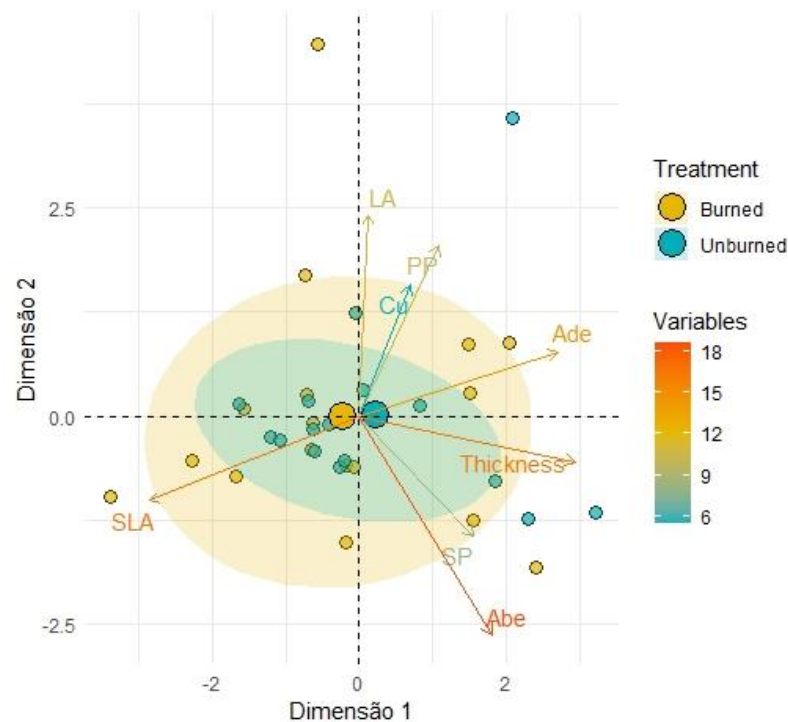


Figure 7. The chart shows a biplot of the Principal Component Analysis (PCA) for different treatments and anatomical leaf traits of plants subjected to fire treatments. Circles represent individuals in the unburned treatment, and triangles represent individuals in the burned treatment. The shaded areas indicate 80% confidence intervals for each group. The arrows represent leaf traits (SLA, LA, Thickness, PP, SP, Ade, Abe, and Cu), and their directions indicate the degree of influence on the first two principal components. The color of the arrows varies according to the magnitude of the variables, as shown in the legend.

Correlation test between variables

Based on the calculated correlations for data from burned and unburned areas, some interesting observations were made. A strong positive correlation was found between palisade parenchyma (PP) and leaf area in burned areas ($r^2 = 0.48$, $p < 0.05$) (Fig. 8a), and this correlation was even stronger in unburned areas ($r^2 = 0.54$, $p < 0.05$) (Fig. 8a), indicating that an increase in PP is consistently linked to an increase in leaf area in both treatments. Spongy parenchyma (SP) exhibited a strong positive correlation with Abe in unburned treatment ($r^2 = 0.50$, $p < 0.05$) (Fig. 8b) but in burned treatments the correlation is not significant in unburned areas ($r^2 = -0.63$, $p < 0.05$).

A negative correlation between Ade and SLA was observed in burned treatments, indicating that when one parenchyma increases, the other tends to decrease ($r^2 = -0.60$, $p < 0.05$) (Fig. 8c). SLA showed a negative relationship with both SLA and LA. In unburned areas, the relationship between SLA and Ade was positive ($r^2 = 0.52$), while the relationship between LA and thickness is negative ($r^2 = -0.60$) (Fig. 9d, e), while the abaxial epidermis (Abe) displayed a moderate positive correlation with SP in burned areas ($r^2 = 0.41$, $p < 0.05$) (Fig. 8f).

These findings suggest intricate relationships among the variables studied, influenced by the treatment (burned vs. unburned). The statistical significance of these correlations underscores the importance of considering the ecological context when interpreting these patterns.

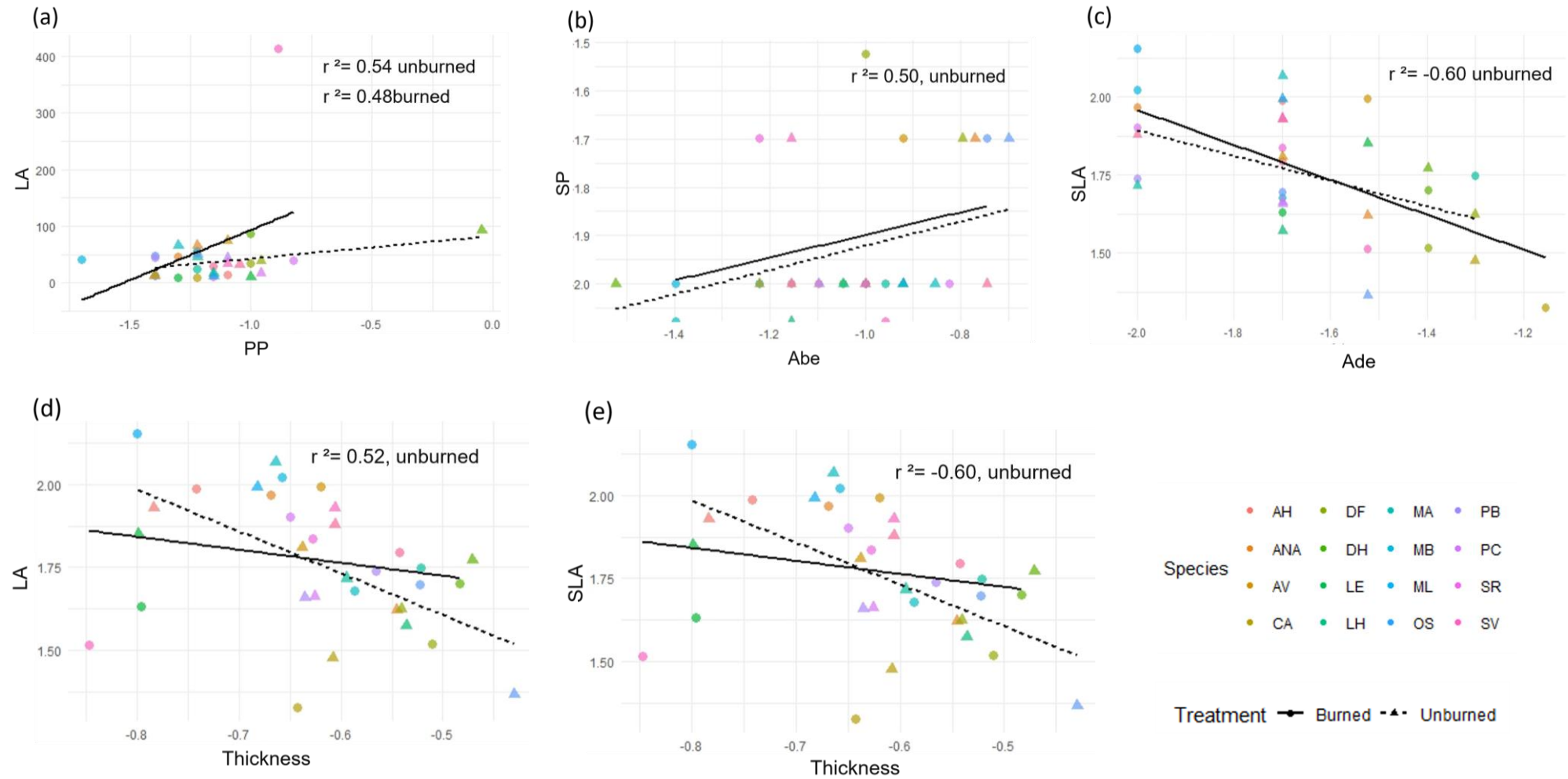


Figure 8. Correlation graphs between leaf traits and the effect of treatments (burned and unburned) and collected species. Each plot represents a different leaf trait, emphasizing the relationships with other leaf traits and the potential impacts of experimental treatments on these traits. (a) Leaf area vs. Palisade parenchyma (b) Spongy parenchyma vs. Abaxial face of epidermis; (c) Specific leaf area vs. Adaxial face of; (d) Leaf area vs. Thickness; (e) Specific leaf area vs. Thickness.

14. Discussion

While we expected to find variations in leaf traits between the burned and unburned treatments, our findings revealed minimal differences among species, regardless of their exposure to annual fire. The variations observed were mainly at the species level and limited to specific leaf traits.

In summary, the responses of different species to the treatments varied considerably, with some species showing little or no significant differences between the burned and unburned treatments. This highlights the variability in how fire affects different species and their leaf traits. For example, species SR exhibited a slight increase in specific leaf area (SLA) in unburned treatments, contrary to our initial hypothesis. We anticipated plants in burned areas to show traits associated with more aggressive resource acquisition, such as higher SLA. The combustion of above-ground biomass often leads to a rapid increase in soil nutrient availability, including essential elements like calcium, magnesium, and potassium in the topsoil layers (Coutinho, 1982; Pivello et al., 2010). This favors plants that employ resource acquisition strategies, which involve producing nutrient-rich leaves, rapid growth, greater height, and higher SLA to maximize light capture and utilization (Diaz et al., 2004; Hoffmann et al., 2012; Pellegrini, 2016). Additionally, species AV had a larger leaf area in unburned sites, while species ML and LE showed higher photosynthetic pigment (PP) values in unburned areas. Overall, the differences observed were less pronounced than expected, with most cases showing higher trait values in the unburned treatments.

Regarding anatomical structure, most species examined displayed typical characteristics of Cerrado species, which generally have a higher proportion of protective tissues, such as the epidermis, often with multiple layers, compared to other tissues (Bieras and Sajo, 2009). The palisade parenchyma is crucial for evenly distributing light across all leaf layers, thereby optimizing photosynthesis (Dickison, 2000; Bieras and Sajo, 2009). Although we initially hypothesized that mesophyll thickness would decrease in areas prone to frequent burning—since thinner mesophyll is often associated with higher specific leaf area (SLA) values—our data did not sufficiently support this expectation. This is demonstrated by species such as ML and LE, which exhibit higher palisade parenchyma (PP) values in unburned areas.

Cerrado leaves are naturally thicker and heavier compared to leaves in other biomes (Bieras and Sajo, 2009; Hoffmann et al., 2005; Rossatto et al., 2015). They typically feature woody plants with sclerophyllous leaves, which are hard and rigid, with a thick waxy cuticular layer, a significant amount of supporting tissue, and developed palisade parenchyma (Sarmiento et al., 1985; Bieras and Sajo, 2009; Pinheiro et al., 2017). Leaf anatomical characteristics are highly sensitive and adapt to the surrounding environment (Royer et al., 2009; Li and Bao, 2014), showing variations among species, geographic regions, and biomes, influenced by phylogeny and adaptations developed over time (He et al., 2018). Additionally, variations in leaf anatomy occur according to growth form; for example, non-arboreal species exhibit a wide range of leaf anatomical traits, from thinner mesomorphic leaves to leaves with some scleromorphic traits (Rossatto et al., 2015). In recent years, numerous studies have explored how plants respond and adapt to environmental changes, focusing on morphological, anatomical, and physiological processes of leaves under different light, moisture, and temperature conditions (Gregoriou et al., 2007; Li and Bao, 2014). However, few studies directly address the impact of fire in savannas.

Studies, such as those conducted by Rossatto et al. (2015) on the leaf anatomy of trees and shrubs, have revealed that savanna tree leaves are typically dorsiventral, a common characteristic among most species, and exhibit a well-developed cuticle. For example, the species CA exhibited a notably thicker cuticle in burned areas compared to unburned areas, where the cuticle was nearly absent, indicating that this feature may function as a defense mechanism against high temperatures. The significant presence of a cuticle in woody savanna plants is considered an adaptation to protect against the intense solar radiation prevalent in these environments (Bieras and Sajo, 2009). In their examination of *Caryocar brasiliense*, Ramos et al. (2015) observed a thick cuticle, a typical trait of xeromorphic plants that have adapted to savanna habitats with distinct seasonal variations (Dickison, 2000).

In the PCA analysis, we observed contrasting trends between SLA and leaf thickness, with thinner leaves showing higher SLA values. This pattern is common in species found in resource-rich environments, which typically have higher SLA values compared to those in nutrient-poor environments (Pérez-Harguindeguy et al., 2013). Species with higher SLA values are often associated with "acquisitive" strategies, characterized by larger leaves and higher SLA values (Díaz et al., 2004). Conversely, lower SLA values indicate a significant investment in structural and

chemical defenses, as well as longer leaf lifespan, typical of species following "conservative" strategies with smaller, thicker, and more resistant leaves (Pérez-Harguindeguy et al., 2013; Díaz et al., 2004).

Leaf area (LA), cuticle thickness (Cu), and palisade parenchyma (PP) are significant variables in the second axis of the principal components in PCA, indicating that leaf area and protection also play a crucial role in distinguishing treatments. Plants with larger leaf areas and thicker cuticles may be better adapted to fire-free environments with intense competition for light and water, displaying characteristics of conservative strategies. Our data suggest that the strategies adopted by plants are species-specific rather than generalizable to the entire community. Wigley et al. (2016) suggest that differences in strategies are influenced by the types of vegetation and growth forms studied. For example, the study by Díaz et al. (2004) included xeric grasses and sclerophyllous species with various lineages and growth forms, such as grasses and monocotyledons. In our study, we examined a diverse range of plant forms from subshrubs to trees, which can encompass a broader set of strategies.

Correlation analysis has revealed a positive relationship between palisade parenchyma (PP) and leaf thickness in both burned and unburned areas. This indicates that an increase in PP is consistently associated with an increase in leaf thickness, potentially serving as an adaptation to improve photosynthetic efficiency in environments with varying levels of fire-induced stress. This structural adjustment may assist plants in optimizing their leaf functions in response to specific environmental challenges, such as those triggered by fires (He et al., 2018).

In the correlation analysis of the burned treatment, a negative correlation was found between spongy parenchyma (SP) and leaf thickness, indicating that as one of these traits increases, the other tends to decrease. Lower leaf thickness is associated with higher specific leaf area (SLA) values, leading to increased photosynthesis and gas exchange. The spongy parenchyma contains larger intercellular spaces, which enhance gas transport, while leaves with thicker palisade parenchyma have more chloroplasts to maximize light absorption. This emphasizes the importance of palisade and spongy tissues in optimizing photosynthesis (Terashima et al., 2011). According to Vogelmann (1993), a well-developed palisade parenchyma is essential for sun leaves, such as those found in woody plants in the Cerrado, as it ensures even distribution of light across all layers of the leaf. These findings underscore the complexity of plant responses to fires

and emphasize the importance of a comprehensive approach to understanding how leaf characteristics adapt and respond to environmental stresses in diverse ecosystems.

Both the upper and lower epidermis showed negative correlations with other leaf traits, such as specific leaf area (SLA) and the spongy parenchyma (SP) itself. The negative correlation between the adaxial epidermis and SLA in unburned areas suggests that a thicker epidermis may serve as a defense mechanism against dehydration or excessive sun exposure. Ramos et al., (2015) observed a thicker epidermis in *Caryocar brasiliense*, influenced by environmental factors such as low soil nutrient content, fire frequency, and high light and temperature intensities; the authors highlighted the impact of fires on vegetation as a contributing factor to the thickening of cellular tissues. Fire plays a significant role in shaping savanna dynamics, impacting floristic composition, the balance between woody and herbaceous plants, and the phytophysiognomic characteristics of Cerrado vegetation (Moreira, 2000; Miranda et al., 2009). The moderate positive correlation between the abaxial epidermis and SP in burned areas suggests that these traits may play a crucial role in leaf protection. While not all our hypotheses were confirmed, the molecular mechanisms governing leaf morphological traits, which can impact photosynthetic rates and leaf anatomy, remain poorly understood (Tholen et al., 2012). This data is essential for comprehending how plants adapt to environments prone to frequent disturbances and can aid in the development of management and conservation strategies for fire-prone ecosystems.

15. Conclusion

This study examined the relationship between fire, leaf traits, and adaptive responses in Cerrado species. The research revealed that there were minimal differences in leaf traits between environments with and without fire. Surprisingly, the traits did not exhibit a consistent trend towards acquisitive characteristics in areas with frequent fire; instead, they varied between conservative and acquisitive traits, indicating a range of responses. These findings highlight the significance of considering fire as a pivotal factor in shaping leaf traits and adaptive strategies in Cerrado ecosystems. They also underscore the necessity of employing comprehensive approaches to comprehend plant reactions to disturbances, essential for devising effective management and conservation strategies in fire-prone ecosystems.

16. References

- Abreu, R.C., Hoffmann, W.A., Vasconcelos, H.L., Pilon, N.A., Rossatto, D.R., & Durigan, G. (2017). The biodiversity cost of carbon sequestration in tropical savanna. *Science Advances*, 3(8), e1701284. <https://doi.org/10.1126/sciadv.1701284>
- Archibald, S., Lehmann, C. E. R., Belcher, C. M., Bond, W. J., Bradstock, R. A., Daniau, A.-L., Dexter, K. G., Forrestel, E. J., Greve, M., He, T., Higgins, S. I., Hoffmann, W. A., Lamont, B. B., McGlenn, D. J., Moncrieff, G. R., Osborne, C. P., Pausas, J. G., Price, O., Ripley, B. S., Rogers, B. M., Schwilk, D. W., Simon, M. F., Turetsky, M. R., Van der Werf, G. R., and Zanne, A. E. (2018). Biological and geophysical feedbacks with fire in the Earth system. *Environmental Research Letters*, 13. doi: 10.1088/1748-9326/aa9ead
- Bieras, A. C., Sajo, M. G. (2009). Leaf structure of the cerrado (Brazilian savanna) woody plants. *Trees*, 23: 451–471. doi:10. 1007/s00468-008-0295-7.
- Clarke, P. J., Lawes, M. J., & Midgley, J. J. (2013). Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist*, 197(3), 837-839. doi: 10.1111/nph.12001
- Coutinho, L. M. (1982). Ecological effects of fire in Brazilian cerrado. *Fire in Tropical Biota*. Springer-Verlag, Berlin, Germany, 273-291.
- Coutinho, L. M. (1990). Fire in the ecology of the Brazilian Cerrado. In J. G. Goldammer (Ed.), *Fire in the tropical biota: ecosystem processes and global challenges* (pp. 82-105). Springer.
- Crang, R. F. E., Lyons-Sobaski, S., Wise, R. (2018). *Plant anatomy: A concept-based approach to the structure of seed plants*. Springer International Publishing.
- Cutler, D. R., T. C. Edwards Jr., K. H. Beard, A. Cutler, K. T. Hess, J. Gibson, J., Lawler, J. J. (2007). Random forests for classification in ecology. *Ecology, Ecological Society of America*. 88 (11). <https://doi.org/10.1890/07-0539.1>
- Díaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., Montserrat-Martí, G., Grime, J. P., Zarrinkamar, F., Asri, Y., Band, S. R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M., Pérez-Harguindeguy, N., Pérez-Rontomé, M. C., Shirvany, F. A., Vendramini, F., Yazdani, S. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, 15(3), 295-304.
- Dickison, W. C. (2000). *Integrative plant anatomy*. Academic Press.
- Dijkstra, P., Lambers, H. (1989). A physiological analysis of genetic variation in relative growth rate within *Plantago major* L. *Functional Ecology*, 3(1), 61-74.

Durigan, G., and Ratter, J. A. (2006). Successional changes in cerrado and cerrado/forest ecotonal vegetation in western São Paulo State, Brazil, 1962-2000. *Edinburgh Journal of Botany*, 63(1), 119-130. <https://doi.org/10.1017/S0960428606000357>

Durigan, G. (2020). Fire management in the Brazilian savanna. *Fire Ecology*, 16, 11. <https://doi.org/10.1016/j.flora.2020.151612>

Evans, J. R., Kaldenhoff, R., Genty, B., Terashima, I. (2009). Resistances along the CO₂ diffusion pathway inside leaves. *Journal of Experimental Botany*, 60, 2235-2248.

Fahn, A. (1986). Plant anatomy. *Pergamon Press*.

Flake, R., Anten, N. P. R., Werger, M. J. A. (2021). Savannas are not old fields: Functional trajectories of forest expansion in a fire- suppressed Brazilian savanna are driven by habitat generalists. *Functional Ecology*, 00:1–13. doi: 10.1111/1365-2435.13818

Gomes, L., Ribeiro, K. T., Durigan, G., Cochrane, M. A. (2018). How can we advance the knowledge on the behavior and effects of fire in the Cerrado biome? *Forest Ecology and Management* 417 (2018) 281–290. <https://doi.org/10.1016/j.foreco.2018.02.032>

Gotelli, N. J., Ellison, A. M. (2011). A primer of ecological statistics. *Sinauer Associates*.

Gregoriou, K., Pontikis, K., Vemmos, S., Ouzounidou, G. (2007). Effects of reduced irradiance on leaf morphology, photosynthetic capacity, and fruit yield in olive (*Olea europaea* L.) *Photosynthetica* 45, 172–181. <https://doi.org/10.1007/s11099-007-0029-x>

Hardesty, J., Myers, R., Fulks, W. (2005). Fire, ecosystems and people: A preliminary assessment of fire as a global conservation issue. *Fire Management*, 22, 78-87. <http://www.jstor.org/stable/43597968>.

He, N., Liu, C., Tian, M., Li, M., Yang, H., Yu, G., Guo, D., Smith, M. D., Yu, Q., Hou, J. (2018). Variation in leaf anatomical traits from tropical to cold-temperate forests and linkage to ecosystem functions. *Functional Ecology*, 32(1). <https://doi.org/10.1111/1365-2435.12934>

Hoffmann, W. A., Franco, A. C., Moreira, A. G., Haridasan, M. (2005). Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. *Functional Ecology*, 19(6), 932-940.

Hoffmann, W. A., Geiger, E. L., Gotsch, S. G., Rossatto, D. R., Silva, L. C. R., Lau, O. L. (2012). Ecological thresholds at the savanna-forest boundary: How plant traits, resources and fire govern the distribution of tropical biomes. *Ecology Letters*, 15(7), 759-768.

Johansen, D. A. (1940). Plant microtechnique. *McGraw-Hill Book Company, Inc.*

Keeley, J. E., Pausas, J. G., Rundel, P. W., Bond, W. J., Bradstock, R. A. (2011). Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science*, 16(8), 406-411. <https://doi.org/10.1016/j.tplants.2011.04.002>

Keeley, J. E., Pausas, J. G. (2022). Evolutionary Ecology of Fire. *Annu. Rev. Ecol. Evol. Syst.* 53:203–25. <https://doi.org/10.1146/annurev-ecolsys-102320-095612>

Li, F.L, Bao, W.K. (2014). Elevational trends in leaf size of *Campylotropis polyantha* in the arid Minjiang River valley, SW China. *Journal of Arid Environments*. 109, 1-9. <https://doi.org/10.1016/j.jaridenv.2014.04.011>

Melo, A.C.G. and Durigan, G. (2011). Encarte 3: Análise da Estação Ecológica de Santa Bárbara. In: ACG Melo, G Durigan (coordinators.) Estação Ecológica de Santa Bárbara: Plano de Manejo, Águas de Santa Bárbara, Instituto Florestal, SP. 66-100

Miranda, A. C., Miranda, H.S, Dias, I.F.P.O, and Dias, B.F.S. (1993). Soil and air temperatures during prescribed cerrado fires in Central Brazil. *Journal Tropical Ecology*. 9: 313–320.

Miranda, H. S., Bustamante, M. M., and Miranda, A. C. (2002). The fire factor. In P. Oliveira and R. Marquis (Eds.), *The Cerrados of Brazil: Ecology and Natural History of a Neotropical Savanna* (pp. 51–61). *Columbia University Press*.

Miranda, H. S., Sato, M. N., Neto, W. N., & Aires, F. S. (2009). Fires in the cerrado, the Brazilian savanna. In M. A. Cochrane (Ed.), *Tropical fire ecology* (Vol. 2, pp. 427–450). Springer.

Moreira, A.G. (2000). Effects of fire protection on savanna structure. *Journal of Biogeography*, 27(4):1021-1029. <https://doi.org/10.1046/j.1365-2699.2000.00422.x>

Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853–858. <https://doi.org/10.1038/35002501>

Niinemets, Ü. (2001). Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology*, 82(2), 453-469. [https://doi.org/10.1890/0012-9658\(2001\)082\[0453:GSCCOL\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[0453:GSCCOL]2.0.CO;2)

Oguchi, R., Hikosaka, K., Hirose, T. (2006). Leaf anatomy and light acclimation in woody plants. *Tree Physiology*, 31(4), 443-456.

Pausas, J. G. (2015). Evolutionary fire ecology: Lessons learned from pines. *Trends in Plant Science*, 20(5), 318-324.

Pellegrini, A. F. A. (2016). Carbon accumulation and nitrogen availability along a savanna rainfall gradient. *Global Change Biology*, 22(7), 2417-2424.

Pellegrini, A.F.A., Anderegg, L., Pinto-Ledezma, J.N., Cavender-Bares, J., Hobbie, S.E., and Reich, P.B. (2023). Consistent physiological, ecological and evolutionary

effects of fire regime on conservative leaf economics strategies in plant communities. *Ecology Letters*, 26(3), 597–608. <https://doi.org/10.1111/ele.14182>

Penman, T. D., Kavanagh, R. P., Binns, D. L., Stephens, S. L. (2011). Prescribed burning: How can it work to conserve the things we value? *International Journal of Wildland Fire*, 20(6), 721-733. <https://doi.org/10.1071/WF09131>

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., de Vos, A. C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J. G., Thompson, K., Morgan, H. D., ter Steege, H., van der Heijden, M. G. A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M. V., Conti, G., Staver, A. C., Aquino, S., Cornelissen, J. H. C. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 61(3), 167-234.

Pilon, N. A. L., Gomes, L., Fidelis, A., Machado, E. L. M., Pivello, V. R. (2018). Quantifying the short-term flowering after fire in some plant communities of a cerrado grassland. <https://doi.org/10.1080/17550874.2018.1517396>

Pinheiro, L. F. S., Kolb, R. M., Rossatto, D. R. (2018). Leaf anatomical traits of non-arboreal savanna species along a gradient of tree encroachment. *Acta Botanica Brasilica*, 32(1), 28-36. <https://doi.org/10.1590/0102-33062017abb0280>

Pivello, V. R., Coutinho, L. M. (1996). A qualitative successional model to assist in the management of Brazilian cerrados. *Forest Ecology and Management*, 87(1-3), 127-138.

Pivello V.R., Oliveras I., Miranda H.S., Haridasan M., Sato M.N., Meirelles S.T. (2010). Effect of fires on soil nutrient availability in an open savanna in Central Brazil. *Plant Soil* 337:111–123

Ramos, B.H., Silva, K. L. F., Coimbra, R. R (2015). Anatomy and micromorphometry of Caryocar brasiliense leaves. *Rodriguesia* 66(1), 087-094. doi: 10.1590/2175-7860201566106

Reich, P. B., Walters, M. B., Ellsworth, D. S. (1997). From tropics to tundra: Global convergence in plant functioning. *Proceedings of the National Academy of Sciences*, 94(15), 7215-7222. doi: 10.1073/pnas.94.25.13730

Reich, P. B. (2014). The world-wide ‘fast–slow’ plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275-301. <https://doi.org/10.1111/1365-2745.1221>

Rizzini, C. T. (1976). Influência da temperatura sobre a germinação de diásporos de cerrado. *Rodriguesia* 41: 341–384.

Rossatto, D. R., Silva, L. C. R., Oliveira, R. S. (2015). Leaf anatomy is associated with the type of growth form in Neotropical savanna plants. *Botany* 93: 507–518 [dx.doi.org/10.1139/cjb-2015-0001](https://doi.org/10.1139/cjb-2015-0001)

- Royer, D.L., Meyerson, L.A., Robertson, K.M., Adams, J.M. (2009). Phenotypic plasticity of leaf shape along a temperature gradient in *Acer rubrum*. *PLOS ONE*, 3(12), e4109. <https://doi.org/10.1371/journal.pone.0007653>
- Sarmiento, G., Goldstein, G. and Meinzer, F. 1985. Adaptative strategies of woody species in neotropical savannas. *Biological Review*, 60: 315-355. <https://doi.org/10.1111/j.1469-185X.1985.tb00420.x>
- Scheepens, J. F., Frei, E. S., Stöcklin, J. (2010). Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia* 164, 141–150. <https://doi.org/10.1007/s00442-010-1650-0>
- Scalon, M. C., Rossatto, D. R., Franco, A. C., Moreira, A. G. (2017). Do litter manipulations affect leaf functional traits of savanna woody plants? *Plant Ecology*, 218(10), 1205-1217. <https://doi.org/10.1007/s11258-013-0282-y>
- Scalon, M.C., Domingos, F.M.C.B., Cruz, W.J.A.da, Júnior, B.H.M., Marimon, B.S., Oliveras, I., (2020). Diversity of functional trade-offs enhances survival after fire in Neotropical savanna species. *Journal of Vegetation Science*. 31, 139–150. <https://doi.org/10.1111/jvs.12823>
- Stotz, G. C., Salgado-Luarte, C., Escobedo, V. M., Valladares, F., Gianoli, E. (2022). Phenotypic plasticity and the leaf economics spectrum: plasticity is positively associated with specific leaf area. *Oikos*, 11. <https://doi.org/10.1111/oik.09342>
- Terashima I., Hanba Y.T., Tholen D., Niinemets Ü. (2011). Leaf functional anatomy in relation to photosynthesis. *Plant Physiology*.155(1), 108-16. doi: 10.1104/pp.110.165472.
- Tholen, D., Ethier, G., Genty, B., Pepin, S., Zhu, X.-G. (2012). Variable mesophyll conductance revisited: theoretical background and experimental implications. *Plant, Cell and Environment*, 35(15). <https://doi.org/10.1111/j.1365-3040.2012.02538.x>
- Vogelmann, T. C. (1993). Plant tissue optics. *Annual Review of Plant Biology*, 44(1), 231-251. <http://doi.org/10.1146/annurev.pp.44.060193.001311>
- White, P.S., and Pickett, S.T.A. (1985). Natural disturbance and patch dynamics: An introduction. In S.T.A. Pickett & P.S. White (Eds.), *The ecology of natural disturbance and patch dynamics* (pp. 3–13). *New York: Academic*.
- Wigley, B. J., Slingsby, J. A., Díaz, S., Bond, W. J., Fritz, H., & Coetsee, C. (2016). Leaf traits of African woody savanna species across climate and soil fertility gradients: evidence for conservative versus acquisitive resource-use strategies. *Journal of Ecology*, 104(5), 1357–1369. <https://doi.org/10.1111/1365-2745.12598>
- Witkowski, E.T.F., and Lamont, B.B. (1991). Leaf specific mass confounds leaf density and thickness. *Oecologia*, 88(4), 486-493. doi: 10.1007/BF00317710
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier,

E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M. L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J., Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827.

Xiong, D., Flexas, J., Yu, T., Peng, S., Huang, J. (2016). Leaf anatomy mediates coordination of leaf hydraulic conductance and photosynthesis in rice. *New Phytologist*, 213(2), 572-583. <https://doi.org/10.1111/nph.14186>

Zhu J., He N., Wang Q., Yuan G., Wen D., Yu G., Jia Y. (2015). The composition, spatial patterns, and influencing factors of atmospheric wet nitrogen deposition in Chinese terrestrial ecosystems. *The Science Total Environment*. doi: 10.1016/j.scitotenv.2014.12.038.

Zirondi, H., Ooi, M. K. J., and Fidelis, A. (2021). Fire-triggered flowering is the dominant post-fire strategy in a tropical savanna. *Journal of Vegetation Science*, 32(2). <https://doi.org/10.1111/jvs.12995>. *Journal of Ecology*, 109(3), 1291-1301.

17. Supplementary material

Table 3. Average leaf traits for each species were compared between burned and unburned treatments in cerrado *sensu stricto*.

Treatments	Species	Thickness	LA	SLA	PP	SP	Ade	Abe	Cu
Burned	ANA	0.214 ($\pm$ 0.015)	45.73 ($\pm$ 4.70)	92.76 ($\pm$ 6.16)	151.85 ($\pm$ 3.82)	-----	17.04 ($\pm$ 1.28)	13.7 ($\pm$ 1.28)	6.67 ($\pm$ 0.92)
Unburned	ANA	0.285 ($\pm$ 0.01)	66.01 ($\pm$ 7.99)	41.68 ($\pm$ 12.1)	162.41 ($\pm$ 7.87)	-----	15.19 ($\pm$ 1.44)	13.7 ($\pm$ 1.44)	3.33 ($\pm$ 0.92)
Burned	AH	0.181 ($\pm$ 0.011)	14.36 ($\pm$ 1.81)	97.26 ($\pm$ 7.0)	221.46 ($\pm$ 2.79)	-----	14.07 ($\pm$ 0.96)	11.1 ($\pm$ 0.96)	6.67 ($\pm$ 0.92)
Unburned	AH	0.164 ($\pm$ 0.005)	11.68 ($\pm$ 1.64)	84.84 ($\pm$ 9.47)	147.41 ($\pm$ 4.07)	-----	20.74 ($\pm$ 1.18)	12.22 ($\pm$ 1.1)	6.67 ($\pm$ 0.92)
Burned	AV	0.24 ($\pm$ 0.013)	53.63 ($\pm$ 1.44)	98.586 ($\pm$ 20.0)	55.56 ($\pm$ 1.71)	118.33 ($\pm$ 6.73)	26.67 ($\pm$ 1.14)	16.6 ($\pm$ 1.14)	0.0 ($\pm$ 0.0)
Unburned	AV	0.163 ($\pm$ 0.019)	69.73 ($\pm$ 12.03)	113.74 ($\pm$ 32.0)	62.2 ($\pm$ 3.23)	107.78 ($\pm$ 3.23)	21.1 ($\pm$ 1.11)	10.0 ($\pm$ 1.11)	0.0 ($\pm$ 0.0)
Burned	CA	0.341 ($\pm$ 0.055)	12.64 ($\pm$ 5.56)	29.96 ($\pm$ 4.78)	65.56 ($\pm$ 3.00)	74.07 ($\pm$ 2.80)	71.48 ($\pm$ 2.9)	11.48 ($\pm$ 2.9)	3.33 ($\pm$ 1.05)
Unburned	CA	0.276 ($\pm$ 0.013)	15.68 ($\pm$ 1.38)	31.94 ($\pm$ 5.32)	50.37 ($\pm$ 1.72)	67.78 ($\pm$ 2.94)	54.44 ($\pm$ 1.5)	10.0 ($\pm$ 1.54)	0.0 ($\pm$ 0.0)
Burned	DF	0.297 ($\pm$ 0.013)	39.03 ($\pm$ 5.22)	48.98 ($\pm$ 6.89)	75.19 ($\pm$ 2.94)	122.96 ($\pm$ 6.46)	34.4 ($\pm$ 1.23)	22.2 ($\pm$ 1.23)	1.54 ($\pm$ 0.70)
Unburned	DF	0.311 ($\pm$ 0.009)	38.83 ($\pm$ 8.22)	48.85 ($\pm$ 10.36)	69.63 ($\pm$ 2.53)	120.74 ($\pm$ 7.82)	38.89 ($\pm$ 1.94)	22.2 ($\pm$ 1.94)	0.0 ($\pm$ 0.0)
Burned	DH	0.370 ($\pm$ 0.015)	132.98 ($\pm$ 18.87)	61.21 ($\pm$ 2.839)	70.0 ($\pm$ 1.92)	78.52 ($\pm$ 2.04)	52.2 ($\pm$ 1.79)	13.33 ($\pm$ 1.7)	6.67 ($\pm$ 0.92)
Unburned	DH	0.40 ($\pm$ 0.04)	60.08 ($\pm$ 11.8)	41.34 ($\pm$ 10.86)	64.81 ($\pm$ 4.41)	80.0 ($\pm$ 6.79)	51.48 ($\pm$ 2.0)	10.74 ($\pm$ 2.0)	10.37 ($\pm$ 0.3)
Burned	LE	0.19 ($\pm$ 0.006)	9.909 ($\pm$ 5.08)	79.371 ($\pm$ 6.12)	58.15 ($\pm$ 1.60)	82.22 ($\pm$ 3.47)	22.9 ($\pm$ 1.49)	4.44 ($\pm$ 1.49)	6.67 ($\pm$ 0.92)
Unburned	LE	0.19 ($\pm$ 0.011)	8.54 ($\pm$ 4.57)	87.87 ($\pm$ 3.973)	80.56 ($\pm$ 5.23)	115.56 ($\pm$ 7.09)	45.28 ($\pm$ 1.35)	9.17 ($\pm$ 1.35)	10.0 ($\pm$ 0.0)
Burned	LH	0.31 ($\pm$ 0.03)	25.23 ($\pm$ 1.59)	60.01 ($\pm$ 5.713)	84.44 ($\pm$ 6.42)	147.78 ($\pm$ 7.25)	67.78 ($\pm$ 4.0)	10.0 ($\pm$ 4.00)	10.0 ($\pm$ 0.0)
Unburned	LH	0.30 ($\pm$ 0.015)	24.21 ($\pm$ 1.94)	55.849 ($\pm$ 5.04)	40.56 ($\pm$ 4.237)	96.67 ($\pm$ 5.113)	19.44 ($\pm$ 2.35)	14.44 ($\pm$ 2.35)	0.0 ($\pm$ 0.0)
Burned	MA	0.254 ($\pm$ 0.007)	46.046 ($\pm$ 6.427)	52.15 ($\pm$ 13.6)	80.74 ($\pm$ 4.80)	94.22 ($\pm$ 7.49)	24.07 ($\pm$ 1.10)	10.0 ($\pm$ 1.10)	10.37 ($\pm$ 0.37)
Unburned	MA	0.259 ($\pm$ 0.015)	55.659 ($\pm$ 6.45)	47.72 ($\pm$ 11.6)	66.67 ($\pm$ 3.99)	141.4 ($\pm$ 11.38)	20.37 ($\pm$ 1.55)	8.89 ($\pm$ 1.55)	10.0 ($\pm$ 0.0)
Burned	MB	0.217 ($\pm$ 0.006)	66.244 ($\pm$ 9.243)	116.8 ($\pm$ 27.27)	29.26 ($\pm$ 1.68)	51.11 ($\pm$ 2.15)	11.85 ($\pm$ 0.76)	10.0 ($\pm$ 0.76)	-----
Unburned	MB	0.22 ($\pm$ 0.011)	41.697 ($\pm$ 6.72)	105.39 ($\pm$ 5.59)	33.33 ($\pm$ 0.92)	45.19 ($\pm$ 1.34)	14.07 ($\pm$ 0.96)	10.0 ($\pm$ 0.96)	-----
Burned	ML	0.158 ($\pm$ 0.003)	13.006 ($\pm$ 6.434)	143.13 ($\pm$ 6.66)	43.89 ($\pm$ 1.10)	93.33 ($\pm$ 4.50)	12.22 ($\pm$ 0.85)	10.56 ($\pm$ 0.85)	0.0 ($\pm$ 0.0)

Unburned	ML	0.208 ($\pm$ 0.007)	12.362 ($\pm$ 1.387)	98.28 ($\pm$ 21.36)	77.22 ($\pm$ 1.86)	130.0 ($\pm$ 3.43)	15.0 ($\pm$ 0.96)	10.56 ($\pm$ 0.96)	5.0 ($\pm$ 0.92)
Burned	OS	0.3 ($\pm$ 0.01)	47.564 ($\pm$ 4.34)	49.72 ($\pm$ 9.733)	50.56 ($\pm$ 2.32)	194.44 ($\pm$ 1.42)	20.56 ($\pm$ 0.37)	19.44 ($\pm$ 0.37)	10.0 ($\pm$ 0.0)
Unburned	OS	0.372 ($\pm$ 0.008)	51.273 ($\pm$ 4.434)	23.19 ($\pm$ 14.04)	58.89 ($\pm$ 3.88)	193.33 ($\pm$ 2.88)	25.56 ($\pm$ 1.75)	20.0 ($\pm$ 1.75)	10.0 ($\pm$ 0.0)
Burned	PB	0.237 ($\pm$ 0.009)	42.113 ($\pm$ 2.117)	76.362 ($\pm$ 3.64)	50.0 ($\pm$ 3.15)	71.11 ($\pm$ 2.46)	12.22 ($\pm$ 0.94)	10.56 ($\pm$ 0.94)	-----
Unburned	PB	0.268 ($\pm$ 0.011)	33.757 ($\pm$ 2.58)	72.38 ($\pm$ 3.56)	65.19 ($\pm$ 2.22)	93.7 ($\pm$ 3.85)	16.6 ($\pm$ 1.06)	10.0 ($\pm$ 1.06)	-----
Burned	PC	0.258 ($\pm$ 0.016)	15.017 ($\pm$ 1.86)	62.45 ($\pm$ 27.19)	105.56 ($\pm$ 3.39)	152.22 ($\pm$ 6.23)	13.7 ($\pm$ 0.94)	10.37 ($\pm$ 0.94)	5.56 ($\pm$ 0.97)
Unburned	PC	0.217 ($\pm$ 0.021)	18.621 ($\pm$ 2.41)	74.17 ($\pm$ 27.78)	84.44 ($\pm$ 4.58)	125.0 ($\pm$ 11.32)	21.1 ($\pm$ 1.96)	12.2 ($\pm$ 1.96)	5.0 ($\pm$ 1.21)
Burned	SR	0.154 ($\pm$ 0.004)	1.155 ($\pm$ 0.273)	72.554 ($\pm$ 23.35)	71.48 ($\pm$ 2.24)	71.85 ($\pm$ 3.06)	19.26 ($\pm$ 0.51)	17.4 ($\pm$ 0.51)	8.15 ($\pm$ 0.76)
Unburned	SR	0.123 ($\pm$ 0.005)	1.55 ($\pm$ 0.193)	19.78 ($\pm$ 15.43)	64.23 ($\pm$ 2.72)	86.15 ($\pm$ 4.60)	15.77 ($\pm$ 1.0)	11.1 ($\pm$ 1.00)	3.46 ($\pm$ 0.97)
Burned	SV	0.248 ($\pm$ 0.008)	33.516 ($\pm$ 1.973)	85.21 ($\pm$ 15.83)	104.4 ($\pm$ 6.96)	126.67 ($\pm$ 5.65)	28.33 ($\pm$ 1.2)	8.89 ($\pm$ 1.21)	10.56 ($\pm$ 0.55)
Unburned	SV	0.287 ($\pm$ 0.023)	29.27 ($\pm$ 3.317)	62.254 ($\pm$ 9.29)	111.8 ($\pm$ 8.16)	114.81 ($\pm$ 3.47)	24.81 ($\pm$ 0.98)	12.2 ($\pm$ 0.98)	10.0 ($\pm$ 0.0)
Burned	TM	0.262 ($\pm$ 0.019)	20.89 ($\pm$ 4.87)	77.28 ($\pm$ 12.19)	66.67 ($\pm$ 2.35)	92.22 ($\pm$ 2.77)	17.78 ($\pm$ 1.47)	15.56 ($\pm$ 1.47)	0.0 ($\pm$ 0.0)
Unburned	TM	0.292 ($\pm$ 0.037)	14.96 ($\pm$ 2.70)	61.96 ($\pm$ 10.36)	90.0 ($\pm$ 1.98)	131.6 ($\pm$ 13.17)	15.56 ($\pm$ 1.45)	6.67 ($\pm$ 1.451)	10.0 ($\pm$ 0.0)

Note: LA: leaf area; SLA: specific leaf area; PP: Palisade parenchyma; SP: Spongy parenchyma; Ade: Adaxial face of epidermis; Abe: Abaxial face of epidermis

Table 4. Table presenting the results of a MANOVA for leaf traits, with F and p-values for treatments (burned and unburned). Variables include PP (palisade tissue thickness), SP (spongy parenchyma thickness), Ade (adaxial epidermis thickness), Abe (abaxial epidermis thickness), Cu (cuticle), Thickness (leaf thickness), LA (leaf area), and SLA (specific leaf area).

Variables	F (Treatments)	p (Treatments)
PP	1.087	0.3104
SP	0.808	0.3893
Ade	0.028	0.8681
Abe	0.483	0.4988
Cu	0.189	0.6662
Thickness	0.440	0.5135
LA	0.649	0.4276
SLA	0.038	0.8491

Statistical significance is indicated with asterisks: ** $p < 0.05$.

Table 5. Means of the measured traits between the burned and unburned treatments.

Variables	Burned	Unburned
PP	0.067	0.125
SP	0.094	0.110
Ade	0.025	0.025
Abe	0.011	0.012
Cu	0.005	0.007
Thickness	0.238	0.251
LA	57.281	39.064
SLA	66.845	60.846

Table 6. Correlation coefficients (r^2) and their corresponding p-values were calculated for leaf traits under both burned and unburned treatment conditions.

Leaf Traits	Correlation - Burned (r, p)	Correlation - Unburned (r, p)
PP and SP	0.36, 0.1545	-0.42, 0.1084
PP and Ade	0.16, 0.5357	0.32, 0.2316
PP and Abe	-0.07, 0.7844	-0.08, 0.7694
PP and Cu	0.39, 0.1262	0.17, 0.5269
PP and Thickness	-0.03, 0.9107	0.41, 0.1113
PP and LA	0.48, 0.0494*	0.54, 0.0293*
PP and SLA	-0.30, 0.2461	-0.03, 0.9160
SP and Ade	0.17, 0.5025	-0.06, 0.8347
SP and Abe	0.30, 0.2425	0.50, 0.0462*
SP and Cu	0.25, 0.3350	0.17, 0.5241
SP and Thickness	0.20, 0.4526	0.20, 0.4496
SP and LA	0.13, 0.6179	0.07, 0.7999
SP and SLA	-0.27, 0.3015	-0.04, 0.8713
Ade and Abe	0.12, 0.6338	0.34, 0.2036
Ade and Cu	0.35, 0.1702	-0.25, 0.3466
Ade and Thickness	0.31, 0.2305	0.33, 0.2107
Ade and LA	0.05, 0.8417	0.08, 0.7630
Ade and SLA	-0.60, 0.0114*	-0.43, 0.0943
Abe and Cu	-0.30, 0.2440	-0.02, 0.9531
Abe and Thickness	0.42, 0.0941	0.33, 0.2171
Abe and LA	-0.42, 0.0948	0.26, 0.3234
Abe and SLA	0.01, 0.9826	-0.13, 0.6318
Cu and Thickness	-0.06, 0.8121	0.01, 0.9654
Cu and LA	0.18, 0.4863	-0.11, 0.6875
Cu and SLA	-0.47, 0.0562	0.37, 0.1538
Thickness and LA	-0.31, 0.2250	0.52, 0.0390*
Thickness and SLA	-0.36, 0.1586	-0.60, 0.0134*
LA and SLA	-0.29, 0.2674	-0.01, 0.9733

Statistical significance is indicated with asterisks: ** $p < 0.05$.

Chapter III

Leaf Traits Responses of Cerrado Plants to Fire Presence and Absence



18. Introduction

The Cerrado, a vast savanna ecosystem in central Brazil, covers about 23% of the country's land area (Ratter, 1997), being known by its rich biodiversity and high endemism of plant species (Klink and Machado, 2005; Silva and Larcher, 2020). Its distribution, composition, and structure are influenced by key environmental factors like climate, soil fertility, water availability, fire frequency, and herbivory (Oliveira-Filho and Ratter, 2002; Dantas et al., 2013; Lehmann et al., 2014; Pellegrini, 2016; Bueno et al., 2018). The dominance of each factor varies by location, with climate and soil often playing crucial roles at geographical scales (Lehmann et al., 2014). While climate impacts savannas on a large scale (Hirota et al., 2011), soil and fire have more localized effects (Coutinho, 1990; Lehmann et al., 2014; Pausas, 2014; Pellegrini, 2016). Cerrado soils are known for being nutrient-poor, and acidic, with low cation exchange capacity, and high aluminum saturation levels (Haridasan, 2008).

In the Cerrado, like in other tropical savannas, fires are common, and most species have traits that allows persistence and survival against these frequent events (Eiten, 1972; Hofmann, 2002; Durigan and Ratter, 2006; Miranda et al., 2009; Simon et al., 2009). Many plant species have strategies to deal with disturbances caused by fires (Coutinho, 1990; Miranda et al., 2009; Zupo et al., 2020). These strategies include features such as thick barks and underground storage organs that aid in post-fire resprout (Appenzato-da-Glória et al., 2008; Souchie et al., 2017; Souza et al., 2017), as well as the presence of numerous protected buds that allow for aerial resprouting of the lost organs (Fidelis et al., 2014; Gottsberger and Silberbauer-Gottsberger, 2006).

Fire can have a significant impact on soil properties and nutrient cycling, directly affecting plant recovery (Miranda et al., 2009; Costa and Rodrigues, 2015; Magallanes et al., 2020). Changes in soil nutrient composition, such as calcium (Ca), magnesium (Mg), aluminum, potassium (K), and phosphorus, as well as organic matter content, can be particularly significant (Bustamante et al., 2012). While fires can have noticeable effects on local plant communities, the burning of above-ground biomass can lead to immediate increases in nutrient availability, especially Ca, Mg, and K (Alexis et al., 2012; Pivello et al., 2010). These events also impact species composition, plant growth, and soil biota, and can enhance leaching and erosion processes (Grogan et al., 2000).

Frequent vegetation removal in the Cerrado through fire increases light availability in the understory, leading to higher photosynthesis rates at this level (Ries and Shugart, 2008;

Scalon and Rossatto, 2021). Fire, as a rapid and potent environmental force, significantly and lastingly impacts nutrient cycles in ecosystems, altering the form, distribution, and quantity of available nutrients (Rogers et al., 2020; Scalon and Rossatto, 2021). Plants exposed to fire may change their gas exchange rates, as shown by Scalon and Rossatto (2021), who discovered that understory plant communities in periodically burned areas had higher maximum gas exchange rates compared to those in unburned areas. Even after medium-term fire events, these communities adapted to the environmental changes by maintaining higher carbon assimilation rates despite increased stomatal conductance. Higher carbon assimilation rates can boost growth, making plants more resilient in fire-adapted ecosystems (Ficken and Wright, 2019). Furthermore, achieving high photosynthetic rates (A_{max}) during the rainy season is crucial for replenishing carbon reserves used to grow above-ground biomass after disturbances (Niinemets, 2007). This process is influenced by variations in fire regimes (Keeley, 2009; Beringer et al., 2015), where the intensity, temperature, and duration of fires are crucial factors for post-fire recruitment and directly impact the survival of plant tissues (Coutinho, 1990; Bova and Dickinson, 2005; Bilbao et al., 2006).

Theoretically, adjustments in plant functional traits occur in response to resource availability, leading to predictable changes in traits along environmental gradients (Aerts and Chapin, 1999; Hobbie, 2015). This theory suggests that the functional traits of plant communities and species should reflect variations in available resources resulting from disturbances. Environments with limited resources favor more conservative strategies, while environments richer in resources favour more acquisitive strategies (Poorter and Bongers, 2006; Cavender-Bares and Reich, 2012; Wigley et al., 2016). The leaf economic spectrum exemplifies these variations by integrating features such as high nutrient levels (N, P, K), specific leaf area, turnover rate, and low carbon concentration in contrast to leaves that exhibit a more costly physical structure (density and thickness), thus having a more conservative strategy (Westoby et al., 2002; Wright et al., 2004; Craine, 2005; Poorter et al., 2009; Fortunel et al., 2012).

However, due to the complex interplay of environmental factors in comparing highly distinct habitats, it remains unclear how these relationships vary predictably among different habitat types (Rogers et al., 2020). Environments with varying fire regimes can trigger diverse species responses (Dantas et al., 2013; Pellegrini et al., 2023). For example, some studies have suggested that different fire patterns may influence the selection of trees with distinct

functional traits, particularly those related to the trunk, such as resprouting capacity and persistence (Stevens et al., 2020; Scalon et al., 2019; Pellegrini et al., 2021). Moreover, these fire patterns can impact plant diversity, which has been linked to changes in light availability and soil nitrogen content (Pellegrini et al., 2023). However, limited research has delved into gas exchange rates and leaf nutrient content under varying fire regime conditions.

To investigate this, we assessed leaf traits in species located in areas subject to frequent annual burns and areas without fire occurrences for over 20 years. Our focus was on leaf traits, including concentrations of nitrogen (N), phosphorus (P), magnesium (Mg), calcium (Ca), and potassium (K) in leaves, as well as maximum carbon assimilation (A_{max}), stomatal conductance of water (G_s), leaf transpiration (E), and intrinsic water use efficiency (IWUE), along with changes in soil nutrients. In this context, our research focuses on how maximum gas exchange rates and leaf nutrient concentrations in different Cerrado species vary under two distinct fire regimes: frequent (annual) burning and absence of burning. We anticipate that burned areas will exhibit higher soil macronutrient and organic matter content but lower canopy coverage compared to unburned areas. Specifically, we expect soil nutrients to be greater in burned areas compared to unburned areas, and this to be reflected in higher maximum photosynthetic capacity higher A_{max} values, stomatal conductance, leaf transpiration, and greater leaf nutrient concentrations.

19. Materials and Methods

19.1 Study site

The study was conducted at the Santa Bárbara Ecological Station (EESB) in Águas de Santa Bárbara, São Paulo, near km 288 on the Castelo Branco Highway. The region has a Cwa climate according to the Köppen classification, characterized by warm and humid conditions with dry winters. Annual precipitation ranges from 1000 to 1300 mm, as reported by Melo and Durigan (2011). The EESB is known for preserving remnants of Cerrado grasslands and savannas, which are rare in São Paulo (Melo and Durigan, 2011).

The soils at the EESB are primarily latosols with a medium texture and high aluminum concentration, as detailed by Abreu et al. (2017). The vegetation in the area ranges from campo cerrado, with a continuous grassy layer and few scattered trees, to cerrado *sensu stricto*, which also has a grassy layer but with more dispersed trees (Oliveira-Filho and Ratter, 2002).

19.2 Experimental Design

Since 2014, Professor Davi Rodrigo Rossatto, in collaboration with Dr. William Hoffmann and Dr. Giselda Durigan, has been experimenting with controlled burning in the area (Figure 2). The project involves treatment measuring 20 x 50 m, totaling 6 in grassland formations and 8 in savanna formations, subjected to different fire regimes: some without burning (half of the plots) and others with annual burning (the other half). The frequently burned treatment have been set on fire annually since 2014, at the end of the dry season, between August and September. For data collection, we selected treatment of cerrado *sensu stricto*, including three treatment with annual burning and three without fire intervention. The treatment that are burned annually will be referred to as "burned," and those that are not burned will be referred to as "unburned."

19.3 Species Selection

Based on the species list provided by Abreu et al. (2017) and confirming the presence of individuals on the treatment, we selected 17 species that are found in areas where treatments involving the absence of fire (unburned) and the presence of annual fire were implemented (burned). We included species from various families and functional groups (trees, shrubs, and herbaceous plants) to create a diverse group with different characteristics.

Table 1. List of selected species utilized in the thesis development, adapted from Abreu et al. (2017).

Family	Species	Abbreviation	Growth form
Anacardiaceae	<i>Anacardium humile</i> A.St.-Hil	ANA	Subshrub
Annonaceae	<i>Duguetia furfuracea</i> (A.St.-Hil.) Saff.	DF	Shrub
Araliaceae	<i>Schefflera vinosa</i> (Cham. and Schltdl.) Frodin and Fiaschi	SV	Tree
Asteraceae	<i>Moquiniastrum barrosoae</i> (Cabrera) G. Sancho	MB	Shrub
Celestraceae	<i>Peritassa campestris</i> (Cambess.) A.C.Sm.	PC	Shrub
	<i>Tontelea micrantha</i> (Mart.) A.C.Sm	TM	Shrub
Chrysobalanaceae	<i>Licania humilis</i> Cham. and Schltdl.	LH	Subshrub
Ebenaceae	<i>Diospyros hispida</i> A.DC.	DH	Shrub
Fabaceae	<i>Stryphnodendron rotundifolium</i> Mart.	SR	Tree
	<i>Leptolobium elegans</i> Vogel	LE	Tree
	<i>Andira humilis</i> Mart. ex Benth.	AH	Subshrub
Lamiaceae	<i>Aegiphila verticillata</i> Vell.	AV	Shrub
Melastomataceae	<i>Miconia albicans</i> (Sw.) Triana	MA	Shrub
	<i>Miconia ligustroides</i> (DC.) Naudin	ML	Tree
Myrtaceae	<i>Campomanesia adamantium</i> (Cambess.) O.Berg	CA	Shrub
Ochnaceae	<i>Ouratea spectabilis</i> (Mart.) Engl.	OS	Tree
Sapotaceae	<i>Pradosia brevipes</i> (Pierre) T.D.Penn	PB	Subshrub

Selected species is based on Abreu et al. (2017) and field observations. Growth form classification is based on Durigan et al. (2004); Souza et al. (2018) and observation in the field.

19.4 Gas exchange and nutrient utilization

In all cases, measurements of parameters related to gas exchange were performed under saturating light and conducted during the rainy season in February 2021, six months after the 2020 annual burn. This timing was chosen as it corresponds to the period when the leaves of the Cerrado species exhibit their maximum physiological activity, as reported by Franco et al. (2005), Rossatto et al. (2013) and Rossatto and Franco (2017). The analyses were carried out on fully expanded leaves of the same age, and without any signs of damage.

Gas exchange measurements were performed using a portable system (LcPro, ADC BioScientific Ltd., Hoddesdon, United Kingdom) to measure CO₂ and H₂O exchange. Three leaves per individual of each species were used for the measurements. Maximum CO₂ assimilation rates (A_{max}) under saturating light conditions were determined at a constant light intensity of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This light intensity range is known to saturate the photosynthetic apparatus of most cerrado plants (Prado and Moraes 1997, Franco et al. 2005,

Rossatto et al. 2013). The chamber temperature was maintained at 25°C, and natural CO₂ concentrations in the air were used during the measurements. Maximum CO₂ assimilation rates (A_{max}), leaf transpiration (E), and stomatal conductance (g_s) readings were recorded and analyzed. Additionally, the instantaneous water use efficiency (iWUE, calculated as A_{max}/g_s) was determined based on these measurements.

For nutrient analysis, fully expanded leaves were collected from the same individuals where gas exchange measurements were taken, preferably from leaves adjacent to those used for gas exchange measurements. Leaf samples for nutrient analysis were collected in 2020 before the burn and in February 2021, six months after the burn. After collection, plant samples were washed in running water, dried, and then placed in an oven for 72 hours at 70°C for drying, followed by grinding. The concentrations of nitrogen (N), phosphorus (P), calcium (Ca), magnesium (Mg), and potassium (K) were determined. The N content in the leaves was determined using the micro-Kjeldahl method (Allen et al., 1989). For the determination of P, Ca, Mg, and K content, leaf samples were digested using a triacid mixture (nitric, sulfuric, and perchloric acids) in a ratio of 10:1:2 (Allen et al., 1989). The P content in the leaf extract was determined by colorimetry at 410 nm using a mixture of ammonium vanadate and molybdate solutions in a 1:1 ratio. The concentrations of the other nutrients were determined by atomic absorption spectrophotometry (Allen et al., 1989). All analyses were conducted at the Plant Ecology Laboratory, Department of Applied Biology to Agriculture, UNESP campus of Jaboticabal – SP, under the supervision of Prof. Dr. Davi Rodrigo Rossatto

19.5 Soil Properties and Canopy Cover

The chemical analysis of the soil focuses on determining nutrient levels and characteristics that can affect the availability of these nutrients to plants (Prezotti and Guarçoni, 2013). For this study, two soil samples per plot were collected from both annually burned (3 burned) and control treatment (3 unburned), resulting in a total of 12 soil samples. The soil was collected from the surface layer (0-20cm) using a gardening spade and stored in labeled "zip-lock" plastic bags before being sent to the Soil Laboratory of ESALQ – USP for chemical analysis. The elements potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), manganese (Mn), and aluminum (Al) were quantified, along with pH and organic matter content.

To evaluate canopy cover in the study treatment, the Leaf Area Index (LAI) was measured using hemispherical photographs. Five photos were taken per area using a plant canopy

imager CI-110–24P-ID (CID Bioscience, Camas, WA, USA). The photographs were captured at the end of the day to take advantage of diffuse light.

19.6 Statistical Analysis

We utilized the Student's t-test to compare environmental disparities in luminosity (LAI) and soil chemical characteristics between areas affected by fire and those unaffected. A multivariate analysis of variance (MANOVA) was performed to determine if the treatments (burned and unburned) had a significant overall impact on the dependent variables (leaf traits), considering the species as well. Subsequently, the permutation test Adonis was used to validate the effects of treatments and species. After conducting the analysis, a univariate ANOVA was carried out for each leaf trait (Amax, E, gs, iWUE, N, P, Ca, Mg, and K) about the treatments. The Tukey post hoc test was then used to examine specific treatment effects on each variable individually.

A principal component analysis (PCA) was carried out to analyze the variability structure in the leaf trait data after standardizing the data. In this analysis, we also included the leaf trait SLA (specific leaf area) from Chapter II. These methods were utilized to explore the connections between treatments and leaf traits, offering a comprehensive insight into the variations and patterns present in the data. All statistical analyses were performed using R software (version 4.1.1) and its relevant packages. A significance level of $\alpha = 0.05$ was set for all analyses, and the results were interpreted based on this threshold.

20. Results

The Student's *t*-test results for soil variables revealed that there were no significant differences in pH values between burned and unburned treatments ($t = 0.530$, $p = 0.603$). Similarly, organic matter (O.M) showed no significant differences ($t = 1.45$, $p = 0.166$). On the other hand, statistically significant differences were noted in the phosphorus content ($t = 1.45$, $p < 0.05$), indicating higher phosphorus concentrations in the burned treatment (Fig. 1f). For calcium and magnesium, the analyses revealed no significant differences, with $t = 1.84$, $p = 0.144$ and $t = 0.144$, $p = 0.0847$, respectively. Similarly, no significant differences were found in potassium ($t = -0.149$, $p = 0.883$) and aluminum levels ($t = -0.461$, $p = 0.651$). Although most nutrients do not show statistically significant differences, there is an observed trend of higher concentrations in treatment subjected to prescribed burning (Fig. 1).

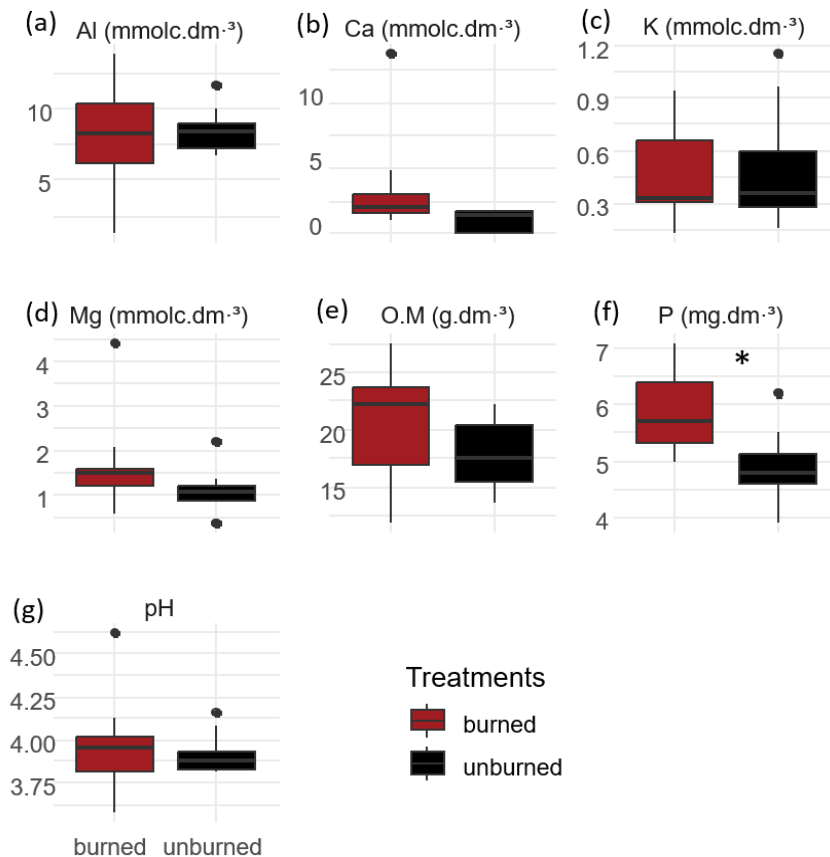


Figure 1. Boxplot comparing the effects of location (burned and unburned) on soil nutrient traits: (a) Aluminum; (b) Calcium; (c) Potassium; (d) Magnesium; (e) Organic matter; (f) Phosphorus; (g) pH. The small solid circles represent the outliers. The symbol * indicates that there is a significant difference when $p < 0.05$ (t-test).

In terms of leaf area index (LAI), there is a noticeable trend where the unburned group may exhibit a slightly higher LAI compared to the burned treatment, although the difference is not statistically significant (Fig. 2) ($t = -1.817$, $p = 0.079$).

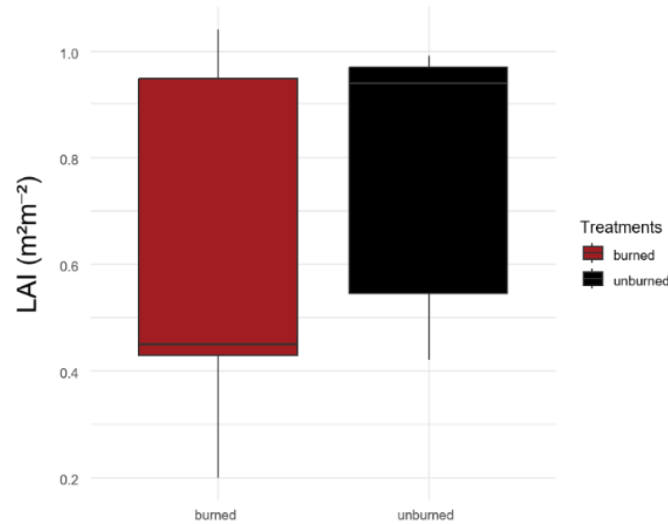


Figure 2. Boxplot comparing the effects of location burned and unburned on the Leaf Area Index (LAI). The small solid circles represent the outliers. The symbol * indicates that there is a significant difference when $p < 0.05$ (t-test).

The MANOVA showed a significant effect of treatments (burned and unburned) (Pillai = 0.39, $F = 5.24$, $df = 10, 81$, $p < 0.001$), while the effect of species was not significant ($F=0.81$, $p =0.77$). These findings suggest that the burned and unburned treatments had distinct impacts on multiple foliar and physiological variables, reflecting a global response of plants to these burning regimes.

The analysis results showed significant differences between the burned and unburned treatments in various leaf and physiological variables. However, there were no significant differences in nitrogen (N) content between the treatments (ANOVA: $F = 0.488$, $p = 0.486$) (Fig. 3a), although there was a slight trend of higher nitrogen concentration in the leaves from unburned areas. A slightly higher concentration of potassium (K) was observed in the leaves from the burned treatment, which was statistically significant (ANOVA: $F = 5.555$, $p < 0.05$) (Fig. 4e). On the other hand, calcium (Ca), magnesium (Mg), and phosphorus did not show significant differences between treatments (refer to Table 2) (Fig. 3).

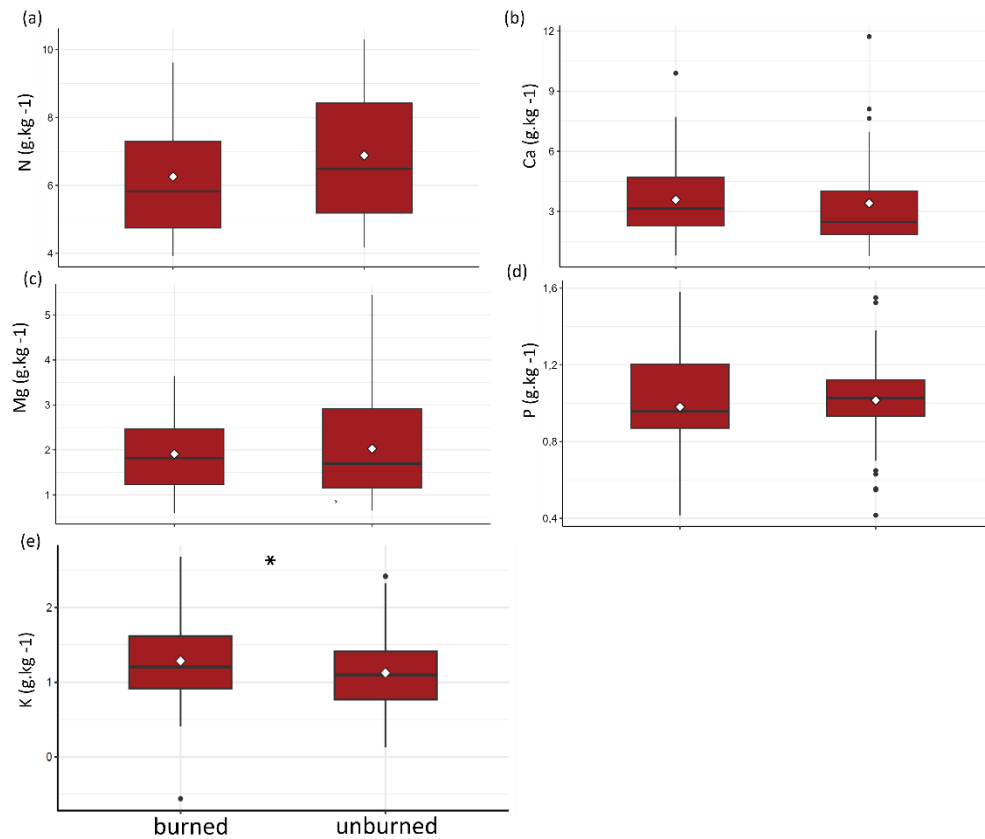


Figure 3. Paired comparisons between species from areas with burned and unburned in cerrado *sensu stricto*, in terms of leaf nutrient availability: (a) nitrogen concentration; (b) calcium concentration; (c) magnesium concentration; (d) phosphorus concentration; (e) potassium concentration. The small solid circles represent outliers. The symbol * indicates a significant difference when $p < 0.05$ (ANOVA).

Table 2. Table presenting the results of an ANOVA for leaf traits, with F and p-values for treatments (burned and unburned). Variables include: N (nitrogen g.kg^{-1}), P (phosphorus g.kg^{-1}), K (potassium g.kg^{-1}), Ca (calcium g.kg^{-1}), Mg (magnesium g.kg^{-1}), E (leaf transpiration), gs (stomatal conductance), Amax (maximum CO_2 assimilation), iWUE (intrinsic water use efficiency). Statistical significance is indicated with asterisks: ** $p < 0.05$.

Leaf traits	F	p-value
N	0.488	0.486
P	11.27	0.220
K	5.555	$p < 0.05^{**}$
Ca	3.236	0.075
Mg	0.904	0.344
E	18.26	$p < 0.05^{**}$
gs	0.155	0.694
Amax	2.825	$p < 0.05^{**}$
iWUE	3.894	$p < 0.05^{**}$

It was expected that regions with frequent burning would have higher photosynthetic rates compared to areas without fire. The data confirm this expectation, showing that photosynthetic rates were indeed higher in most of the assessed traits, such as carbon assimilation (A_{max}) and leaf transpiration (E), which exhibited a significant difference between burned and unburned areas (Fig. 4 a, d). These rates were higher in the burned areas, as expected (ANOVA: $F = 2.825$, $p < 0.05$; $F = 18.26$, $p < 0.05$, respectively). In terms of physiological variables, water use efficiency ($iWUE$) was significantly higher in the burned treatment (ANOVA: $F = 3.894$, $p < 0.05$) (Fig. 4c). The only variable that did not show significant differences between treatments was stomatal conductance (g_s) (ANOVA: $F = 0.155$, $p = 0.694$) (Fig. 4b).

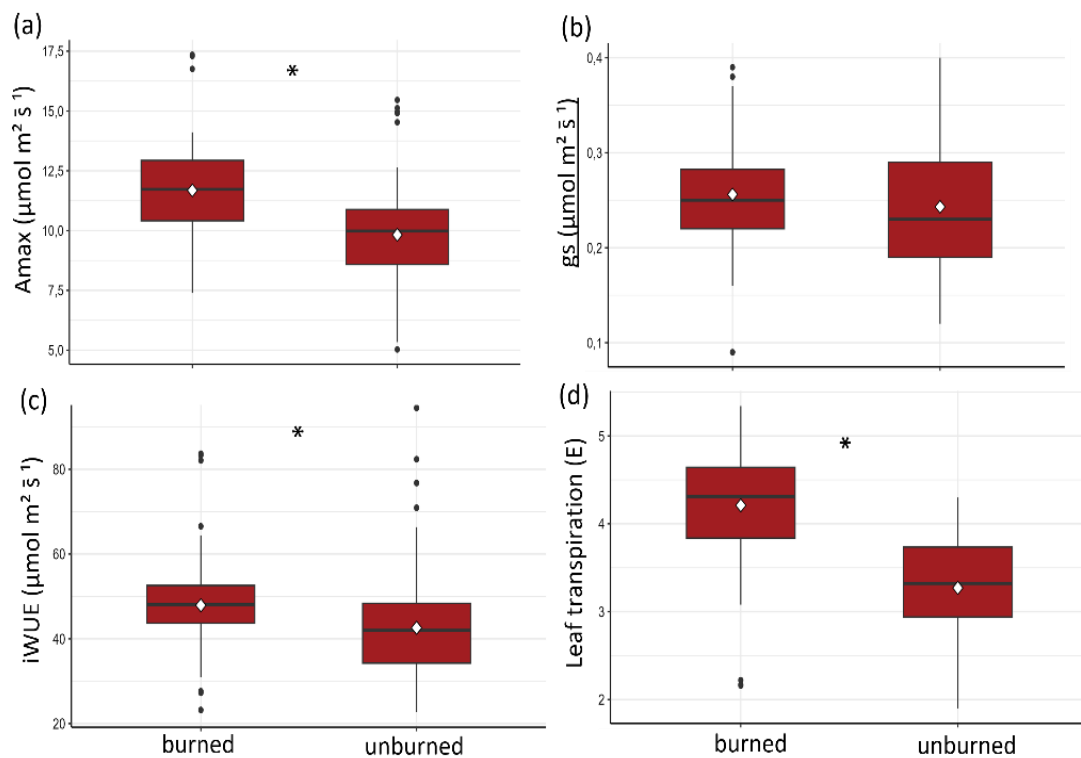


Figure 4. Paired comparison between species from annually burned (burned) and unburned areas, regarding photosynthetic leaf traits: (a) maximum carbon assimilation (A_{max}); (b) stomatal conductance (g_s); (c) leaf transpiration (E); (d) intrinsic water use efficiency ($iWUE$) in cerrado *sensu stricto*. Solid small circles represent outliers. The symbol * indicates a significant difference when $P < 0.05$ (ANOVA).

Principal Component Analysis (PCA) was utilized to explore the relationships among leaf traits under burned and unburned treatment conditions (Fig. 6). The first principal component (Dim1) accounts for 25.5% of the variation in the data, while the second principal component (Dim2) explains 20%. Dimension 3 (Dim-3) contributes an additional 17.04%, resulting in a cumulative variation of 63.10% when considering the first three dimensions. Along the Dim1 axis, a positive correlation is observed between Amax, gs, and SLA, while iWUE shows a negative correlation. This indicates that samples with higher values of Amax, gs, and SLA tend to have lower iWUE. On the Dim2 axis, the leaf nutrients Ca and Mg are positively correlated, standing out from the other variables (Ca: 0.844; Mg: 0.788), highlighting the significant influence of leaf mineral content on this dimension (Fig. 6).

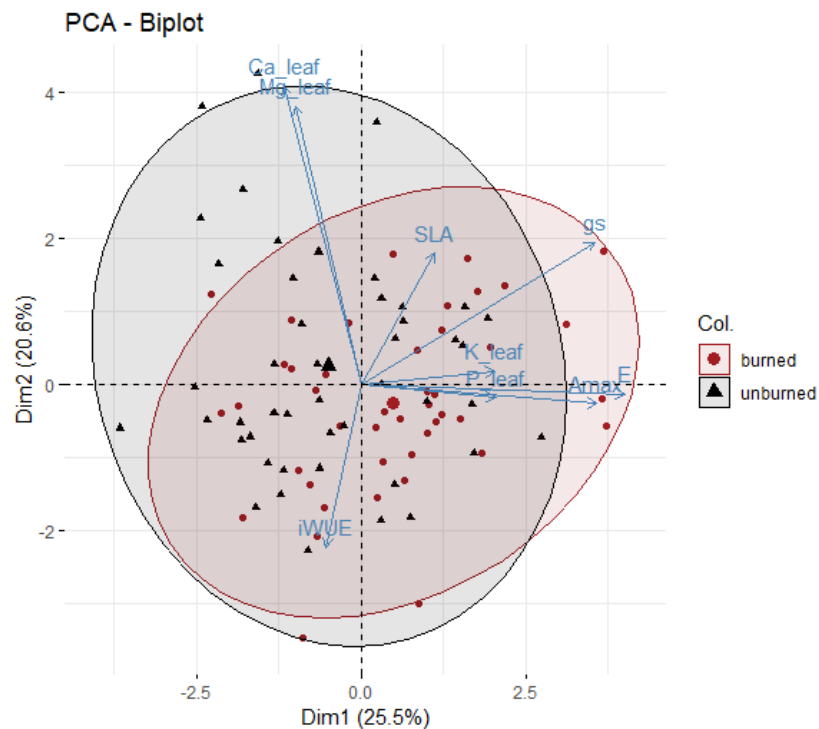


Figure 6. Principal Component Analysis (PCA) of leaf nutrient and photosynthetic traits under different treatment conditions (burned and unburned). The vectors represent the following: Nutrients – magnesium, calcium, potassium, phosphorus. Photosynthetic rates – maximum CO₂ assimilation rate (Amax), leaf transpiration (E), stomatal conductance, (gs) intrinsic water use efficiency (iWUE). Morphological: specific leaf area (SLA).

21. Discussion

When evaluating the impact of frequent burning on a set of cerrado species, we observed significant differences in leaf traits between burned and unburned treatment. The results indicate that burning treatments have a distinct effect on some of the analyzed traits.

Concerning soil nutrients and organic matter, we observed an increase in phosphorus (P) concentrations in burned treatment. This nutrient plays a crucial role in terrestrial and aquatic productivity and is essential for the biodiversity and biomass of ecosystems (Cramer, 2010). The availability of P is influenced by soil microbiota, which can either compete with plants for this nutrient or form beneficial symbioses, such as mycorrhizae, to enhance its absorption (López-Arredondo et al., 2014). Unlike nitrogen, which is abundant in the atmosphere, rock phosphate reserves are limited (Khan et al., 2023), underscoring the significance of P recycling in ecosystems.

In contrast to nitrogen and phosphorus, potassium (K) exhibited significant differences in treatments, with higher concentrations in leaves from burned treatments. This increase may be attributed to the ash deposited by the fire, which could temporarily enrich the soil with potassium (Stromgaard, 1984). Potassium replenishment after a wildfire can take 1.6 to 4.1 years, as indicated by Pivello and Coutinho (1992). Potassium is one of the most susceptible nutrients to volatilization during fires, potentially increasing soil fertility in burned areas and leading to higher foliar concentrations. These patterns suggest an interesting dynamic in which potassium, though initially increased due to ashes and fire heat, tends to be rapidly lost from surface soil, possibly due to its solubility and percolation by rainwater (Stromgaard, 1984). This could explain the higher K levels found in leaves but not in soil, as data collection occurred months after the burn. Potassium is predominantly found as a free cation (K^+) in plant cells and is the second most abundant nutrient in foliar biomass after nitrogen, highlighting its great importance for plant functioning (Bhatla and Lal, 2018; Sardans and Peñuelas, 2021). Plant ash contains primarily potassium, constituting nearly 50% of its total weight (Bhatla and Lal, 2018). Recent studies have shown that potassium directly impacts photosynthetic capacity (Tranker et al., 2018) and plant growth (Tripler et al., 2006), as it activates enzymes used in photosynthesis and respiration (Bhatla and Lal, 2018), responding complexly to different stresses (Dreyer et al., 2017).

Scalon and Rossatto (2021) also noted that there is little variation in nutrient concentrations in leaves between environments with and without burning. However, Kauffman et al. (1994) and Pivello et al. (2010) reported significant nutrient losses associated

with fire. One possible explanation is that in the Cerrado, a significant portion of plant biomass is located below ground, suggesting that nutrient reservoirs may be minimally affected by fire. The lack of significant differences in other nutrients, such as calcium, magnesium, and phosphorus, also supports the notion that fire does not greatly alter the long-term availability of nutrients in leaves. This could be attributed to plants' ability to adjust their absorption strategies or the effectiveness of the ecosystem's nutrient recycling mechanisms.

Comparative analyses between areas (burned vs. unburned) revealed that photosynthetic rates are higher in locations with annual fire frequency compared to those without disturbance. These results are consistent with the literature (Scalon and Rossatto, 2021) and support our initial expectations. The foliar physiological traits that exhibited the most significant differences were leaf transpiration (E) and maximum carbon assimilation (A_{max}), both of which were higher in regularly burned areas. One possible explanation for the higher photosynthetic rates in areas with frequent burning is that cerrado species, with adaptive characteristics such as robust underground organs (Apezzato-da-Glória et al., 2008), can resprout after fires (Fidelis et al., 2014). This process involves a high-energy cost trade-off (Clarke et al., 2013; da Silva and Rossatto, 2019), as plants must achieve high photosynthetic rates (A_{max}) during the rainy season to recover carbon reserves used in above-ground biomass regeneration post-fire (Niinemets, 2007).

Moreover, the increased light availability in areas with frequent fires may contribute to higher photosynthetic rates. Recurrent fires tend to result in savanna physiognomies dominated by grasses, reducing the canopy formed by woody species, a phenomenon that in savannas with lower fire incidence could lead to vegetation densification (Sankaran et al., 2005; Abreu et al., 2017). Therefore, locations with higher fire frequency receive higher levels of radiation (Rossatto et al., 2018), promoting an increase in photosynthetic rates post-burning (Goorman et al., 2011), which supports a more acquisitive strategy in gas exchange characteristics (Ficken and Wright, 2019), as hypothesized. The elevated transpiration rates may be a response to increased solar exposure and the need for leaf cooling, as well as a mechanism to enhance the absorption of nutrients mobilized in the soil.

Although there is a well-established long-term correlation between photosynthesis and stomatal conductance (g_s), strategies aimed at reducing g_s to enhance intrinsic water use efficiency ($iWUE$) often result in decreased carbon assimilation rate (A_{net}) and productivity (Blum, 2009; Ghannoum, 2016). Despite the crucial role of g_s , we did not observe significant

differences between treatments, suggesting that plants may be adopting a conservative water management strategy, possibly to prevent dehydration in the face of high variability in water availability after fires. However, we did find a strong positive correlation between maximum CO₂ assimilation rates (A_{max}), g_s , and specific leaf area (SLA). Plants with higher photosynthetic and transpiration capacities also tend to have larger leaves, a characteristic of an acquisitive strategy, especially in environments with higher nutrient abundance (Wright et al., 2004; Maracahipes et al., 2018), contrasting with areas unaffected by fire.

The trend of higher nutrient concentrations in burned soil may result in plants developing thinner leaves with higher SLA, characteristic of acquisitive strategies (Wright et al., 2004). Conversely, lower SLA and/or maximum photosynthesis rates (A_{max}) in unburned treatment suggest a more conservative resource use approach, linked to limited phenotypic plasticity (Westoby et al., 2002; Wright et al., 2004; Poorter et al., 2009; Díaz et al., 2016; Stotz, 2022). Our findings suggest that cerrado species exposed to fire may exhibit a combination of conservative and acquisitive strategies, demonstrating the diversity of responses among species to environmental stimuli, influenced by evolutionary factors (Resco de Dios, 2020).

In a study conducted in Brasília, Scalon and Rossatto (2021) found that herbaceous plants are capable of transitioning to a more acquisitive resource strategy in the medium term and at the community scale. This transition involves exhibiting a higher maximum photosynthetic capacity at the expense of increased stomatal conductance, regardless of their growth form. The study encompassed a range of plant forms from subshrubs to small trees, and similar results were obtained, including increased maximum photosynthetic capacity and minimal differentiation in leaf nutrient concentrations. These findings support the hypothesis that species in disturbed areas tend to exhibit mesomorphic traits, while leaves from undisturbed areas show more scleromorphic characteristics, aligning with common Cerrado strategies and not requiring resprouting strategies after fire events (Clarke et al., 2013). The traits observed are likely adaptations in response to fire, influencing post-fire recruitment and persistence (Resco de Dios, 2020). Our research provides evidence that fire impacts on plant communities lead to changes in key functional traits related to carbon uptake potential. While nutrient use appears to be more resilient and constrained, similar to observations in the ground

layer (Oliveras et al., 2013; Scalon and Rossatto, 2021), maximum gas exchange rates can adapt to changes in resource availability.

22. Conclusion

In conclusion, this study highlights the varied responses of different plant species with diverse growth forms to frequent fire occurrences. Despite more than five years of annual burning, the analysis showed a trend towards increased nutrient concentrations in the burned treatment, particularly potassium, indicating a potential positive impact on nutrient availability. However, this was not sufficient to significantly alter mineral resource use strategies. Annual burning was observed to potentially enhance CO₂ assimilation and photosynthetic rates without a significant reduction in canopy cover, leading to increased sun exposure. This could result in higher water demand, as evidenced by the negative correlation between intrinsic water-use efficiency (iWUE) and maximum CO₂ assimilation (A_{max}). The findings underscore the adaptive strategies employed by Cerrado species to adapt to frequent disturbances like fire, showcasing a flexible response that varies based on post-fire conditions. This adaptive complexity demonstrates the plants' ability to adjust resource use strategies in response to environmental changes, crucial for the resilience and sustainability of Cerrado ecosystems amidst evolving fire regimes.

23. References

- Abreu, R.C., Hoffmann, W.A., Vasconcelos, H.L., Pilon, N.A., Rossatto, D.R., and Durigan, G. (2017). The biodiversity cost of carbon sequestration in tropical savanna. *Science Advances*, 3(8), e1701284. <https://doi.org/10.1126/sciadv.1701284>
- Allen, S.E., Grimshaw H.M., Parkinson J.Á., Quarmby C. (1989). Chemical analysis of ecological materials. *Oxford, Blackwell Scientific Publications*. 2end editon, 565p.
- Alexis, M. A., Rasse, D. P., Knicker, H., Anquetil, C., and Rumpel, C. (2012). Evolution of soil organic matter after prescribed fire: A 20-year chronosequence. *Geoderma*, 189–190, 98–107. <https://doi.org/10.1016/j.geoderma.2012.05.003>
- Appezzato-da-Glória, B., Cury, G., Soares, M.K.M., Rocha, R., Hayashi, A.H. (2008). Underground systems of Asteraceae species from the Brazilian Cerrado. *J. Torrey Botan. Soc.*, 135:103–113
- Aerts, R., and Chapin, F. S. (1999). The mineral nutrition of wild plants revisited: A reevaluation of processes and patterns. *Advances in Ecological Research*, 30, 1-67. [https://doi.org/10.1016/S0065-2504\(08\)60016-1](https://doi.org/10.1016/S0065-2504(08)60016-1)
- Beringer, J., Hutley, L. B., Abramson, D., Arndt, S. K., Briggs, P., Bristow, M., Canadell, J. G., Cernusak, L. A., Eamus, D., Edwards, A. C., Evans, B. J., Fest, B., Goergen, K., Grover, S. P., Hacker, J., Haverd, V., Kanniah, K., Livesley, S. J., Lynch, A., Maier, S., Moore, C., Raupach, M., Russell-Smith, J., Scheiter, S., Tapper, N. J., Uotila, P. (2015). Fire in Australian savannas: from leaf to landscape. *Global Change Biology*. 21, 62–81. <https://doi.org/10.1111/gcb.12686>
- Bhatla, S. C., and Lal, M. A. (2018). Plant physiology, development and metabolism. Springer. doi: 10.1007/978-981-13-2023-1
- Bilbao B., Méndez C., Delgado M., Moreno J.M. (2006). Fire behavior in experimental savanna burnings in Gran Sabana, Canaima National Park, Venezuela. In: *Viegas DX (Ed.), Proceedings of 5th International Conference on Forest Fire Research*, p.
- Bova, A.S., Dickinson, M.B. (2005). Linking surface-fire behavior, stem heating, and tissue necrosis. *Can. J. For. Res.*, 35(4), 814–822
- Bueno, M. L., Dexter, K. G., Pennington, R. T., Pontara, V., Neves, D. M., and Oliveira-Filho, A. T. (2018). The environmental triangle of tree life history strategies in tropical forests: Structure and interpretation. *Ecological Monographs*, 87(3), 335-347. <https://doi.org/10.1111/1365-2745.12969>
- Bustamante, M. M. C., Nardoto, G. B., Pinto, A. S., Klink, C. A., Harley, P. (2012). Potential impacts of climate change on biogeochemical functioning of Cerrado ecosystems. *Brazilian Journal of Biology*, 72(3), 655-671. <https://doi.org/10.1590/S1519-69842012000400005>
- Cavender-Bares, J., and Reich, P. B. (2012). Shocks to the system: community assembly of the oak savanna in a 40-year fire frequency experimente. *Ecology: Ecological Society of America*. 93(8), S52-S69. <https://doi.org/10.1890/11-0502.1>
- Clarke, P.J., Lawes, M.J., Midgley, J.J., Lamont, B.B., Ojeda, F., Burrows, G.E., Enright, N.J., and Knox, K.J.E. (2013). Resprouting as a key functional trait: How buds, protection and

resources drive persistence after fire. *New Phytologist*, 197(1), 19–35. <https://doi.org/10.1111/nph.12001>

Costa, Y.T., and Rodrigues, S.C. (2015). Efeito do fogo sobre vegetação e solo a partir de estudo experimental em ambiente de cerrado. *Revista do Departamento de Geografia*, 30, 149-65. <https://doi.org/10.11606/rdg.v30i0.101686>

Coutinho, L. M. (1990). Fire in the ecology of the Brazilian Cerrado. In J. G. Goldammer (Ed.), *Fire in the tropical biota: ecosystem processes and global challenges* (pp. 82-105). *Springer*.

Craine, J. M. (2005). Reconciling plant strategy theories of Grime and Tilman. *Journal of Ecology*, 93(6), 1041-1052. <https://doi.org/10.1111/j.1365-2745.2005.01043.x>

Cramer, M. D., van Cauter, A., and Bond, W. J. (2010). Growth of N₂-fixing African savanna *Acacia* species is constrained by below-ground competition with grass. *Journal of Ecology*, 98(1), 156-167. doi:10.1111/j.1365-2745.2009.01594.x

da Silva, E. C., and Rossatto, D. R. (2019). Are underground organs able to store water and nutrients? A study case in non-arboreal species from the Brazilian Cerrado. *Theo. Exp. Plant Phys.* 31, 413–421.

Dantas, V. L., Pausas, J. G., Batalha, M. A., Loiola, P. P., and Cianciaruso, M. V. (2013). The role of fire in structuring trait variability in Neotropical savannas. *Oecologia*, 171(2), 487-494. <https://doi.org/10.1007/s00442-012-2431-8>

de Souza M.C., Rossatto D.R., Cook G.D., Fujinuma R., Menzies N.W., Morellato L.P.C., Habermann G. (2016). Mineral nutrition and specific leaf area of plants under contrasting long-term fire frequencies: a case study in a mesic savanna in Australia. *Trees*, 30(1): 329-335. doi: 10.1007/s00468-015-1285-1

Dreyer, J., Gomez-Porras, J.L., Riedelsberger, J. (2017). The potassium battery: A mobile energy source for transport processes in plant vascular tissues. *New Phytologist.*, 216, 1049–1053. <https://doi.org/10.1111/nph.14667>

Durigan, G., Baitello, J. B., Corrêa Franco, G. A. D., and de Siqueira, M. F. (2004). Plantas do Cerrado paulista: Imagens de uma paisagem ameaçada. *Páginas & Letras*.

Eiten, G. (1972). The cerrado vegetation of Brazil. *Botanical Review*, 38(2), 201-341.

Ficken, C. D., and Wright, I. J. (2019). Nitrogen uptake and biomass resprouting show contrasting relationships with resource acquisitive and conservative plant traits. *Journal Vegetation Science*. 30 (1), 65–74. <https://doi.org/10.1111/jvs.12705>

Fidelis A., Appezzato-da-Glória B., Pillar V.D., Pfadenhauer, J. (2014). Does disturbance affect bud bank size and belowground structures diversity in Brazilian subtropical grasslands? *Flora*, 209:110–116. <https://doi.org/10.1016/j.flora.2013.12.003>

Fortunel, C., Paine, C. E., Fine, P. V., Kraft, N. J., Baraloto, C. (2012). Environmental factors predict community functional composition in Amazonian forests. *Journal of Ecology*, 100(1), 145-154. <https://doi.org/10.1111/1365-2745.12160>

- Franco A.C., Bustamante M., Caldas L.S., Goldstein G., Meinzer F.C., Kozovits A.R., Rundel P., Coradin V.T.R. (2005). Leaf functional traits of Neotropical savanna trees in relation to seasonal water deficit. *Trees*, 19, 326-335. <https://doi.org/10.1007/s00468-004-0394-z>
- Ghannoum, O. (2016). How can we breed for more water use-efficient sugarcane? *Journal of Experimental Botany*. 67 (3), 557–559. <https://doi.org/10.1093/jxb/erw009>
- Goorman, R., Bartual, A., Paula, S., Ojeda, F., 2011. Enhancement of photosynthesis in post-disturbance resprouts of two co-occurring Mediterranean Erica species. *Plant Ecology*. 212 (12), 2023–2033. <https://doi.org/10.1007/s11258-011-9967-2>
- Gottsberger, G., and Silberbauer-Gottsberger, I. (2006). Life in the Cerrado: A South American tropical seasonal ecosystem. Vol. I: Origin, Structure, Dynamics and Plant Use. *Reta Verlag, Ulm*.
- Grogan, P., Burns, T. D., Chapin, F. S. (2000). Fire effects on ecosystem nitrogen cycling in a Californian bishop pine forest. *Oecologia*, 122(4), 537-544. <https://doi.org/10.1007/s004420050977>
- Haridasan, M. (2008). Nutritional adaptations of native plants of the Cerrado biome in acid soils. *Brazilian Journal of Plant Physiology*, 20(3), 183-195. <https://doi.org/10.1590/S1677-04202008000300003>
- Hirota, M., Holmgren, M., Van Nes, E. H., Scheffer, M. (2011). Global resilience of tropical forest and savanna to critical transitions. *Science*, 334(6053), 232-235. DOI: 10.1126/science.1210657
- Hobbie, S. E. (2015). Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends in Ecology & Evolution*, 30(6), 357-363. <https://doi.org/10.1016/j.tree.2015.03.015>
- Kauffman, J. B., Cummings, D. L., and Ward, D. E. (1994). Relationships of fire, biomass and nutrient dynamics along a vegetation gradient in the Brazilian Cerrado. *Journal of Ecology*, 82(3), 519-531.
- Keeley JE. (2009). Fire intensity, fire severity and burn severity: A brief review and suggested usage. *International Journal of Wildland Fire*, 18(1), 116–126. doi: 10.1071/WF09049
- Fahad Khan, F., Siddique, A.B., Shabala, S., Zhou, M. and Zhao, C. (2023). Phosphorus Plays Key Roles in Regulating Plants' Physiological Responses to Abiotic Stresses. *Plants*. 12(15), 2861. <https://doi.org/10.3390/plants12152861>
- Klink, C. A., and Machado, R. B. (2005). Conservation of the Brazilian Cerrado. *Conservation Biology*, 19(3), 707-713.
- Lehmann, C. E. R., Anderson, T. M., Sankaran, M., Higgins, S. I., Archibald, S., Hoffmann, W. A., Hanan, N. P., Williams, R. J., Fensham, R. J., Felfili, J., Hutley, L. B., Ratnam, J., San Jose, J., Montes, R., Franklin, D., Russell-Smith, J., Ryan, C. M., Durigan, G., Hiernaux, P., Haidar, R., Bowman, D. M. J. S., and Bond, W. J. (2014). Savanna vegetation-fire-climate relationships differ among continents. *Science*, 343(6170), 548-552. doi: 10.1126/science.1247355
- López-Arredondo, D. L., Leyva-González, M. A., González-Morales, S. I., López-Bucio, J., Herrera-Estrella, L. (2014). Phosphate nutrition: improving low-phosphate tolerance in crops.

Annual Review of Plant Biology, 65, 95-123. <https://doi.org/10.1146/annurev-arplant-050213-035949>

Magallanes, S. R. S., Giongo, M., Carvalho, E. V. de Rotili, E. A., Fernandes, A. C., Cachoeira, J. N., & Santos, G. R. (2020). Immediate effects of prescribed burning on chemical properties of the Cerrado soil. *Floresta e Ambiente*, 27(3), e20180253. <https://doi.org/10.1590/2179-8087.025318>

Maracahipes, L., Carlucci, M.B., Lenza, E., Marimon, B.S., Marimon, B.H., Guimarães, F.A.G., Cianciaruso, M.V. (2018). How to live in contrasting habitats? Acquisitive and conservative strategies emerge at inter- and intraspecific levels in savanna and forest woody plants. *Perspect. Plant Ecol. Evol. Syst.*, 34. doi: 10.1016/j.ppees.2018.07.006

Melo, A.C.G. and Durigan G. (2011). Encarte 3: Análise da Estação Ecológica de Santa Bárbara. In: ACG Melo, G Durigan (coordinators.) Estação Ecológica de Santa Bárbara: Plano de Manejo, Águas de Santa Bárbara, Instituto Florestal, SP. 66-100

Miranda, H. S., Bustamante, M. M. C., Miranda, A. C. (2009). The fire factor. In A. Oliveira R. Marquis (Eds.), *The cerrados of Brazil: Ecology and natural history of a neotropical savanna* (pp. 51-68). *Columbia University Press*.

Niinemets, U. (2007). Photosynthesis and resource distribution through plant canopies. *Plant Cell Environ.*, 30(9), 1052–1071.

Oliveras, I., Meirelles, S.T., Hirakuri, V.L., Freitas, C.R., Miranda, H.S., Pivello, V.R., (2013). Effects of fire regimes on herbaceous biomass and nutrient dynamics in the Brazilian savanna. *Int. J. Wild. Fire* 22, 368–380

Oliveira-Filho, A.T., and Ratter, J.A. (2002). Vegetation physiognomies and woody flora of the Cerrado Biome. In: *The Cerrados of Brazil: ecology and natural history of a neotropical savanna*. P.S. Oliveira & R.J. Marquis (eds). *Columbia University Press*, New York

Pausas, J. G. (2014). Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist*, 204, 55- 65., 9(1), e82692. doi: 10.1111/nph.12921.

Pellegrini, A. F. A. (2016). Nutrient limitation in tropical savannas across multiple scales and mechanisms. *Ecology*, 97(12), 3139-3150. doi: 10.1890/15-0869.1

Pellegrini, A.F.A., Anderegg, L., Pinto-Ledezma, J.N., Cavender-Bares, J., Hobbie, S.E., and Reich, P.B. (2023). Consistent physiological, ecological and evolutionary effects of fire regime on conservative leaf economics strategies in plant communities. *Ecology Letters*, 26(3), 597–608. <https://doi.org/10.1111/ele.14182>

Scalon, M. C., and Rossatto, D. R. (2021). Ground layer Cerrado plants sustain higher maximum photosynthetic rates after medium-term fire events. *Flora: Morphology, Distribution, Functional Ecology of Plants*, 285(November). <https://doi.org/10.1016/j.flora.2021.151962>

Pivello, V. R., and Coutinho, L. M. (1992). Transfer of macronutrients to the atmosphere during experimental burns in an open cerrado (Brazilian savanna). *Journal of Tropical Ecology*, 8(4), 487-497.

- Pivello, V.R., Oliveras, I., Miranda, H.S., Haridasan, M., Sato, M.N., and Meirelles, S.T. (2010). Effect of fires on soil nutrient availability in an open savanna in Central Brazil. *Plant and Soil*, 337(1-2), 111-123. <https://doi.org/10.1007/s11104-010-0518-7>
- Poorter, L., and Bongers, F. (2006). Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology*, 87(7), 1733-1743.
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist*, 182(3), 565-588.
- Ratter, J. A. (1997). The Brazilian Cerrado Vegetation and Threats to Its Biodiversity. *Annals of Botany*, 80(3), 223-230.
- R Core Team. (2020). R: A Language and Environment for Statistical Computing. Viena: R Foundation for Statistical Computing. <https://www.r-project.org>
- Reich, P. B., Tilman, D., Isbell, F., Mueller, K., Hobbie, S. E., Flynn, D. F. B., & Eisenhauer, N. (2012). Impacts of biodiversity loss escalate through time as redundancy fades. *Science*, 336(6081), 589-592. <https://doi.org/10.1126/science.1217909>
- Resco de Dios, V. (2020). Plant Carbon Economies and the Dynamics of Wildland Fuels. In Managing Forest Ecosystems. Plant Fire Interactions 36. *Springer Nature* Switzerland. DOI: 10.1007/978-3-030-41192-3_6
- Ries, L., and Shugart, H. H. (2008). Nutrient limitations on understory grass productivity and carbon assimilation in an African woodland savanna. *Journal of Arid Environments*. 72 (8), 1423–1430.
- Rossatto, D. R., Hoffmann, W. A., Silva, L. C. R., Haridasan, M., Sternberg, L. S. L., and Franco, A. C. (2013). Seasonal variation in leaf traits between congeneric savanna and forest trees in Central Brazil: implications for forest expansion into savana. *Trees* (2013) 27:1139–1150. doi: 10.1007/s00468-013-0864-2
- Rossatto, D.R., Franco, A.C. (2017). Expanding our understanding of leaf functional syndromes in savanna systems: the role of plant growth form. *Oecologia*, 183(4): 953-962
- Rossatto, D. R., de Araújo, P. E., da Silva, B. H. P., Franco, A. C. (2018). Photosynthetic responses of understory savanna plants: Implications for plant persistence in savannas under tree encroachment. *Flora* 240, 34–39. <https://doi.org/10.1016/j.flora.2017.12.009>
- Sankaran, M., Hanan, N. P., Scholes, R. J., Ratnam, J., Augustine, D. J., Cade, B. S., and Zambatis, N. (2005). Determinants of woody cover in African savannas. *Nature*, 438(7069), 846-849.
- Sardans, J., and Peñuelas, J. (2021). Potassium Control of Plant Functions: Ecological and Agricultural Implications. *Plants*, 10(419). <https://doi.org/10.3390/plants10020419>
- Scalon, M.C., Domingos, F.M.C.B., Cruz, W.J.A.da, Júnior, B.H.M., Marimon, B.S., Oliveras, I., (2019). Diversity of functional trade-offs enhances survival after fire in Neotropical savanna species. *Journal of Vegetation Science*. 31, 139–150. <https://doi.org/10.1111/jvs.12823>

- Scalon, M. C., and Rossatto, D. R. (2021). Ground layer Cerrado plants sustain higher maximum photosynthetic rates after medium-term fire events. *Flora: Morphology, Distribution, Functional Ecology of Plants*, 285. <https://doi.org/10.1016/j.flora.2021.15196>
- Simon, M. F., Grether, R., de Queiroz, L. P., Skema, C., Pennington, R. T., and Hughes, C. E. (2009). Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by *in situ* evolution of adaptations to fire. *Proceedings of the National Academy of Sciences*, 106(48), 20359-20364.
- Stevens, N., Lehmann, C. E. R., Murphy, B. P., and Durigan, G. (2020). Savanna woody encroachment is widespread across three continents. *Global Change Biology*, 26(5), 2051-2063.
- Stromgaard, P. (1984). The immediate effect of burning and ash-fertilization. *Plant and Soil*, 80(1), 307-320.
- Stotz, G. C., Salgado-Luarte, C., Escobedo, V. M., Valladares, F., Gianoli, E. (2022). Phenotypic plasticity and the leaf economics spectrum: plasticity is positively associated with specific leaf area. *Oikos*, 11. <https://doi.org/10.1111/oik.09342>
- Tripler, C. E., Kaushal, S. S., Likens, G. E., and Walter, M. T. (2006). Patterns in potassium dynamics in forest ecosystems. *Ecology Letters*, 9(4), 451-466.
- Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A., and Wright, I. J. (2002). Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics*, 33, 125-159. <https://doi.org/10.1146/annurev.ecolsys.33.010802.150452>
- Wigley, B.J., Slingsby, J.A., Díaz, S., Bond, W.J., Fritz, H., & Coetsee, C. (2016). Leaf traits of African woody savanna species across climate and soil fertility gradients: Evidence for conservative versus acquisitive resource-use strategies. *Journal of Ecology*, 104(5), 1357-1369. <https://doi.org/10.1111/1365-2745.12593>
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., Midgley, J. J., Navas, M. L., Niinemets, Ü., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V. I., Roumet, C., Thomas, S. C., Tjoelker, M. G., Veneklaas, E. J., Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821-827.
- Zupo, T., Fidelis, A., Pausas, J., Fidelis, A. and Overbeck, G. E. (2020). Post-fire regeneration strategies in a frequently burned Cerrado Community. *J Veg Sci.* 2021;32:e12968. doi: 10.1111/jvs.12968 . *Journal of Vegetation Science*, 31(3), 458-467.

24. Conclusão geral

A investigação sobre as respostas das espécies do Cerrado aos distúrbios anuais de fogo revelou insights significativos sobre as estratégias ecofisiológicas dessas plantas em um dos biomas mais ricos e biodiversos do mundo. Este estudo demonstrou que o fogo, um fator ecológico predominante no Cerrado, pode influenciar características como a SLA e as taxas fotossintéticas. No entanto, mesmo após anos de queimadas anuais, poucas mudanças foram observadas nos atributos coletados, indicando que as espécies são resilientes. Talvez seja necessário uma queima de maior intensidade para que mudanças significativas ocorram, pois as queimadas anuais tendem a ser de baixa intensidade e muitas vezes não queimam todas as espécies presentes nas parcelas por completo. Esses insights fornecem uma compreensão crucial das adaptações que permitem às espécies não apenas sobreviver, mas também prosperar em um ambiente frequentemente perturbado. Os resultados fenológicos mostraram que o fogo pouco altera a fenologia e a longevidade foliar, sugerindo uma resiliência notável das plantas em manter funções vitais apesar da ocorrência regular de incêndios e a manutenção de suas estratégias conservativas.

A investigação das características morfológicas e anatômicas das folhas entre áreas queimadas e não queimadas demonstraram que não ocorrem diferenças no desenvolvimento de traços mesomórficos em resposta ao fogo, esta resposta pode estar relacionada a mudanças que possam ocorrer e se manifestar a longo prazo.

Além disso, foi observado que plantas em áreas queimadas exibem maiores taxas de troca gasosa e concentrações de alguns nutrientes, como o potássio confirmando que o fogo pode incrementar a disponibilidade de recursos, mas os outros nutrientes avaliados, não apresentara, grandes diferenças. Esse fenômeno, por sua vez, potencializa a capacidade das plantas de utilizar esses aumentos para uma recuperação e crescimento rápidos após os distúrbios, neste caso confirmamos nossa hipótese de que as espécies teriam tendência a exibir estratégias aquisitivas.

Essas descobertas mostram que as espécies tendem a flutuar dentro do espectro conservativo e aquisitivo, demonstrando características que podem estar dentro destes dois patamares, além disso, sublinham a importância do fogo como um fator ecológico chave no Cerrado, influenciando não apenas a dinâmica e estrutura das comunidades vegetais, mas também promovendo adaptações essenciais que sustentam a resiliência e a adaptação das espécies a longo prazo. Portanto, é necessário que as estratégias de manejo do fogo sejam cuidadosamente planejadas e implementadas para manter a biodiversidade do Cerrado,

especialmente em face das mudanças climáticas globais. Recomenda-se que pesquisas futuras continuem a explorar as respostas das espécies de diferentes formas de crescimento aos diferentes regimes de fogo e sua interação com outros fatores ecológicos e climáticos, com o objetivo de otimizar as práticas de conservação e manejo.