UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

A COMUNIDADE HERBÁCEO-ARBUSTIVA DE CERRADO *SENSU STRICTO* FRENTE ÀS ALTERAÇÕES DE LUMINOSIDADE E DE SERAPILHEIRA EM CONDIÇÃO DE ADENSAMENTO: DINÂMICA DE ESPÉCIES, GERMINAÇÃO E RESPOSTAS FUNCIONAIS

LUIZ FELIPE SOUZA PINHEIRO

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 Ciências Biológicas (Biologia Vegetal).

Rio Claro
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AUTOR: LUIZ FELIPE SOUZA PINHEIRO
ORIENTADORA: ROSANA MARTA KOLB
COORIENTADOR: DAVI RODRIGO ROSSATTO

Aprovado como parte das exigências para obtenção do Título de Doutor em CIÊNCIAS BIOLÓGICAS (BIOLOGIA VEGETAL), área: Biologia Vegetal pela Comissão Examinadora:

Rosana Marta Kolb

Profa. Dra. ROSANA MARTA KOLB (Participaçao Virtual)
Departamento de Ciências Biológicas / UNESP/FCL-Assis

Profa. Dra. ALESSANDRA TOMASELLI FIDELIS (Participaçao Virtual)
Departamento de Biodiversidade / UNESP - Instituto de Biociências de Rio Claro - SP

Prof. Dr. AUGUSTO CESAR FRANCO (Participaçao Virtual)
Departamento de Biologia Vegetal / Universidade de Brasília

Pesquisadora Dr.ª NATASHI APARECIDA LIMA PILON (Participaçao Virtual) Natashi A. L. Pilon
Universidade Estadual de Campinas

Profª. Drª. ANA PAULA DE OLIVEIRA (Participaçao Virtual) Ana Paula de Oliveira
IESA - Instituto de Estudos Socioambientais / Universidade Federal de Goiás

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RESUMO

O adensamento vegetacional consiste no aumento da densidade e da cobertura de espécies lenhosas em vegetações com um expressivo estrato graminoso. Este processo vem acontecendo em ritmo acelerado nas savanas da América do Sul. Particularmente no Brasil, muitas áreas de cerrado *stricto sensu* (CSS) vêm apresentando perda de espécies herbáceo-arbustivas típicas de áreas abertas em áreas adensadas. Assim, as mudanças microambientais causadas pelo adensamento podem estar filtrando essas espécies. Tais mudanças envolvem, por exemplo, a diminuição da luminosidade incidente no sub-bosque e o aumento na espessura da camada de serapilheira; juntos esses dois fatores podem causar redução na flutuação de temperatura no solo superficial e diminuição na razão de vermelho e vermelho-extremo (V:VE). Por meio de experimentação em campo, este estudo objetivou investigar o papel da redução da luminosidade e do aumento da camada de serapilheira causados pelo adensamento sobre a diversidade, estrutura e fisiologia da comunidade herbáceo-arbustiva de CSS. Além disso, foram estudados aspectos da regeneração por sementes de espécies de CSS relacionados às alterações no regime de temperatura, na razão de V:VE e espessura da camada de serapilheira. Os efeitos da serapilheira sobre a estrutura e a diversidade florística e funcional da comunidade herbáceo-arbustiva foram discretos, assim como para os parâmetros foliares funcionais. Entretanto, a presença de uma camada de serapilheira diminuiu drasticamente a emergência de plântulas de espécies herbáceo-arbustivas. Por outro lado, o sombreamento foi um fator de grande importância na estrutura e florística da comunidade do estrato rasteiro, causando redução nas riquezas florística e funcional desse estrato, assim como na densidade, cobertura e biomassa de partes aéreas ao longo do tempo. Dentre as formas de crescimento, as plantas herbáceas foram as mais forte e rapidamente impactadas pelo sombreamento. Ainda, com o sombreamento imposto, as espécies herbáceo-arbustivas foram capazes de apresentar alguns ajustes relacionados à melhora da captura luminosa, tais como o aumento na área foliar específica e nos teores de clorofilas e carotenoides. Estes ajustes, no entanto, não foram suficientes para a manutenção do desempenho fotoquímico e da assimilação de carbono destas espécies. Com relação aos aspectos da regeneração por sementes, além dos amplos efeitos da serapilheira mencionados anteriormente, a diminuição da razão de V:VE e do regime de amplitude térmica também afetaram negativamente a germinação de algumas espécies. Os resultados indicam que as alterações ambientais causadas pelo adensamento representam fortes filtros ambientais, moldando a montagem da comunidade herbáceo-arbustiva. Essas alterações atuam limitando tanto a sobrevivência como a regeneração de espécies herbáceo-arbustivas típicas de CSS em condições adensadas.

Palavras-chave: atributos funcionais, filtros ambientais, fotossíntese, germinação, luz, savana.

ABSTRACT

Woody plant encroachment refers to the increase in density and cover of woody species in vegetation with an expressive herbaceous-grassy layer. This process is going rapidly in South America savannas, where many Brazilian savanna areas have undergone losses of herbaceous-shrubby species in encroached sites. Thus, environmental changes caused by woody encroachment may be filtering these species. Such changes comprise understory light reduction and higher litter deposition in encroached sites; these two factors combined can induce narrowing of the soil surface temperature alternation and red and far-red ratio (R:FR) decrease. By means of field manipulation, this study investigated the role of light reduction and litter layer increase caused by woody encroachment in diversity, structure and physiology of savanna ground layer community. Furthermore, seed regeneration aspects of savanna ground layer species were evaluated according to alterations in temperature alternation, R:FR ratio and litter layer thickness. We found that the litter had only small effects in floristic and functional diversity of savanna ground layer community as well as structural and functional parameters. However, litter layer presence drastically reduced seedling emergence of savanna ground layer species. In the other hand, shading was an important factor for savanna ground layer structure and floristic. Shading caused significant reductions in floristic and functional richness as well as in density, cover and aboveground biomass over time. Among growth forms, herbaceous plants were most strongly and rapidly affected by shading. Moreover, savanna ground layer species were capable of some adjusts related to light capture improvement in shaded conditions, such as higher leaf specific area values and chlorophyll and carotenoid content. However, these adjustments were not enough to allow the photochemical and carbon assimilation performance maintenance. Regarding seed regeneration aspects, besides the large litter effects mentioned before, reductions in R:FR and temperature alternation regime also negatively affected some species. In conclusion, results indicate that environmental changes caused by woody encroachment represent strong environmental filters shaping ground layer community assembly. These changes act limiting both survival and regeneration of savanna ground layer species in encroached situations.

Keywords: environmental filters, functional traits, germination, photosynthesis, light, savanna.

SUMÁRIO

INTRODUÇÃO GERAL

O adensamento das savanas

Savanas e florestas são os tipos vegetacionais mais dominantes nas regiões tropicais. Fatores climáticos como a precipitação e a duração da estação seca, edáficos como a disponibilidade nutricional e a profundidade dos solos, e os distúrbios como o fogo e a herbivoria são os principais aspectos que governam a dinâmica savana-floresta (MURPHY; BOWMAN, 2012). Curiosamente, regiões com condições edafo-climáticas semelhantes podem apresentar vegetação savânica ou florestal a depender dos regimes de distúrbio, especialmente o fogo; assim, savana e floresta podem ser considerados estágios alternativos estáveis (MURPHY; BOWMAN, 2012; STAVER; ARCHIBALD; LEVIN, 2011).

De fato, as savanas tropicais têm apresentado uma tendência de aumento na sua biomassa lenhosa, sendo cada vez mais dominadas por árvores e arbustos, tanto em termos de densidade como de cobertura (STEVENS et al., 2017). Entre os fatores que contribuem para este adensamento da vegetação estão as mudanças climáticas – ligadas aos aumentos na concentração de CO_2 e alteração nos regimes de precipitação – e especialmente a supressão dos distúrbios, como o fogo (GARCIA-CRIADO et al., 2020; ROSAN et al., 2019; STEVENS et al., 2017). Embora o adensamento em regiões de savanas tropicais possa ser visto como uma oportunidade de sequestro de carbono, isso se dá ao custo da biodiversidade encontrada nestes ambientes (ABREU et al., 2017). Tal fato é especialmente verdadeiro para as savanas brasileiras, as quais possuem um estrato rasteiro herbáceo-arbustivo composto por diversas formas de crescimento, com alta diversidade florística e funcional (BUSTAMANTE et al., 2012; GOTTSBERGER; SILBERBAUER-GOTTSBERGER, 2006; MENDONÇA et al., 2008; ROSSATTO et al., 2014; ROSSATTO; FRANCO, 2017; ROSSATTO; KOLB; FRANCO, 2015; ROSSATTO; TONIATO; DURIGAN, 2008; WALTER, 2006). Comparativamente ao estrato arbóreo, o estrato herbáceo-arbustivo tem se mostrado mais negativamente afetado pelo adensamento, apresentando perda de espécies e de funções, possivelmente devido às mudanças nos filtros ambientais (ABREU et al., 2017; PILON et al., 2020; PINHEIRO; KOLB; ROSSATTO, 2016).

A supressão do fogo tem sido determinante para o adensamento lenhoso no Cerrado (DURIGAN; RATTER, 2016; ROSAN et al., 2019; STEVENS et al., 2017). Nesta condição, o cerrado *sensu stricto* – fisionomia savânica mais comum e amplamente distribuída do Cerrado – pode ficar mais denso ou até mesmo adquirir características florestais, principalmente nos limites mais periféricos da distribuição do bioma (DURIGAN; RATTER, 2006; PINHEIRO; DURIGAN, 2009). Isto é possível porque algumas espécies tipicamente florestais podem se estabelecer nestes locais na ausência de fogo (HOFFMANN et al., 2009; SILVA et al., 2008). O recrutamento das espécies florestais juntamente com aumentos na densidade e cobertura de algumas espécies lenhosas próprias do Cerrado contribuem para o fechamento do dossel (HOFFMANN et al., 2012), que leva à diminuição da luminosidade que chega ao sub-bosque, e ao aumento da camada de serapilheira (PAIVA; SILVA; HARIDASAN, et al. 2015; PINHEIRO; KOLB; ROSSATTO, 2016; ROSSATTO; RIGOBELO, 2016). Estas transformações estão colocando a vegetação em uma situação de menor propensão a eventos de fogo, pela redução da biomassa do estrato rasteiro, principalmente das gramíneas, que são a maior fonte de combustão em savanas (HOFFMANN et al., 2012; NEWBERRY et al., 2020).

Efeitos do adensamento sobre a vegetação herbáceo-arbustiva

Filtros ambientais expressam a capacidade dos hábitats selecionarem um certo conjunto de atributos adequados para a sobrevivência naquele determinado ambiente, selecionando assim um conjunto de espécies dentre o *pool* local (*sensu* KEEDY, 1992). Deste modo, as modificações nos filtros ambientais causadas pela maior cobertura de dossel (redução de luz que chega ao solo e maior deposição de serapilheira) parecem ser responsáveis por filtrar as espécies do estrato rasteiro típicas de cerrado *stricto sensu* (PINHEIRO; KOLB; ROSSATTO, 2016), afetando a sobrevivência e a capacidade de estabelecimento destas espécies nos locais mais adensados. Em outras palavras, as alterações nos filtros ambientais causadas pelo adensamento podem tornar o ambiente original inadequado aos requerimentos dos nichos de habitat e de regeneração dessas espécies do estrato herbáceo-arbustivo, afetando assim a montagem desta comunidade (GRUBB, 1987; KEEDY, 1992).

Como evidência disso, temos a redução da diversidade e riqueza de espécies herbáceo-arbustivas em regiões que adensaram para um cerradão em comparação com áreas ainda abertas de cerrado *sensu stricto* (PINHEIRO; KOLB; ROSSATTO, 2016). Além disso, o adensamento acarreta uma série de alterações estruturais para a comunidade herbáceo-arbustiva. Em um curto período de apenas quatro anos de acompanhamento da comunidade herbáceo-arbustiva, em um cenário de supressão do fogo, Pilon (2019) encontrou incrementos na riqueza e na densidade dos regenerantes de espécies arbóreas e lianescentes, assim como de espécies generalistas. Além disso, houve reduções na densidade e cobertura de elementos típicos de savana associadas a hábitats com maior cobertura de copas (PILON, 2019).

Especificamente, a cobertura de gramíneas foi severamente reduzida em áreas com maior sombreamento (PILON, 2019; PILON et al., 2020). Tudo isso ressalta que os efeitos causados pelo adensamento lenhoso sobre a diversidade e a estrutura do estrato herbáceo-arbustivo bem como para o funcionamento ecossistêmico não podem ser menosprezados, principalmente em razão das evidências em relação ao curto espaço de tempo em que eles se impõem (ABREU et al., 2017; DURIGAN; RATTER, 2006).

As características morfofuncionais das espécies herbáceo-arbustivas típicas de cerrado *stricto sensu* podem estar relacionadas com a provável falta de capacidade de se ajustar e sobreviver em ambientes mais sombreados. Estudos apontam que, no geral, tais espécies apresentam folhas com baixos valores de área foliar específica e altas taxas de transpiração, condutância estomática e assimilação de carbono (CARLOS; ROSSATTO, 2018; ROSSATTO et al., 2018; ROSSATTO; FRANCO, 2017); características consideradas típicas de plantas de sol e não relacionadas com a tolerância ao sombreamento (VALLADARES; NIINEMETS, 2008). Somado a isso, há evidência de que plantas típicas de ambientes com grande incidência luminosa e com solos distróficos apresentem menos plasticidade frente às variantes ambientais, sendo assim mais conservativas nos seus atributos foliares (POWER et al. 2019; VALLADARES et al., 2000).

Mesmo assim, é possível que estas plantas do estrato rasteiro apresentem algum grau de plasticidade fenotípica nos atributos morfofuncionais frente ao sombreamento imposto pelo adensamento, contribuindo para a permanência destas espécies (MARRONI, 2019). Alterações nas estruturas e funcionamento foliares que poderiam favorecer as plantas típicas de cerrado *sensu stricto* a lidar com o sombreamento envolvem a diminuição na espessura dos tecidos foliares, levando a maiores valores de área foliar específica, bem como alterações na distribuição dos cloroplastos e aumentos nos teores de clorofila, especialmente clorofila *b*, que podem aumentar a capacidade de absorção de luz, e manter assim, a eficiência da fotossíntese (BARROS et al., 2012; MOREIRA et al., 2013; VALLADARES; NIINEMETS, 2008).

Algumas espécies arbóreas, por exemplo, são generalistas, apresentando uma amplitude em seus nichos de germinação, além de possuírem uma capacidade de plasticidade morfofuncional que as permite prosperar tanto em ambientes de floresta como de savana (BARROS et al., 2012; MENDONÇA et al., 2020; RIBEIRO; KOLB, 2016; ROSSATTO; KOLB, 2012). Além disso, há evidência de algumas espécies herbáceo-arbustivas capazes de produzir novas folhas com alterações morfológicas e funcionais para lidar com a menor disponibilidade de luz (BEDETTI et al., 2011; MARRONI, 2019). Marroni (2019) estudando a aclimatação de três espécies herbáceo-arbustivas ao sombreamento encontrou que folhas aclimatadas destas espécies são capazes de atingir os seus pontos de compensação luminosa em condições de disponibilidade luminosa semelhante à de áreas adensadas, porém sem atingir a saturação da fotossíntese. Assim, o ganho de carbono nestas espécies pode não ser o suficiente para a sua persistência em condições adensadas, ainda mais considerando as sucessivas rebrotas que essas espécies apresentaram, possivelmente, na tentativa de encontrar condições ambientais mais favoráveis à fotossíntese (MARRONI, 2019).

Isto aliado com a ausência de muitas das espécies herbáceo-arbustivas típicas de cerrado *sensu stricto* em fisionomias que passaram por adensamento a mais de 30 anos (PINHEIRO; ROSSATTO; KOLB, 2016), leva a crer que tais espécies não apresentam plasticidade à luz suficiente para tolerar o sombreamento em longo prazo. Além do sombreamento por si só, a homogeneização do ambiente luminoso encontrada no cerradão (PINHEIRO; ROSSATTO; KOLB, 2016), pode reduzir os nichos necessários para a ocorrência das características morfofuncionais diversificadas presentes em espécies herbáceo-arbustivas de cerrado *sensu stricto* (PINHEIRO; ROSSATTO; KOLB, 2018; ROSSATTO; FRANCO, 2017; ROSSATTO; KOLB; FRANCO, 2015).

Em relação à serapilheira, constatou-se que sua presença influencia a diversidade e a produtividade em vegetações do mundo inteiro (XIONG; NILSSON, 1999). A camada de serapilheira pode dificultar o estabelecimento de plântulas e a rebrota (CARSON; PETERSON, 1990; FACELLI; PICKETT, 1991; SALAZAR et al., 2012). Adicionalmente, um maior acúmulo de matéria vegetal morta sobre o solo pode levar a alterações na disponibilidade hídrica e nutricional (ROSSATTO; RIGOBELO, 2016; VILLALOBOS-VEGA et al., 2011), as quais poderiam favorecer espécies de crescimento rápido, em detrimento de espécies savânicas adaptadas à baixa disponibilidade nutricional (BOBBINK et al., 2010; BUSTAMANTE et al., 2012). Assim, espera-se que o aumento da camada de serapilheira ocasionada pelo adensamento lenhoso também influencie a diversidade e a produtividade das espécies herbáceo-arbustivas típicas do cerrado *sensu stricto*.

O adensamento e os nichos de germinação

O adensamento pode afetar a comunidade do estrato herbáceo-arbustivo de cerrado *sensu stricto* não somente com relação às plantas já estabelecidas – podendo afetar a fisiologia e sobrevivência – mas também em relação às chances de propágulos dos elementos típicos de savana dispersarem e se estabelecerem em áreas adensadas. Assim, o conhecimento sobre as respostas germinativas das espécies herbáceo-arbustivas frente às condições ambientais impostas pelo adensamento poderia fornecer informações importantes para o entendimento da dinâmica deste componente frente a esta situação (JIMÉNEZ-ALFARO et al., 2016) uma vez que a germinação e o estabelecimento de muitas espécies de Cerrado são fortemente influenciados pelas condições luminosas (MARQUES et al., 2014; PEREIRA et al., 2020; ZAIDAN; CARREIRA, 2008).

Além das mudanças na quantidade disponível de luz (PINHEIRO; ROSSATTO; KOLB, 2016), o adensamento altera sua composição espectral, de modo que a maior cobertura de copas diminui a razão dos comprimentos de onda vermelho/vermelho extremo (V:VE) presentes na radiação luminosa que chega ao sub-bosque (SALAZAR et al., 2012). Esta alteração na composição espectral da luz influencia a germinação, por estar envolvida na fotoconversão dos fitocromos (SMITH, 2000). Desta forma, a radiação com alta razão de V:VE favorece a conversão dos fitocromos para sua forma ativa, promovendo a germinação em muitas espécies (SMITH, 2000).

Muitas espécies herbáceo-arbustivas de Cerrado requerem luz para germinar, sendo classificadas como fotoblásticas positivas (ZAIDAN; CARREIRA, 2008). E, em sendo dependentes de luz, estas espécies podem também ser sensíveis às alterações na composição espectral luminosa, requerendo altas razões de V:VE para germinar (CASAL; SÁNCHEZ, 1998). Além das condições luminosas, a alternância de temperaturas também pode favorecer a germinação de espécies herbáceas e de gramíneas de Cerrado (DAIREL; FIDELIS, 2020; MUSSO et al., 2015; KOLB; PILON; DURIGAN, 2016). Deste modo, o sombreamento causado pelo dossel fechado e/ou pela maior espessura da camada de serapilheira no cerradão pode tornar a temperatura do solo superficial mais estável, dificultando a germinação de espécies que precisam de flutuações na temperatura para quebrar a dormência (KOLB; PILON; DURIGAN, 2016).

Além disso, a serapilheira também pode atuar como barreira mecânica para o estabelecimento das plântulas, por impedir a chegada da raiz de sementes germinadas ao solo ou, então, por impedir que a parte aérea das plântulas atinja a superfície, nos casos em que a semente germine sob a camada de serapilheira (FACELLI; PICKETT, 1991; VÁZQUEZ-YANES; OROZCO-SEGOVIA, 1993). Salazar et al. (2012), por exemplo, verificaram experimentalmente que maiores quantidades de serapilheira diminuem a emergência de plântulas de espécies lenhosas de cerrado *sensu stricto*.

Adensamento – estado atual do conhecimento e suas lacunas

Quando se trata de questões relacionadas ao adensamento da vegetação em áreas de Cerrado, ou mesmo da ecologia da transição cerrado-floresta, grande parte dos trabalhos é direcionada para espécies lenhosas, seja no âmbito da florística e da estruturação das comunidades (PINHEIRO; AZEVEDO; MONTEIRO, 2010; PINHEIRO; DURIGAN, 2012; ROITMAN; FELFILI; REZENDE, 2008), da ecofisiologia (BARROS et al., 2012; HABERMANN et al., 2011; HOFFMANN et al., 2005; TOLENTINO et al., 2020), ou de aspectos germinativos e estabelecimento de plântulas (HOFFMANN; ORTHEN; FRANCO, 2004; GEIGER et al., 2011; SALAZAR et al., 2012; RIBEIRO; BORGHETTI, 2014). Pode-se dizer ainda, que o estudo do componente rasteiro herbáceo-arbustivo tem sido relegado a segundo plano num contexto mais geral, tanto no Cerrado como em outras savanas mundiais (DURIGAN et al., 2018; ROSSATTO, 2011; SARMIENTO, 1984; SIMIONI et al., 2004), apesar da sua alta importância florística e para o funcionamento destes ecossistemas.

Atualmente, maior atenção tem sido despendida ao entendimento de como o adensamento lenhoso em áreas de savana afeta negativamente a comunidade vegetal do estrato herbáceo-arbustivo, tanto em termos florísticos como funcionais (MARRONI, 2019; PILON, 2019; PINHEIRO, 2016; ROSSATTO et al., 2018; UTAILE et al., 2020). Entretanto, os efeitos das mudanças nos filtros ambientais causadas pelo adensamento nunca foram empiricamente testados para as espécies herbáceo-arbustivas, tendo os estudos anteriormente citados se utilizado de variações naturais ocorrentes na paisagem. Assim, ainda não está claro como a luminosidade e/ou a serapilheira alteram a dinâmica das espécies do estrato rasteiro e se estas plantas possuem ajustes morfofisiológicos frente às mudanças nestes fatores. Adicionalmente, pouco se sabe a respeito dos nichos de regeneração das espécies herbáceo-arbustivas, especialmente, se estas espécies possuem condições de germinar e de se estabelecer no cerradão. Deste modo, este estudo certamente irá trazer relevante contribuição a respeito das adaptações funcionais e da conservação da comunidade herbáceo-arbustiva de cerrado *sensu stricto*; ainda mais levando em consideração a importância dos regimes de fogo para a manutenção das fisionomias abertas de Cerrado, e que o uso do manejo de fogo ainda é incipiente no Brasil na conservação destes ambientes (SCHMIDT; ELOY, 2020).

Estrutura geral da tese e apresentação dos capítulos

Ao longo dos três capítulos desta tese, nós nos propusemos a experimentalmente investigar os efeitos de mudanças em importantes filtros ambientais (luz e serapilheira) causadas pelo adensamento lenhoso sobre a dinâmica de espécies e respostas funcionais e germinativas da comunidade herbáceo-arbustiva do cerrado *sensu stricto*. Os capítulos apresentados são os seguintes:

- Capítulo 1. Woody plant encroachment consequences for Cerrado ground layer: an experimental approach

Nesse capítulo foram avaliados os efeitos do sombreamento e da presença da camada de serapilheira sobre a diversidade florística e funcional, estruturação e biomassa aérea da comunidade do estrato herbáceo-arbustivo do cerrado *sensu stricto*. Para tanto, foi realizado um experimento com manipulação do sombreamento e adição de serapilheira, simulando condições ambientais encontradas em áreas adensadas sobre a comunidade do estrato herbáceo-arbustivo.

- Capítulo 2. Aclimatação de espécies herbáceo-arbustivas de *cerrado sensu stricto* à redução da luminosidade e ao aumento da serapilheira impostos pelo adensamento

Nesse capítulo foi verificado se espécies típicas da comunidade herbáceo-arbustiva de cerrado *sensu stricto* são capazes de apresentar plasticidade em suas respostas fisiológicas foliares, essencialmente a etapa fotoquímica da fotossíntese, as concentrações de pigmentos foliares e área foliar específica, para sobreviver sob o sombreamento e a maior deposição de serapilheira. Essas avaliações fisiológicas foram realizadas em plantas utilizando o procedimento experimental do Capítulo 1.

- Capítulo 3. Woody plant encroachment constrains seed regeneration of ground layer species in a Neotropical savanna

Neste capítulo, buscou-se responder se espécies herbáceo-arbustivas comuns e exclusivas ao cerrado *sensu stricto* possuem capacidade de regeneração em condições ambientais de áreas adensadas. Foram avaliados os efeitos da qualidade da luz e da variação de temperatura sobre a germinação destas espécies, além da avaliação da influência de camada de serapilheira no processo de emergência de plântulas.

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CAPÍTULO 1

Woody plant encroachment consequences for Cerrado ground layer: an experimental approach

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Abstract Woody plant encroachment is a reality in many savannas around the world leading open areas toward a forest state. As tree density and cover increase, shade and litter deposition may impair savanna ground-layer plant community. By means of field manipulation, we tested shading and litter effects, alone and in combination, on the savanna ground-layer plant community. We investigated how changes in these environmental factors can affect the composition, structure, functional diversity and aboveground biomass in this species-rich layer in a Neotropical savanna. Shade negatively affected all parameters, while litter had only small effects. Herbs and grasses had the major shade-induced reductions in terms of richness, density and cover. The increase in shade promoted a sharp decrease in monocots aboveground biomass. Our results highlight the light as a strong environmental filter shaping ground layer community assembly. With grass cover and biomass reduction, ecosystem function was highly altered attesting woody encroachment as a degradation factor for open savannas. Management decisions need to be taken in order to prevent woody encroachment or mitigate its harmful effects for savanna biodiversity and ecosystem function.

Key-words: crowding, environmental filtering, functional traits, savanna, shade tolerance.

Introduction

Savanna systems are characterized by the coexistence between an herbaceous-shrub layer - or ground layer - and a tree component (SCHOLES; ARCHER, 1997). One major threat to savanna conservation around the world is the woody plant encroachment, which has altered grass-tree dynamics through increases in tree density and biomass (ARCHER et al., 2017; STEVENS et al., 2017). As tree density increases, grasses and other ground layer species adapted to typical savanna environmental conditions can have their persistence hindered and even disappear (ABREU et al., 2017; PINHEIRO; KOLB; ROSSATTO, 2016). Most of the savanna flammability depends on grasses biomass; thus, without fire, typically tree forest species are able to establish and grow in savanna sites providing positive feedback for the encroachment process and negative feedback for fire (HOFFMANN et al., 2012; NEWBERRY et al., 2020). In this scenario, the ground layer component responses to woody encroachment environmental changes are key aspects to understand how woody encroachment impacts savannas functioning, biodiversity and ecosystem services.

Increased canopy cover and litter deposition are the major structural changes produced under encroached savannas (PINHEIRO; KOLB; ROSSATTO, 2016). These changes may be filtering ground layer savanna species and affecting their survival, regeneration ability and thereafter promoting species turnover (PINHEIRO; KOLB; ROSSATTO, 2016). In fact, light is an important environmental factor influencing composition and structure of savannas ground layer community (ABDALLAH et al., 2016; CHARLES-DOMINIQUE et al., 2018), as savanna species are usually adapted to thrive under high irradiances, being many of them shade-intolerant (ROSSATTO et al., 2018). On the other hand a thick litter layer may hinder regeneration ability by negatively affect resprout and seedling emergence (CARSON; PETERSON, 1990; FACELLI; PICKETT, 1991; SALAZAR et al., 2012). Also, the presence of a higher quantity of dead plant material on savanna soil can promote nutrient availability increases (ROSSATTO; RIGOBELO, 2016), which could benefit some faster–growing generalist/ruderal species over low-nutrient adapted species of savanna (BOBBINK et al., 2010; BUSTAMANTE et al., 2012).

Recent research has revealed differences in composition and structure of ground layer communities in relation to increased canopy shading and woody encroachment (ABREU et al., 2017; CHARLES-DOMINIQUE et al., 2018; PINHEIRO; KOLB; ROSSATTO, 2016; UTAILE et al., 2020). Additionally, selection of morpho-physiological traits related to shade tolerance has been reported in encroached conditions, such as higher specific leaf area values and greater amounts of leaf pigment content (PINHEIRO; KOLB; ROSSATTO, 2018;

ROSSATTO et al., 2018; UTAILE et al., 2020). Such findings were obtained using natural variations provided by natural encroachment after 20-30 years of fire absence. However, the dynamics of plant communities under woody encroachment have never been demonstrated as direct effects of environmental filters changing on the ground layer. Thereby, the process of floristic and functional differentiations induced by changes on environmental filtering triggered by woody encroachment on ground layer community remains practically unknown.

Ground layer species loss is a problem when it comes to the Cerrado due to its high floristic and functional diversity. Although the ground layer component has been a neglected issue for a long time, recent attention has been devoted to this vegetation component. Beyond its ecosystem role promoting vegetation flammability, ground layer species represent most part of the Cerrado plant biodiversity comprehending six to seven species for each arboreal one (MENDONÇA et al., 2008; ZAPPI et al., 2015). The ground layer is also rich in growth forms and functional strategies given Cerrado environmental heterogeneity (BUSTAMANTE et al., 2012; GOTTSBERGER; SILBERBAUER-GOTTSBERGER, 2006; PILON et al., 2020; ROSSATTO; FRANCO, 2017; ROSSATTO; KOLB; FRANCO, 2015). This diversity is potentially at risk with the environmental filtering changes promoted by woody encroachment further considering that use of fire management still is scarce in Brazil (SCHMIDT; ELOY, 2020).

Here we experimentally reproduced shade and litter conditions of encroached sites and monitored the ground layer community response in a typical savanna of Cerrado region. We performed a field experiment manipulating shade, litter and promoting the combination of both; we monitored the effects of these changes in the ground layer plant species throughout time. Ground layer composition and structure were annually assessed during three years. Functional diversity and aboveground biomass were evaluated at the end of the experiment. Specifically, we intended to disentangle which factors (shade, litter or both) are involved in shoot disappearance of the high species-rich ground-layer in the woody encroachment process. In addition, we investigated which components of this layer are promptly affected by the changes in these environmental components. For this, we monitored changes in taxonomic and functional diversity, and also in the vegetation structure and biomass. We expected that shading and litter will promote short-time negative effects on savanna ground layer, such as loss of taxonomic and functional diversity as well as decreases in density, cover and biomass.

Methods

Study area

This study was conducted at Assis Ecological Station (AES), a Cerrado protected area located in southeastern Brazil (22º33’20’’S e 50º21’27’’W). AES is more than 50 years protected from fire, due to the Brazilian policies of fire suppression (DURIGAN; RATTER, 2016). The protected area has been undergoing a fast woody encroachment process, with great forest advance (these encroached areas are henceforth called encroached savannas, also locally known as *cerradões*), and so the study was developed in the few remnants of typical savannas (PINHEIRO; DURIGAN, 2009). AES Köppen climate classification is Cfa (ÁLVARES et al., 2013), with dry winters and rainy summers; annual precipitation is about 1,400 mm. Soils are dystrophic, acid and sandy oxisols with high aluminum content (JUHÁSZ et al., 2006).

Environmental characterization in encroached areas

To simulate the shade condition and litter accumulation of encroached areas, we measured photosynthetically active radiation (PAR), litter layer thickness and litter dry mass in 30 1-m² plots haphazardly distributed along AES encroached savanna with a minimal distance of one kilometer among them. We avoided to set the plots in localities near to edges or uneven ground surface. PAR was measured at 70 cm from the soil, between 8h00 a.m. and 12h00 p.m., with a CI-110-24P-ID plant canopy imager (CID Bioscience, Camas, WA, USA), in December 2017. In each plot, litter thickness was measured using a graduated ruler and then all the litter present in each plot was collected and oven-dried at 60°C during three days and weighted. Encroached savanna plots mean PAR was 46.93 ± 2.31 µmol $m^{-2}s^{-1}$. Plots presented a mean litter thickness of 3.93 ± 1.42 cm and a mean dry mass of 1.63 ± 0.49 Kg m^{-2}. These were the parameters that were taken into account for the field manipulation experiment explained in the next section.

Field experiment: experimental design and data collection

The experiment simulating woody encroachment effects was done in typical savanna patches at AES and consisted of four treatments: shading (S), litter addition (L), shading plus litter addition (S+L) and control (C, no intervention). These treatments were distributed in 10 blocks bearing four 1 m^2 plots with a maximum distance of 1 m among plots in the same block. As typical savanna areas are currently scarce in AES, due to woody encroachment and forest expansion (PINHEIRO; DURIGAN, 2009), blocks were set in eight typical savanna patches: six patches bearing one block each and two patches bearing two blocks each; for the patches with two block with the distance between block was approximately 10 m. Each typical savanna patch was located relatively distant from another patch (more than 200 m). We avoided to set the plots in localities with soil surface irregularities or near to anthills. Each block had theirs plots randomly assigned for the four treatments mentioned before. In order to simulate shade conditions of the encroached savanna, plots were covered with shade screen (70%) in an amount that simulated the light intensity of encroached savanna understory (PAR: 40-50 µmol m^{-2} s^{-1}). Litter was added in the plots until it reached similar thickness of the encroached savanna (4 cm). More litter was added to maintain litter layer thickness whenever necessary.

Before litter and shading treatments implantation, a preliminary survey was carried out in the middle of the rainy season (T0, January 2017). Treatments were established in July 2017. After the preliminary survey, three surveys were performed always in January months to verify treatment effect in the ground layer community: T1 (six months of treatment effect), T2: (1.5 years) and T3 (2.5 years). In all surveys, the following growth forms were considered: (a) herbaceous species: small plants which stem has no woody tissue, this category includes grasses and forbs; (b) subshrub: plant with a woody basis and herbaceous branches; (c) shrub: wooded stem plant with a trunk that ramify near the basis; for the present study only the small shrubs were considered (less than 1m height); (d) climber: herbaceous or woody plant with long, slender and flexible branches usually growing supported on another plant (adapted from DURIGAN et al., 2004). The growth form classification was done on the basis of field observation and specialized literature (DURIGAN et al., 2004; 2018; ZAPPI et al., 2015).

All individuals of the cited growth forms with height of more than 10 cm were identified and counted in each plot, and each ramet was considered as a single individual. Also, all individuals had their cover estimated. Each individual had crown longitudinal and transversal diameter measured with a measuring tape. Crown projection was calculated as an ellipse using the longitudinal and transversal crown measurements of each individual. Species cover percentage was given by the division of crown projection area by plot area.

Aboveground biomass was evaluated at the end of the experiment (2.5 years of treatment effect) in five plots from each treatment. All individuals' shoots were cut at the soil level and the material was separated between monocots and eudicots, and between dead and living biomass. Then, plant material was oven-dried at 60°C during 3 days and weighted.

Functional diversity assessment

Functional diversity can be briefly described as the extent of functional traits found among species of a certain community (PETCHEY; GASTON, 2002). To give an idea of how shade conditions and litter accumulation can influence the functional diversity, a physiological assessment was performed during the rainy season, close to the end of the experiment (February 2020) in all individuals of the plots.

From leaves of the 3-4th node, leaf pigment content and specific leaf area (SLA) were measured in all species per plot, one individual of each species per plot. Leaf pigments content (chlorophylls *a* and *b*) were obtained from 1 cm^2 leaf discs. Discs were placed in 2-ml amber tubes filled with DMF (N,N-dimethylformamide) (PORRA; THOMPSON; KRIEDEMANN, 1989). Tubes were kept in the dark at 10°C for pigment extraction for 48 h. Leaf pigment content was determined using a spectrophotometer (Quimis, model Q898DRM), according to WELLBURN (1994) using absorbance values of 647 and 664 nm. SLA was obtained from the ratio of leaf area digitalized (obtained with free software ImageJ) and leaf dry mass obtained after leaf oven-drying at 60°C for three days. Components of functional diversity - *i.e.*, functional richness (FRic), evenness (FEve) and divergence indices (FDiv) (VILLÉGER; MASON; MOUILLOT, 2008) - were calculated using chlorophyll *b* and SLA traits. Analysis was done in the R environment (3.6.3) using the *FD* package (LALIBERTÉ; LEGENDRE; SHIPLEY, 2014).

Statistical analyses

Non-metric multidimensional scaling (nMDS) analyses were performed in order to evaluate the composition of the ground layer community among different treatments through time. nMDS analyses were done using species abundance and cover data as weights, and Bray-Curtis distance. Grouping significance was verified by similarity analysis (ANOSIM). Analyses were done in the R environment (3.6.3) using *vegan*, *lattice* and *permute* packages (OKSANEN et al., 2019).

Richness, density and cover of the ground layer plant community were analyzed by linear mixed models (LMM); treatments and time were considered fixed factors and blocks were considered random factors. LMMs were also performed for the growth forms, separately. Data transformations were necessary for the following variables: total cover and subshrub cover (arcsine square root transformation), and subshrub density (log+1 transformation). Aboveground biomass and functional diversity indexes differences among treatments were also compared by LMMs. Models were performed using *nlme* package (PINHEIRO et al., 2020). When significant differences were detected, we performed contrast analysis by *emmeans* function from the *emmeans* package (RUSSEL, 2019). All analyses were performed using R program (R DEVELOPMENT CORE TEAM, 2020).

Results

Ground layer community composition and structure

We found 111 species which belong to 77 genera and 34 families over the course of the experiment. The grass *Axonopus pressus* (Nees ex Steud.) Parodi (Poaceae) was the species with the most notorious shoot disappearance under shaded conditions through time. However, other typical savanna species commonly found in the study area also had a noticeable decrease in number of individuals, such as *Byrsonima intermedia* A.Juss. (Malpighiaceae), *Campomanesia adamantium* (Cambess.) O.Berg (Myrtaceae), *Chamaecrista desvauxii* (Collad.) Killip var. desvauxii (Fabaceae), *Chamaecrista flexuosa* (L.) Greene (Fabaceae), *Galactia decumbens* (Benth.) Chodat & Hassl. (Fabaceae), *Hyptis campestris* Harley & J.F.B. Pastore (Lamiaceae), *Miconia fallax* DC. (Melastomataceae) and *Lippia origanoides* Kunth (Verbenaceae) (Supplementary Table 1).

Ordination by nMDS suggests a gradual differentiation in composition of the ground layer plant community through time for both species abundance and species cover (Figure 1). For T0 and T1 ANOSIMs were not significant for both, species abundance and cover. Starting from T2 (one year and six months after installing the treatments), ANOSIM showed significance ($P < 0.0001$), indicating some degree of dissimilarity among treatments (Table 1). Species cover differentiation among treatments was more evident than for species abundance in T2 (Figure 1E and F). In T3, we observed two groups in both ordinations (Fig 1G and I): (i) control and litter plots composition was similar, while (ii) shade and shade plus litter (S+L) ended up with more similar plant communities.

Table 1 – Similarity analysis (ANOSIM) considering the abundance and cover of the cerrado ground layer community under control and woody encroachment simulated conditions - litter addition, shading and shading plus litter - through time. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect.

	Species abundance		**Species cover**	
	R	***P***	**R**	***P***
T0	-0.06419	0.96561	-0.03761	0.82204
T1	-0.04328	0.87882	0.001583	0.43711
T2	0.1626	0.001	0.1891	0.0002
T3	0.2206	0.0004	0.2359	0.0002

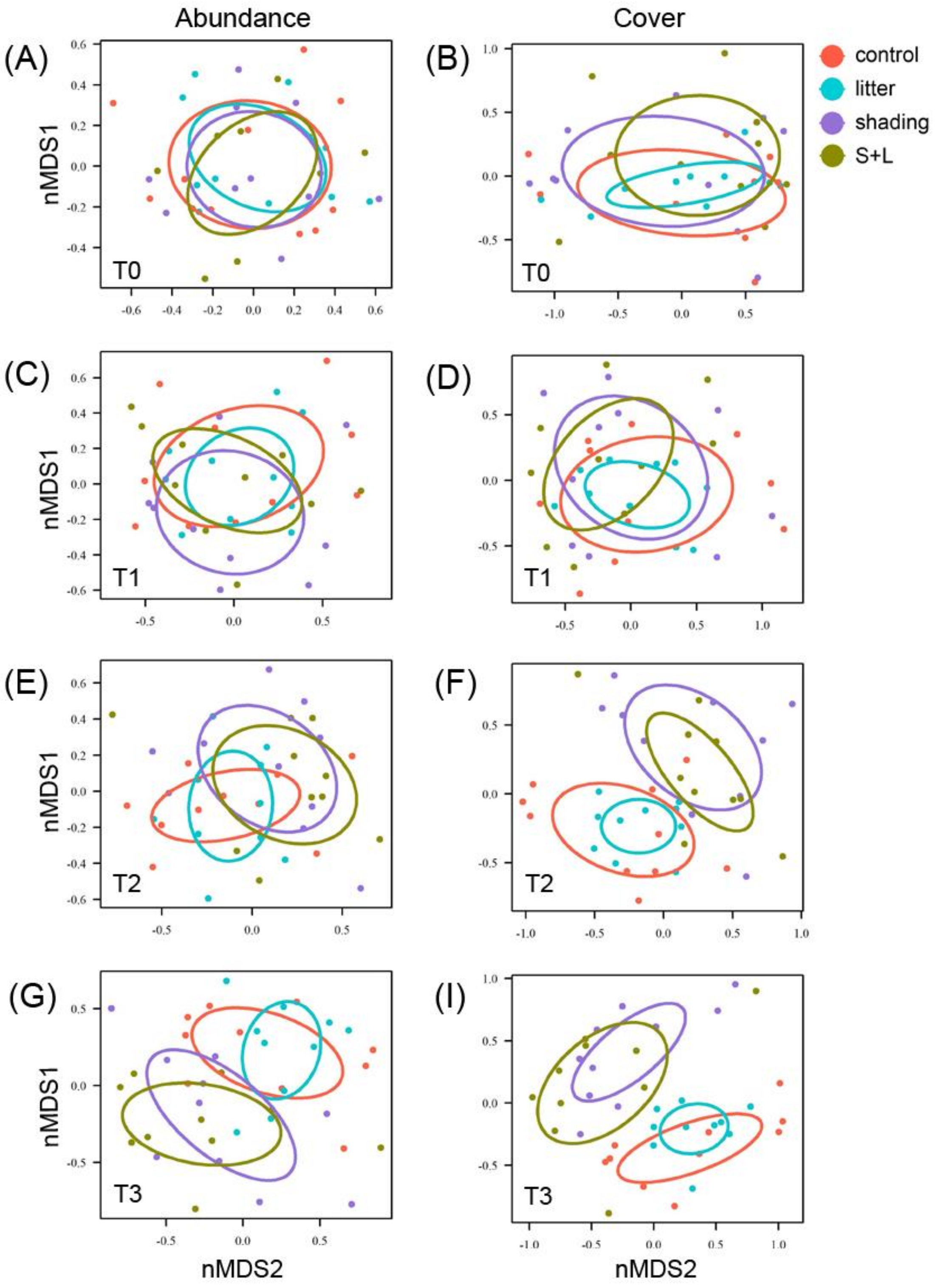

Figure 1 – Non-metric multidimensional scaling (nMDS) ordination of ground layer composition and cover under control and simulated woody encroachment conditions - litter addition, shading and shading plus litter (S+L) – through time. Ellipses represent 95% confidence intervals. Stress values: 0.19, 0.11, 0.18, 0.16, 0.19, 0.18, 0.18 and 0.16. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect.

Species richness, density and cover of ground layer community were affected by treatments, time and treatment-time interaction (Table 2). Richness for control and litter treatments increased 51% and 37% in T2, compared with T0 ($P = 0.03$ and 0.01, respectively). Shading treatment presented reduction of 36% in T3 compared with T1 ($P = 0.01$), and S+L presented reduction of 51% compared with T0 ($P < 0.0001$). These alterations led to changes in which shading and S+L treatments had lower richness than control and litter starting from T2 ($P < 0.0001$). As observed by the composition, richness in the control and litter plots did not differ from each other in the last survey ($P = 0.99$) as well as richness in the shade and S+L plots ($P = 1$). In addition, these shaded plots differed from control and litter plots in number of species ($P < 0.0001$; Figure 2A).

Density followed a similar pattern to that observed for richness. Shading and S+L treatments presented reduction of 43% and 50% in density in T3 in comparison with T0 ($P = 0.004$ and 0.0002, respectively), while litter and control treatments had 54% ($P = 0.0001$) and 46% ($P = 0.0001$) more density in T2 when compared with T0. The same differentiation among treatments observed for richness can be found for density, in which shading and S+L treatments had a lower density than control and litter treatments starting from T2 ($P < 0.0001$; Figure 2B). Regarding ground layer cover, shading and S+L treatments had cover reduction of 52% ($P < 0.0001$) and 39% in T3 ($P < 0.0001$) compared with T0. Cover variation over time caused differences among treatments in T2 and T3. In T2, shading treatment had lower cover than control ($P = 0.047$) and litter in T2 ($P = 0.047$); and in T3, S+L also had lower cover ($P < 0.0001$; Figure 2C).

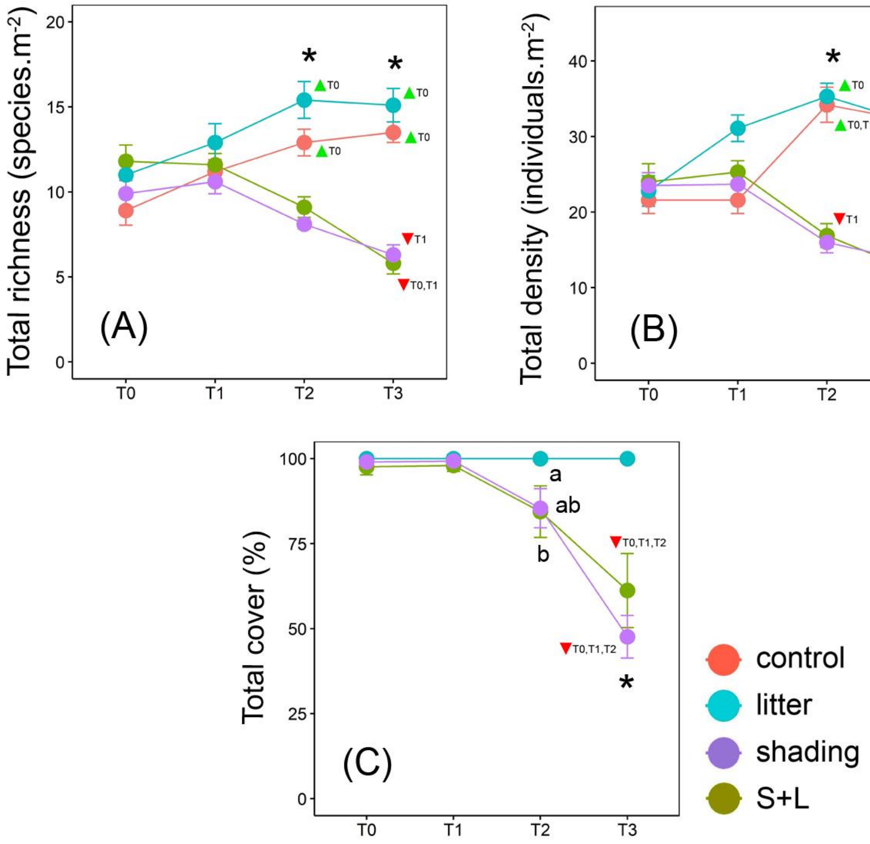

Figure 2 – Changes in ground layer community richness (A), density (B) and cover (C) (mean ± SD) under control and simulated woody encroachment conditions - litter addition, shading and shading plus litter (S+L) – through time. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect. Different letters represent significant differences among treatments and asterisks indicate differentiation between shaded (shading and S+L) and no shaded treatments (control and litter). Green arrowheads indicate significant temporal increments related to the cited time and red arrowheads indicate significant temporal decreases related to the cited time ($P < 0.05$).

Table 2 – Linear mixed models results for ground layer community richness, density and cover comparing control and simulated woody encroachment conditions - litter addition, shading and shading plus litter - through time (for all post-hoc contrasts see ST2 and ST3).

		Richness		**Density**		**Cover**	
		F	***P***	**F**	***P***	**F**	***P***
Total	Treatment	314.596	< 0.001	436.599	< 0.001	29.937	< 0.001
	Time	32.019	0.025	39.922	0.009	30.126	< 0.001
	Interaction	99.657	< 0.001	149.539	< 0.001	10.825	< 0.001
Shrub	Treatment	12.265	< 0.001	64.693	< 0.001	51.132	0.002
	Time	2.099	0.103	30.157	0.032	69.084	0.002
	Interaction	3.808	< 0.001	29.606	0.003	57.539	< 0.001
Subshrub	Treatment	110.678	< 0.001	14.871	< 0.001	119.964	< 0.001
	Time	11.701	0.324	1.388	0.249	27.937	0.043
	Interaction	48.338	< 0.001	5.353	< 0.001	23.058	0.019
Herbaceous	Treatment	22.365	< 0.001	56.054	< 0.001	48.002	< 0.001
	Time	7.132	< 0.001	8.524	< 0.001	31.547	< 0.001
	Interaction	3.743	< 0.001	7.237	< 0.001	5.929	< 0.001
Climbers	Treatment	2.013	0.115	0.283	0.837	2.522	0.061
	Time	3.485	0.018	3.325	0.022	3.631	0.015
	Interaction	0.814	0.604	0.541	0.843	1.221	0.287

Growth forms

Species richness, density and cover of herbaceous species were more reduced and earlier affected by shading treatment than shrubs and subshrubs, while climbers were not significantly affected by treatments (Figure 3; Table 2). Concerning richness, despite shrub and subshrub richness had little difference through time (Figure 3A and B); some differences could be found among treatments. Shrub species richness presented some differences in T2 where litter treatment was richer than shading ($P = 0.018$), and in T3 control plots were richer than S+L plots ($P = 0.01$). In T3, litter plots were richer than shading and S+L plots ($P \leq 0.0001$) and control plots were richer than S+L ($P = 0.01$; Figure 3A). The richness of subshrubs was higher in the control treatment than in shading ($P = 0.0001$) and S+L ($P = 0.003$) starting from T2 (Figure 3B). Herbaceous species richness was reduced by 65% and 61% in T3 for shading ($P = 0.009$) and S+L treatments ($P = 0.002$), respectively, comparing with T0; but differences among treatments can be noted in T2 when litter treatment was richer than shading ($P < 0.0001$) and S+L ($P = 0.0002$) treatments. In T3, beyond litter treatment, control was richer than shading (P = 0.0002) and S+L (P = 0.002; Figure 3C) treatments.

Regarding density, shrub density in the litter treatment was higher than shading ($P = 0.0082$) and S+L ($P = 0.0004$) treatments in T3 (Figure 3D). Subshrub differences among treatments can be observed in T2 when shading and S+L treatments density was lower than control and litter (Figure 3E) treatments, being that the control remained denser than shading ($P = 0.007$) and S+L ($P < 0.0001$) treatments in T3. The density of herbaceous species (grasses and forbs) under shading treatment reduced 59% in T2 ($P = 0.0094$) and 69% in S+L treatment in T3 ($P = 0.0021$) when compared with T0. Differences in herbs density can be noted in T1 when control treatment was denser than shading treatment ($P = 0.003$), and litter plots were denser than shading ($P < 0.0001$) and S+L ($P = 0.006$) plots. As from T2, shading and S+L treatments had less density than control and litter ones ($P < 0.0001$; Figure 3F).

The cover of shrubs almost triplicated in litter plots in T2 ($P = 0.0039$), even so it did not differ from the control plots ($P = 1$). Control plots presented more cover than shading plots in T3 ($P = 0.003$), as litter plots presented more cover than shading ($P = 0.0001$) and S+L ($P = 0.002$; Figure 3G) plots. Subshrubs had control treatment with more cover than shading ($P = 0.0001$) and S+L ($P = 0.002$) treatments in T2, but this could not be observed in T3 (Figure 3H). In the other hand, the cover of herbaceous species was more affected, shading caused 43% reduction in T1 compared to T0 ($P = 0.0165$) and 80% reduction in T3 compared with T1 ($P = 0.0018$). S+L treatment had cover reduction in T2 ($P = 0.0013$) compared with T0. In T3, treatments with shading had cover reduction of around 88% compared to initial values ($P < 0.0001$). These changes led to an earlier distinction among treatments. Shading treatment cover was lower than control ($P = 0.039$) and litter ($P = 0.023$) treatments in T1. As from T2, S+L treatment also had lower cover than control and litter treatments ($P < 0.0001$; Figure 3I).

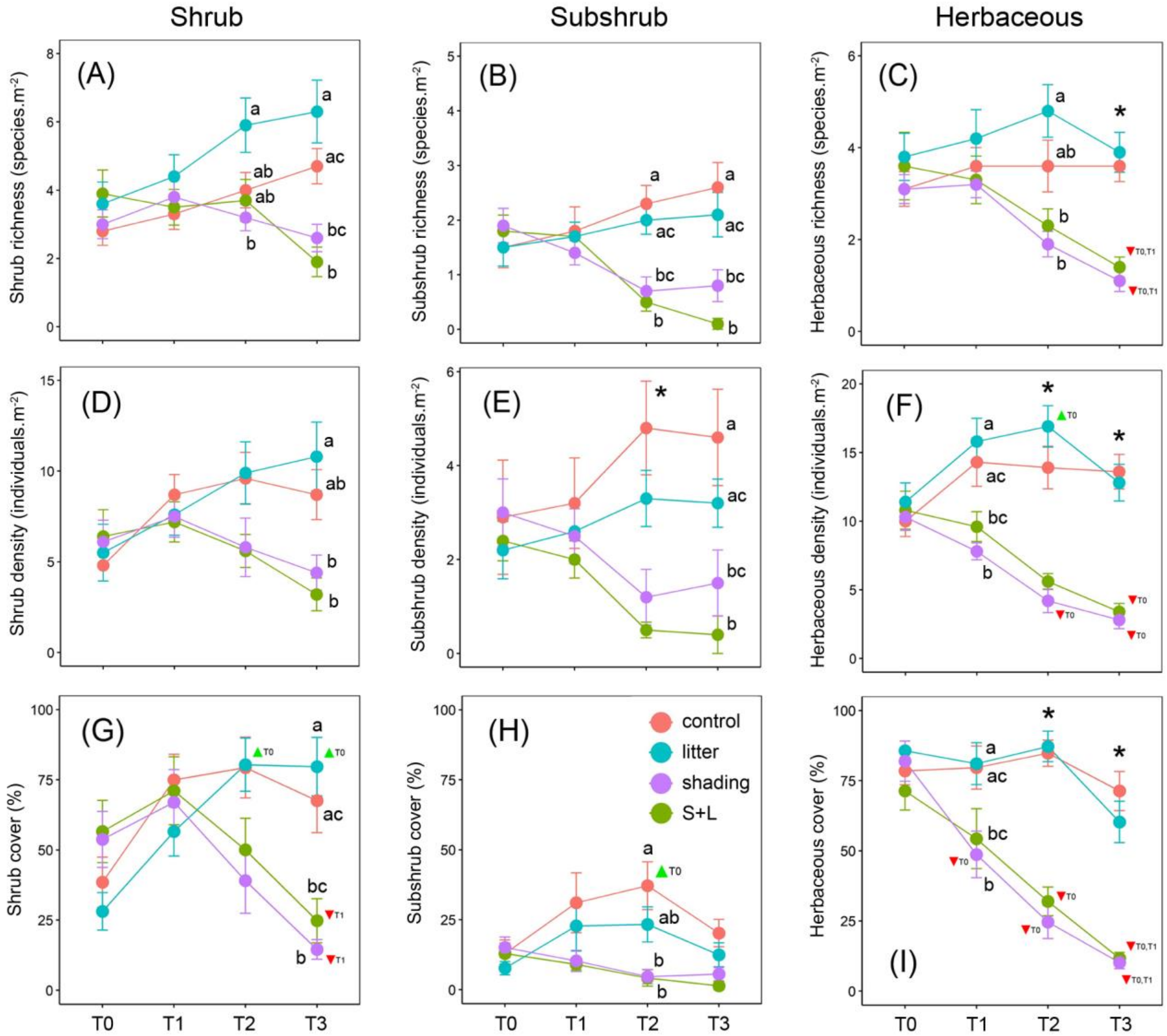

Figure 3 – Richness (A-C), density (D-F) and cover (G-I) (mean ± SD) of shrub, subshrub and herbaceous species under control and simulated woody encroachment conditions - litter addition, shading and shading plus litter (S+L) – through time. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect. Different letters represent significant differences among treatments and asterisks indicate differentiation between shaded (shading and S+L) and no shaded treatments (control and litter). Green arrowheads indicate significant temporal increments related to the cited time

and red arrowheads indicate significant temporal decreases related to the cited time (*P* < 0.05).

Ground layer community structure – aboveground biomass

Monocot dead biomass was affected by treatments (F = 6.69; *P* = 0.007), as well as both eudicot and monocot living biomass (F = 3.56; *P* = 0.047, and F = 8.79; *P* = 0.002). Eudicot living biomass under shading was reduced in 81% compared with the control treatment (*P* = 0.0472; Figure 4A). Monocot living biomass of shading and S+L plots was drastically reduced of around 87 and 91%, respectively, compared with the control plots (*P* < 0.005; Figure 4B), as well as the monocot dead biomass that was drastically reduced of around 94% in both shading and S+L plots compared with the control treatment (*P* < 0.011; Figure 4C).

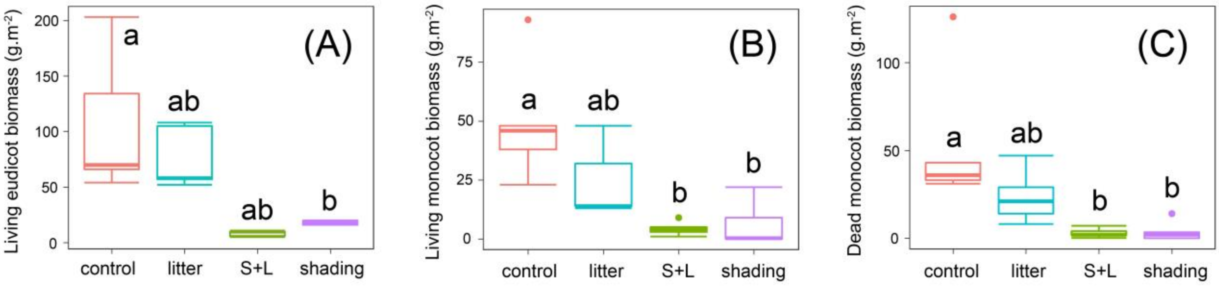

Figure 4 – Aboveground eudicot living dry biomass (A) and monocot living and dead dry biomass (A-B) of ground layer community under 2.5 years of control and woody encroachment simulated conditions - litter addition, shading and shading plus litter (S+L). Boxplots represent median and quartiles; filled circles represent outliers. Different letters represent significant differences among treatments (*P* < 0.05).

Woody encroachment effect on functional diversity

Regarding the functional diversity, functional richness (FRic) was significantly affected by the treatments (F = 4.26; *P* = 0.01), while functional evenness and divergence were not significantly affected (F = 1.57; *P* = 0.22, and F = 0.95; *P* = 0.43, respectively). FRic of the shading treatment presented a reduction of approximately 50% in relation to control and litter treatments (*P* = 0.019 and 0.046, respectively; Figure 5).

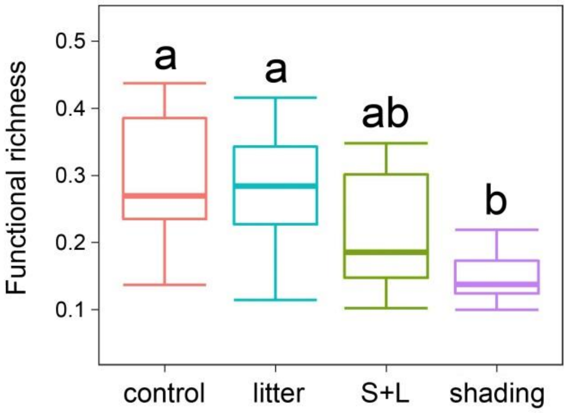

Figure 5 – Functional richness of ground layer community under 2.5 years of control and woody encroachment simulated conditions - litter addition, shading and shading plus litter (S+L). Boxplots represent median and quartiles. Different letters represent significant differences among treatments ($P < 0.05$).

Discussion

Savanna ground layer community was highly affected by shading, confirming light as a strong environmental filter for ground layer community assembly (PINHEIRO; KOLB; ROSSATTO, 2016; ROSSATTO et al., 2018). On the other hand, litter did not significantly affect the ground layer community being not related to floristic or functional losses. The ground layer community underwent compositional and structural distinction between plots under shading and full light over time. Richness, density and cover were also negatively affected under shading, with all growth forms - except climbers - undergoing floristic and structural alterations to some degree. Herbaceous species were particularly the most affected group among growth forms. Lastly, shading promoted functional richness loss and eudicot and reduction of monocot aboveground biomass. All these effects occurred in a short time (six months to 2.5 years), which indicates how inhospitable encroached light conditions are to typical savanna ground layer species.

Contrasting with the overall pattern observed for ground layer, many studies have reported increases in species richness with woody encroachment for the tree component, due to savanna species persistence and colonization of forest and the presence of generalist species (PELLEGRINI et al., 2016; PINHEIRO; DURIGAN, 2012; ROITMAN; FELFILI; REZENDE, 2008). Our results indicate that subshrubs and mainly shrubs seem to be a little more persistent when shading condition is experimentally imposed. Phenotypic plasticity may contribute to shade persistence as seen for some species occurring in both encroached and non-encroached conditions (PINHEIRO; KOLB; ROSSATTO, 2018). Shrubs presented a trend for cover increase in T1 in shaded treatments (Fig. 1G), which may represent the production of leaves with larger surface, as a response to low light levels (VALLADARES; NIINEMETS, 2008). *Miconia albicans*, a common Cerrado shrub, is able to produce mesomorphic leaves with larger leaf area and specific leaf area values in response to shade conditions (BEDETTI et al., 2011). However, most savanna ground layer species are uncommonly found in encroached sites, which indicate that most of them did not have enough morpho-functional plasticity to thrive in low light environments (PINHEIRO; KOLB; ROSSATTO, 2016; ROSSATTO et al., 2018).

Ground layer species usually need high irradiances to perform light-saturated high photosynthesis rates (ROSSATTO et al., 2018; ROSSATTO; FRANCO, 2017), leading us to believe that, under shade condition, underground storage organs are a way ensuring persistence for a limited time. In fact, presence of underground storage organs was associated with persistence in shaded areas for savanna grass community (PILON et al., 2020). Several ground layer species possess well-developed complex subterranean systems often thickened and able to store water, nutrients and carbohydrates (APPEZZATO-DA-GLÓRIA et al., 2008; DA SILVA; ROSSATTO, 2019; DE MORAES et al., 2016; RIZZINI; HERINGER, 1961). , Although the experimentation time of the present work may have been too short to induce losses in the bank of underground organs (bud bank), it is possible that long periods of woody encroachment can affect the underground savanna system along with its resilience capacity; for instance, the number of bud-bearing underground organs (such as xylopodia and rhizomes) was greatly reduced in a Cerrado area under 50-years *Pinus elliottii* afforestation (FERRARO et al., 2020).

Herbaceous component (forbs and grasses) was the most affected by simulated shading among the growth forms. C4 grasses were proved to be highly susceptible to shading presenting considerable richness and cover reduction in environments under leaf area index above 1 (PILON et al., 2020). In turn, C4 grass exclusion is a key factor enabling woody encroachment of savanna areas by reducing ecosystem flammability (HOFFMANN et al., 2012; NEWBERRY et al., 2020). Here, we not only attested structural and compositional alterations of herbaceous component but also monocots aboveground biomass reduction, corroborating a rapid transition to a lower flammable state. This is probably the first experimental report of savanna ground layer aboveground biomass reduction caused by shading, even that shading had been artificially imposed simulating an encroached condition. Due to an experimental design issue, biomass was evaluated only with 2.5 years under shading, however, given that the herbaceous cover reduction has already been observed with six months of shading (Figure 2I), it is likely that biomass reduction could occur even before. Visually, aboveground biomass in shading and S+L treatments was already quite prejudiced at 1.5 years of the experiment (L.F.S. PINHEIRO, personal observation).

Functional richness index expresses the amount of functional volume occupied by the community; thus, lower functional richness values indicate a more limited range of functional trait combinations in the functional space possible (VILLÉGER; MASON; MOUILLOT, 2008). Functional richness loss in encroached light conditions probably results from light environment homogenization caused by the shaded treatment, leading to reduction in species richness along with a lower variation in leaf physiological traits. Indeed, variable tree density and cover provide a light environment quite heterogeneous for savannas (SCHOLES; ARCHER, 1997; HOFFMANN et al., 2005). Furthermore, woody encroachment selects a set of traits related to shade tolerance as increased SLA values (PINHEIRO; KOLB; ROSSATTO, 2018; UTAILE et al., 2020) and leaf pigments content for ground layer species (ROSSATTO et al., 2018). Functional richness was also lower in encroached sites in a 50-year fire suppressed area than in typical savanna areas (D.R. ROSSATTO, unpublished data).

Contrary to our expectations, savanna ground layer richness and density increased with litter treatment; also, litter had some positive effects on shrubs cover and herbaceous density. However, ground layer community presented great variation over time, even for the control condition; consequently, the litter effect was not enough to distinguish between control and litter treatments richness, density and cover. Instead litter plots seemed to follow the dynamics of the control ones. Compositional and structural differences found in control and litter treatments over time may be attributed to a natural variation that Cerrado ground layer communities can present (MUNHOZ; FELFILI, 2007). Nevertheless, it is possible that litter could exert a latter effect on savanna ground layer community altering soil nutritional aspects and species competition dynamics (BUSTAMANTE et al., 2012). Even so, we argue that litter would exert a lower effect on savanna ground layer communities under woody encroachment given the more immediate effects of light decrease.

Changes in light environment promoted by woody encroachment alter savanna ground layer community assembly as well as ecosystem maintenance. These short time alterations emphasize the importance of the formulation of conservation strategies appropriate to savanna environment. Woody plant encroachment consequences for savannas biodiversity and ecosystem functioning have been alerted for some time, as well as the need for fire management to conserve Neotropical savannas (DURIGAN; RATTER, 2016; FIDELIS; PIVELLO, 2011; PARR et al., 2014). This alert remains valid for areas that are not yet undergoing woody encroachment or in which it is still in incipient stages. However, where woody encroachment is in advanced stages, with high tree density and already lost the typical herbaceous-layer, restoration may be more challenging, due to the reduced possibility to reintroducing fire management and uncertainties in real chances to recover the high species-rich savanna ground layer (BUISSON et al., 2019). Also, remains to be understood the recovery capacity of savanna ground-layer species if typical luminosity conditions and fire management were reestablished in already encroached areas. While science still searches for these answers, conservation action should focus in the maintenance of open savanna remnants performing fire management in regions were woody encroachment is possible and is in early steps because once trees shade out the area, the loss of typical ground layer components can occur in fast pace.

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Supplementary material

Supplementary Table 1 – Abundances of savanna ground layer species found in the plots of the field experiment simulating encroached conditions. C: control treatment; S: shading treatment; L: litter addition treatment; S+L: shading plus litter treatment.

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
AMARANTHACEAE																	
Froelichia procera (Seub.) Pedersen	forb	0	0	1	0	0	0	2	0	0	0	1	0	0	0	2	0
ANACARDIACEAE																	
Anacardium humile A.St.-Hil.	subshrub	0	2	1	2	0	6	1	0	0	2	2	0	0	2	3	0
ANNONACEAE																	
Annona coriacea Mart.	shrub	1	5	3	0	1	3	1	0	1	3	3	0	1	3	3	0
Annona dioica A.St.-Hil.	shrub	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0	1
Duguetia furfuracea (A.St.-Hil.) Saff.	shrub	0	0	0	2	0	2	0	1	0	0	0	1	1	0	0	1
APOCYNACEAE																	
Blepharodon bicuspidatum E.Fourn.	climber	2	3	3	3	2	1	2	4	1	0	3	4	0	0	1	0
Forsteronia glabrescens Müll.Arg.	climber	0	1	0	0	0	0	1	1	2	1	1	3	3	0	3	2
Forsteronia pubescens A.DC.	climber	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Temnadenia violacea (Vell.) Miers	climber	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
ARECACEAE																	
Arecaceae sp1	palm	0	0	0	0	4	0	0	0	1	0	0	0	1	0	0	0

Species/Family	Growth form	T0 (January 2017)				T1 (January 2018)				T2 (January 2019)				T3 (January 2020)			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
ASTERACEAE																	
Asteraceae sp1	subshrub	0	0	2	0	1	0	4	0	0	0	0	0	0	0	0	0
Baccharis crispa Spreng.	forb	0	1	1	3	0	2	1	0	0	0	2	0	0	0	1	0
Bidens gardneri Baker	forb	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
Bidens segetum Mart. ex Colla	subshrub	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
Chromolaena oxylepis (DC.) R.M.King & H.Rob.	shrub	6	5	4	4	4	7	5	4	5	5	13	3	7	3	11	2
Chromolaena sp.	shrub	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysolaena obovata (Less.) Dematt.	forb	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	0
Lepidaploa chamissonis (Less.) H.Rob.	shrub	5	1	1	6	17	1	6	6	12	2	5	2	5	0	5	0
Lessingianthus bardanoides (Less.) H.Rob.	shrub	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Lessingianthus tomentellus (Mart. ex DC.) H.Rob.	subshrub	1	0	1	0	2	0	2	0	2	0	2	0	2	0	1	0
Moquiniastrum barrosoae (Cabrera) G. Sancho	shrub	5	6	9	3	3	5	4	4	10	6	10	4	7	3	8	1
Vernonanthura oligolepis (Sch.Bip. ex Baker) H.Rob.	subshrub	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
BIGNONIACEAE																	
Amphilophium elongatum (Vahl) L.G.Lohmann	climber	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0
Anemopaegma arvense (Vell.) Stellfeld ex de Souza	subshrub	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Cuspidaria pulchella (Cham.) K.Schum.	climber	3	11	2	4	3	11	2	5	5	14	2	2	4	10	5	0
BROMELIACEAE																	
Ananas ananassoides (Baker) L.B.Sm.	forb	2	2	1	1	3	2	1	1	3	1	2	1	3	2	1	0
Dyckia tuberosa (Vell.) Beer	forb	0	3	0	0	0	2	0	0	0	2	0	0	0	0	0	0

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
CELASTRACEAE																	
Peritassa campestris (Cambess.) A.C.Sm.	subshrub	1	0	0	2	0	0	0	3	0	0	0	0	1	0	0	0
CHRYSOBALANACEAE																	
Leptobalanus humilis (Cham. & Schltdl.) Sothers & Prance	subshrub	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	3
COMMELINACEAE																	
Commelina erecta L.	forb	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1
CONVOLVULACEAE																	
Evolvulus sericeus Sw.	forb	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ipomoea procurrens Meisn.	climber	0	0	0	0	0	0	0	0	3	1	1	0	2	0	1	0
CYPERACEAE																	
Cyperus aggregatus (Willd.) Endl.	graminoid	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fimbristylis sp.	graminoid	1	2	0	2	1	1	2	1	2	1	2	0	5	0	3	0
Rhynchospora exaltata Kunth	graminoid	46	37	41	39	58	39	53	47	44	22	63	42	53	23	45	27
DILLENIACEAE																	
Davilla elliptica A.St.-Hil.	shrub	0	0	0	1	1	0	2	2	0	0	1	2	0	0	1	0
EBENACEAE																	
Diospyros lasiocalyx (Mart.) B.Walln.	shrub	0	0	1	0	1	0	1	0	0	0	0	0	0	0	3	0
ERYTHROXYLACEAE																	
Erythroxylum campestre A.St.-Hil.	shrub	4	1	7	2	8	1	7	2	9	0	7	1	11	2	10	0

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
Erythroxylum cuneifolium (Mart.) O.E.Schulz	shrub	2	2	6	2	1	3	6	3	8	1	8	1	7	5	12	0
Erythroxylum pelleterianum A.St.-Hil.	shrub	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythroxylum suberosum A.St.-Hil.	shrub	0	0	0	0	0	1	0	2	0	1	0	2	0	1	0	0
EUPHORBIACEAE																	
Croton campestris A.St.-Hil.	subshrub	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0
Croton glandulosus L.	forb	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0
Microstachys hispida (Mart. & Zucc.) Govaerts	forb	2	2	0	4	2	0	1	0	0	0	2	1	4	0	0	1
Microstachys serrulata (Mart. & Zucc.) Müll.Arg.	forb	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0
FABACEAE																	
Andira humilis Mart. ex Benth.	subshrub	8	6	4	5	1	7	2	1	7	3	3	0	7	6	1	4
Bauhinia rufa (Bong.) Steud.	shrub	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1
Chamaecrista desvauxii (Collad.) Killip var. *desvauxii*	forb/ subshrub	4	1	4	3	3	1	3	2	5	0	2	1	5	0	3	0
Chamaecrista fagonioides (Vogel) H.S.Irwin & Barneby	forb	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Chamaecrista flexuosa (L.) Greene	forb/ subshrub	2	2	1	5	16	1	7	5	16	0	6	1	10	0	4	0
Desmodium barbatum (L.) Benth.	subshrub	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eriosema campestre Benth.	subshrub	0	1	1	0	0	1	1	4	1	0	3	1	0	0	0	1
Galactia decumbens (Benth.) Chodat & Hassl.	forb	8	5	14	5	12	8	8	3	15	4	15	3	15	0	14	2
Mimosa debilis Humb. & Bonpl. ex Willd.	subshrub	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Mimosa xanthocentra Mart.	subshrub	3	5	1	0	1	4	1	2	3	1	1	0	3	1	2	0
Senna rugosa (G.Don) H.S.Irwin & Barneby	shrub	2	1	1	0	1	1	0	0	0	1	1	0	1	0	0	0
Stylosanthes acuminata M.B.Ferreira & Sousa Costa	subshrub	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Stylosanthes bracteata Vogel	subshrub	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
LAMIACEAE																	
Aegiphila verticillata Vell.	shrub	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Gymneia interrupta (Pohl ex Benth.) Harley & J.F.B.Pastore	forb	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Hyptis campestris Harley & J.F.B. Pastore	forb	8	6	6	6	17	5	14	6	13	6	16	2	10	3	12	0
MALPIGHIACEAE																	
Banisteriopsis campestris (A.Juss.) Little	shrub	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0
Banisteriopsis sp	shrub	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Banisteriopsis sp2	climber	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Banisteriopsis stellaris (Griseb.) B.Gates	shrub/ climber	11	9	12	9	14	13	15	12	22	12	17	14	24	17	15	17
Banisteriopsis variabilis B.Gates	climber	2	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0
Byrsonima intermedia A.Juss.	shrub	4	11	6	11	15	15	19	15	17	14	20	16	10	8	20	8
Mascagnia cordifolia (A.Juss.) Griseb.	climber	0	8	5	9	10	16	11	20	7	10	10	14	3	14	6	17
MALVACEAE																	
Peltaea polymorpha (A.St.-Hil.) Krapov. & Cristóbal	forb	0	1	2	5	0	1	2	1	0	0	3	0	0	0	3	0
MELASTOMATACEAE																	
Leandra aurea (Cham.) Cogn.	shrub	0	0	1	7	0	0	2	5	0	1	2	5	0	0	2	3
Miconia albicans (Sw.) Triana	shrub	3	5	1	2	3	4	1	2	3	4	1	2	5	5	1	2
Miconia fallax DC.	shrub	5	8	3	10	10	9	2	6	12	3	8	3	7	2	10	2
Miconia paucidens DC.	shrub	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0
MENISPERMACEAE																	
Cissampelos ovalifolia DC.	subshrub	0	3	3	7	0	1	3	6	0	0	2	5	0	0	3	4

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
MYRTACEAE																	
Campomanesia adamantium (Cambess.) O.Berg	shrub	6	11	12	6	16	13	8	9	7	13	8	4	10	8	12	4
Eugenia anomala D.Legrand	subshrub	0	0	2	0	0	0	5	0	0	0	6	0	0	0	5	0
Eugenia bimarginata DC.	shrub	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Eugenia dysenterica (Mart.) DC.	shrub	3	0	0	0	4	0	0	0	9	0	0	0	7	0	1	0
Eugenia livida O.Berg	shrub	0	0	0	0	0	0	0	3	1	0	3	2	0	0	0	0
Eugenia pitanga (O.Berg) Nied.	subshrub	2	3	1	0	2	3	0	0	0	0	0	0	0	0	0	0
Eugenia punicifolia (Kunth) DC.	shrub	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Myrcia bella Cambess.	shurb	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Psidium laruotteanum Cambess.	subshrub	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Psidium sp.	shrub	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
ORCHIDACEAE																	
Epidendrum secundum Jacq.	forb	3	0	0	3	3	0	0	1	4	0	0	0	5	0	0	0
POACEAE																	
Axonopus marginatus (Trin.) Chase	graminoid	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Axonopus pressus (Nees ex Steud.) Parodi	graminoid	30	30	32	12	34	12	45	13	36	4	47	2	34	0	37	0
Panicum sellowii Nees	graminoid	0	0	0	4	0	0	0	7	0	0	0	0	0	0	0	2
Paspalum sp	graminoid	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Pennisetum sp	graminoid	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Poaceae sp1	graminoid	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Urochloa decumbens (Stapf) R.D.Webster	graminoid	0	2	4	3	3	1	13	1	0	0	7	0	1	0	4	0
POLYGALACEAE																	
Securidaca rivinifolia A.St.-Hil. & Moq.	climber	0	0	0	0	1	0	1	3	0	1	2	0	1	0	1	1

Species/Family	**Growth form**	**T0 (January 2017)**				**T1 (January 2018)**				**T2 (January 2019)**				**T3 (January 2020)**			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
RUBIACEAE																	
Borreria poaya (A.St.-Hil.) DC.	subshrub	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Borreria verticillata (L.) G.Mey.	subshrub	3	1	2	5	4	0	3	2	3	0	0	0	3	0	1	0
Coccocypselum lanceolatum (Ruiz & Pav.) Pers.	forb	0	0	0	0	0	1	1	0	0	2	0	0	1	0	0	0
Cordiera obtusa (K.Schum.) Kuntze	shrub	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Cuphea micrantha Kunth	forb	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Declieuxia fruticosa (Willd. ex Roem. & Schult.) Kuntze	forb	0	0	1	0	0	0	1	0	1	0	4	0	4	0	3	0
Palicourea marcgravii A.St.-Hil.	shurb	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Palicourea rigida Kunth	shurb	0	0	1	1	0	0	2	2	0	0	2	2	0	0	2	1
Psychotria hoffmannseggiana (Willd. ex Schult.) Müll.Arg.	shrub	3	0	0	0	0	3	0	2	0	2	3	4	3	3	3	3
Tocoyena formosa (Cham. & Schltdl.) K.Schum.	shrub	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
SAPINDACEAE																	
Serjania lethalis A.St.-Hil.	climber	9	6	5	7	9	6	7	8	0	6	8	9	5	5	6	7
SAPOTACEAE																	
Pradosia brevipes (Pierre) T.D.Penn.	subshrub	0	6	0	0	0	2	0	1	0	4	0	1	0	3	0	0
SMILACACEAE																	
Smilax campestris Griseb.	climber	2	1	5	9	2	2	5	5	0	3	8	3	6	1	8	3
SOLANACEAE																	
Schwenckia americana Rooyen ex L. var. americana	forb	0	0	1	0	0	0	2	0	0	0	2	0	0	0	0	0

Species/Family	Growth form	T0 (January 2017)				T1 (January 2018)				T2 (January 2019)				T3 (January 2020)			
		C	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**	**C**	**S**	**L**	**S+L**
TURNERACEAE																	
Piriqueta rosea (Cambess.) Urb.	forb	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
VERBENACEAE																	
Lippia balansae Briq.	shrub	0	5	1	0	0	6	6	0	0	0	0	0	0	0	0	0
Lippia lupulina Cham.	subshrub	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Lippia origanoides Kunth	subshrub	8	3	5	5	4	3	4	5	11	2	6	1	11	2	8	0

Supplementary Table 2 – Post-hoc contrasts significance values (*P*-values) for treatments (control, litter addition, shading and S+L) and time (T0, T1, T2 and T3) interaction of Cerrado ground layer community and shrub, subshrub and herbaceous growth forms. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect; S+L: shading plus litter.

	Total			**Shrubs**			**Subshrubs**			**Herbaceous species**		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
control T0 - S+L T0	0.3847	0.9997	1	0.9731	0.9997	0.984	1	1	1	0.9998	1	1
control T0 - litter T0	0.868	1	1	0.999	1	1	1	1	1	0.9898	0.9999	1
control T0 - shading T0	0.9999	1	1	1	1	0.9972	0.9999	1	1	1	1	1
control T0 - control T1	0.7686	1	1	1	0.5025	0.199	1	1	0.4598	0.9998	0.2459	1
control T0 - S+L T1	0.5126	0.9738	1	0.9998	0.9786	0.3748	1	1	1	1	1	0.2882
control T0 - litter T1	0.0326	0.0102	1	0.6503	0.9223	0.9839	1	1	0.8812	0.6918	0.0153	1
control T0 - shading T1	0.975	1	1	0.9889	0.9414	0.6114	1	1	1	1	0.9844	0.0583
control T0 - control T2	0.0326	0.0001	1	0.9439	0.1688	0.0809	0.8625	0.5293	0.0368	0.9998	0.4075	1
control T0 - S+L T2	1	0.8363	0.0507	0.9962	1	0.9999	0.5519	0.1732	0.9292	0.9651	0.213	< 0.0001
control T0 - litter T2	< 0.0001	< 0.0001	1	0.0023	0.1052	0.0638	0.998	0.9892	0.9327	0.0637	0.001	0.9997
control T0 - shading T2	1	0.5886	0.0475	1	1	1	0.8625	0.7444	0.9641	0.5468	0.0153	< 0.0001
control T0 - control T3	0.0051	0.0026	1	0.349	0.5025	0.5768	0.3815	0.5975	0.9927	0.9998	0.5509	1
control T0 - S+L T3	0.2733	0.0102	< 0.0001	0.9962	0.9997	0.9991	0.0734	0.0172	0.4109	0.0637	0.0022	< 0.0001

	Total			Shrubs			Subshrubs			Herbaceous species		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
control T0 - litter T3	< 0.0001	0.0041	1	0.0002	0.0194	0.075	0.987	0.994	1	0.9651	0.886	0.7605
control T0 - shading T3	0.5794	0.0582	< 0.0001	1	1	0.8471	0.9487	0.9233	0.9936	0.0094	0.0004	< 0.0001
S+L T0 - litter T0	1	1	1	1	1	0.6125	1	1	0.9996	1	1	0.9529
S+L T0 - shading T0	0.9363	1	1	0.9962	1	1	1	1	1	0.9998	1	0.9974
S+L T0 - control T1	1	0.9997	1	1	0.9856	0.9815	1	1	0.7479	1	0.6	0.9998
S+L T0 - S+L T1	1	1	1	1	1	0.9984	1	1	1	1	1	0.8407
S+L T0 - litter T1	0.9998	0.1927	1	1	1	1	1	1	0.9836	0.998	0.0786	0.999
S+L T0 - shading T1	0.9993	1	1	1	1	1	0.9999	1	1	1	0.8209	0.3989
S+L T0 - control T2	0.9998	0.0035	1	1	0.8078	0.892	0.998	0.8525	0.1154	1	0.7823	0.9732
S+L T0 - S+L T2	0.5126	0.1927	0.2573	1	1	1	0.1381	0.0468	0.7275	0.4034	0.0538	0.0013
S+L T0 - litter T2	0.0935	0.0006	1	0.2653	0.6863	0.8537	1	0.9999	0.9939	0.5468	0.0076	0.8985
S+L T0 - shading T2	0.0729	0.0739	0.2457	0.9998	1	0.9882	0.3815	0.3961	0.8139	0.0637	0.0022	< 0.0001
S+L T0 - control T3	0.975	0.0739	1	0.999	0.9856	0.9999	0.8625	0.8942	0.9999	1	0.886	1
S+L T0 - S+L T3	< 0.0001	0.0002	< 0.0001	0.2653	0.8078	0.4159	0.0073	0.003	0.1838	0.0021	0.0003	< 0.0001
S+L T0 - litter T3	0.1844	0.1039	1	0.0659	0.2921	0.8803	1	1	1	1	0.9939	0.9962
S+L T0 - shading T3	0.0002	0.0019	< 0.0001	0.8972	0.9965	0.0615	0.5519	0.655	0.931	0.0002	< 0.0001	< 0.0001
litter T0 - shading T0	0.9998	1	1	1	1	0.7706	0.9999	0.9993	0.9978	0.9898	1	1
litter T0 - control T1	1	1	1	1	0.8078	0.0185	1	0.9997	0.1249	1	0.8556	1
litter T0 - S+L T1	1	0.9996	1	1	0.9994	0.0495	1	1	1	0.9998	0.998	0.0334

	Total			**Shrubs**			**Subshrubs**			**Herbaceous species**		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
litter T0 - litter T1	0.9363	0.0515	1	0.999	0.9942	0.6117	1	1	0.47	1	0.213	1
litter T0 - shading T1	1	1	1	1	0.9965	0.1232	1	1	1	0.998	0.5509	0.0036
litter T0 - control T2	0.9363	0.0005	1	1	0.4126	0.0053	0.8625	0.3303	0.0043	1	0.9518	1
litter T0 - S+L T2	0.9363	0.4962	0.0507	1	1	0.918	0.5519	0.3201	0.9991	0.1803	0.0153	< 0.0001
litter T0 - litter T2	0.0098	0.0001	1	0.0973	0.2921	0.0039	0.998	0.9432	0.5723	0.8183	0.0293	1
litter T0 - shading T2	0.3847	0.2535	0.0475	1	1	0.9999	0.8625	0.8978	0.9998	0.0185	0.0004	< 0.0001
litter T0 - control T3	0.6456	0.0157	1	0.9731	0.8078	0.1092	0.3815	0.3901	0.8259	1	0.9844	0.9531
litter T0 - S+L T3	0.0006	0.0016	< 0.0001	0.5468	0.9856	1	0.0734	0.043	0.8412	0.0004	< 0.0001	< 0.0001
litter T0 - litter T3	0.0244	0.0237	1	0.0178	0.0748	0.0048	0.987	0.9617	1	1	0.9999	0.2111
litter T0 - shading T3	0.0037	0.0118	< 0.0001	0.9889	1	0.9992	0.9487	0.9832	1	< 0.0001	< 0.0001	< 0.0001
shading T0 - control T1	0.9983	1	1	1	0.9569	0.9367	1	1	0.8486	0.9998	0.3631	1
shading T0 - S+L T1	0.975	1	1	1	1	0.9894	1	0.9999	1	1	1	0.1144
shading T0 - litter T1	0.3264	0.1159	1	0.8312	0.9999	1	1	1	0.9953	0.6918	0.0293	1
shading T0 - shading T1	1	1	1	0.999	0.9999	0.9994	0.998	1	1	1	0.9518	0.0165
shading T0 - control T2	0.3264	0.0016	1	0.9889	0.6863	0.7725	0.9999	0.9618	0.1769	0.9998	0.5509	1
shading T0 - S+L T2	1	0.3002	0.1504	0.9998	1	1	0.0734	0.0171	0.6045	0.9651	0.133	< 0.0001
shading T0 - litter T2	0.0002	0.0002	1	0.0067	0.5488	0.7175	1	1	0.9986	0.0637	0.0022	1
shading T0 - shading T2	0.9589	0.1289	0.1425	1	1	0.9981	0.2398	0.2118	0.7044	0.5468	0.0076	< 0.0001
shading T0 - control T3	0.0935	0.0401	1	0.5468	0.9569	0.999	0.9487	0.9772	1	0.9998	0.6954	0.9974

	Total			Shrubs			Subshrubs			Herbaceous species		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
shading T0 - S+L T3	0.0244	0.0005	< 0.0001	0.9731	0.8995	0.5823	0.003	0.0009	0.1204	0.0637	0.001	< 0.0001
shading T0 - litter T3	0.0006	0.0582	1	0.0008	0.1954	0.7551	1	1	1	0.9651	0.9518	0.4739
shading T0 - shading T3	0.0935	0.0041	< 0.0001	1	0.9994	0.1151	0.3815	0.4237	0.8632	0.0094	0.0002	< 0.0001
control T1 - S+L T1	1	0.9738	1	1	0.9999	1	1	1	0.3048	1	0.133	0.2149
control T1 - litter T1	0.975	0.0102	1	0.9731	1	0.9816	1	1	1	0.998	0.9998	1
control T1 - shading T1	1	1	1	1	1	1	0.9999	1	0.2955	1	0.0029	0.0385
control T1 - control T2	0.975	0.0001	1	0.9998	1	1	0.998	0.9417	0.9995	1	1	1
control T1 - S+L T2	0.868	0.8363	0.0507	1	0.8421	0.8061	0.1381	0.0226	0.0036	0.4034	< 0.0001	< 0.0001
control T1 - litter T2	0.0182	< 0.0001	1	0.0282	1	1	1	1	1	0.5468	0.934	1
control T1 - shading T2	0.2733	0.5886	0.0475	1	0.8995	0.2204	0.3815	0.2544	0.006	0.0637	< 0.0001	< 0.0001
control T1 - control T3	0.7686	0.0026	1	0.8312	1	1	0.8625	0.9632	0.9985	1	1	0.9998
control T1 - S+L T3	0.0003	0.0102	< 0.0001	0.8312	0.0521	0.0073	0.0073	0.0012	0.0001	0.0021	< 0.0001	< 0.0001
control T1 - litter T3	0.043	0.0041	1	0.004	0.9942	1	1	1	0.5929	1	0.9998	0.666
control T1 - shading T3	0.0018	0.0582	< 0.0001	0.9998	0.3299	0.0003	0.5519	0.4844	0.0154	0.0002	< 0.0001	< 0.0001
S+L T1 - litter T1	0.9983	0.5269	1	0.9962	1	0.9984	1	1	0.7533	0.9106	0.006	0.1477
S+L T1 - shading T1	0.9999	1	1	1	1	1	1	1	1	1	0.998	1
S+L T1 - control T2	0.9983	0.0237	1	1	0.9786	1	0.987	0.4281	0.0173	1	0.2459	0.0461
S+L T1 - S+L T2	0.6456	0.0455	0.3054	1	0.9997	0.9403	0.2398	0.2374	0.9791	0.8183	0.3631	0.4208
S+L T1 - litter T2	0.0563	0.0048	1	0.0659	0.9414	1	1	0.9737	0.8353	0.1803	0.0003	0.0191

	Total			**Shrubs**			**Subshrubs**			**Herbaceous species**		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
S+L T1 - shading T2	0.1186	0.0136	0.2924	1	0.9999	0.4057	0.5519	0.8284	0.9918	0.2783	0.0361	0.0599
S+L T1 - control T3	0.9363	0.2763	1	0.9439	0.9999	1	0.7234	0.4941	0.967	1	0.3631	0.8404
S+L T1 - S+L T3	0.0001	< 0.0001	< 0.0001	0.6503	0.4569	0.0213	0.0168	0.0272	0.5806	0.0185	0.006	0.0003
S+L T1 - litter T3	0.1186	0.3517	1	0.011	0.6414	1	0.9999	0.9837	1	0.998	0.7403	1
S+L T1 - shading T3	0.0004	0.0002	< 0.0001	0.9962	0.9223	0.001	0.7234	0.9606	0.9992	0.0021	0.0013	0.0001
litter T1 - shading T1	0.7686	0.1431	1	1	1	1	1	1	0.743	0.8183	< 0.0001	0.0231
litter T1 - control T2	1	0.9953	1	1	0.9965	0.8925	0.987	0.8835	0.9392	0.998	0.9964	1
litter T1 - S+L T2	0.0563	< 0.0001	0.0507	0.9998	0.9965	1	0.2398	0.0382	0.0332	0.0185	< 0.0001	< 0.0001
litter T1 - litter T2	0.6456	0.9252	1	0.7473	0.9856	0.8542	1	1	1	0.998	1	1
litter T1 - shading T2	0.0026	< 0.0001	0.0475	0.9439	0.9989	0.9881	0.5519	0.352	0.0507	0.001	< 0.0001	< 0.0001
litter T1 - control T3	1	1	1	1	1	0.9999	0.7234	0.9192	1	0.998	0.9844	0.999
litter T1 - S+L T3	< 0.0001	< 0.0001	< 0.0001	0.0436	0.2921	0.4152	0.0168	0.0023	0.0017	< 0.0001	< 0.0001	< 0.0001
litter T1 - litter T3	0.8219	1	1	0.349	0.8078	0.8808	0.9999	1	0.9445	1	0.8209	0.5482
litter T1 - shading T3	< 0.0001	< 0.0001	< 0.0001	0.4443	0.8078	0.0613	0.7234	0.6064	0.1073	< 0.0001	< 0.0001	< 0.0001
shading T1 - control T2	0.7686	0.0022	1	1	0.9942	0.9997	0.7234	0.7334	0.0165	1	0.0076	0.0053
shading T1 - S+L T2	0.9924	0.2535	0.1272	1	0.998	0.9914	0.7234	0.0839	0.981	0.9106	0.9844	0.8567
shading T1 - litter T2	0.0026	0.0003	1	0.1955	0.9786	0.9993	0.987	0.999	0.8269	0.1101	< 0.0001	0.0018
shading T1 - shading T2	0.6456	0.1039	0.1203	1	0.9994	0.6448	0.9487	0.5417	0.9927	0.4034	0.5509	0.2933
shading T1 - control T3	0.3847	0.0515	1	0.9962	1	1	0.2398	0.7916	0.9641	1	0.0153	0.3985

	Total			**Shrubs**			**Subshrubs**			**Herbaceous species**		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
shading T1 - S+L T3	0.0026	0.0003	< 0.0001	0.349	0.3299	0.059	0.1381	0.0064	0.5923	0.0351	0.213	0.0036
shading T1 - litter T3	0.0071	0.0739	1	0.0436	0.77	0.9996	0.9487	0.9996	1	0.9898	0.0786	0.9937
shading T1 - shading T3	0.0134	0.003	< 0.0001	0.9439	0.8421	0.0037	0.987	0.7904	0.9993	0.0045	0.0786	0.0018
control T2 - S+L T2	0.0563	< 0.0001	0.0507	1	0.4569	0.5619	0.003	< 0.0001	< 0.0001	0.4034	< 0.0001	< 0.0001
control T2 - litter T2	0.6456	1	1	0.349	1	1	1	0.9997	0.8909	0.5468	0.8209	1
control T2 - shading T2	0.0026	< 0.0001	0.0475	0.999	0.5488	0.0917	0.0168	0.0012	0.0001	0.0637	< 0.0001	< 0.0001
control T2 - control T3	1	0.9999	1	0.9998	1	0.9999	1	1	0.6708	1	1	0.9733
control T2 - S+L T3	< 0.0001	< 0.0001	< 0.0001	0.1955	0.0082	0.0019	0.0001	< 0.0001	< 0.0001	0.0021	< 0.0001	< 0.0001
control T2 - litter T3	0.8219	0.9996	1	0.0973	1	1	1	0.9993	0.0632	1	1	0.2645
control T2 - shading T3	< 0.0001	< 0.0001	< 0.0001	0.8312	0.0889	0.0001	0.0363	0.0048	0.0003	0.0002	< 0.0001	< 0.0001
S+L T2 - litter T2	< 0.0001	< 0.0001	0.0507	0.1399	0.3299	0.4997	0.0363	0.0019	0.0507	0.0002	< 0.0001	< 0.0001
S+L T2 - shading T2	0.9999	1	1	1	1	0.9999	1	0.9999	1	1	0.9999	1
S+L T2 - control T3	0.0098	< 0.0001	0.0507	0.9889	0.8421	0.988	0.0002	< 0.0001	0.142	0.4034	< 0.0001	0.0013
S+L T2 - S+L T3	0.1844	0.8135	0.0162	0.4443	0.9786	0.7893	0.9999	1	1	0.9106	0.9844	0.5926
S+L T2 - litter T3	< 0.0001	< 0.0001	0.0507	0.0282	0.0889	0.5416	0.0168	0.0026	0.8565	0.1101	0.0004	0.0939
S+L T2 - shading T3	0.4472	0.9843	< 0.0001	0.9731	1	0.235	1	0.9967	1	0.5468	0.886	0.4622
litter T2 - shading T2	< 0.0001	< 0.0001	0.0475	0.0178	0.4126	0.0727	0.1381	0.0435	0.0757	< 0.0001	< 0.0001	< 0.0001
litter T2 - control T3	0.9363	0.9911	1	0.9439	1	0.9996	0.987	0.9999	1	0.5468	0.6954	0.8987
litter T2 - S+L T3	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0041	0.0014	0.0012	0.0001	0.0029	< 0.0001	< 0.0001	< 0.0001

	Total			**Shrubs**			**Subshrubs**			**Herbaceous species**		
	Richness	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**	**Richness**	**Density**	**Cover**
litter T2 - litter T3	1	0.9796	1	1	1	1	1	1	0.9732	0.9106	0.3211	0.1403
litter T2 - shading T3	< 0.0001	< 0.0001	< 0.0001	0.0008	0.0521	< 0.0001	0.2398	0.1185	0.1526	< 0.0001	< 0.0001	< 0.0001
shading T2 - control T3	0.0003	< 0.0001	0.0475	0.7473	0.8995	0.6106	0.0012	0.0017	0.1976	0.0637	< 0.0001	< 0.0001
shading T2 - S+L T3	0.7686	0.9587	0.0175	0.8972	0.9569	0.9986	0.987	0.9423	0.9998	0.9998	1	0.9814
shading T2 - litter T3	< 0.0001	< 0.0001	0.0475	0.0023	0.1239	0.0852	0.0734	0.055	0.9153	0.0094	< 0.0001	0.0064
shading T2 - shading T3	0.9589	0.9993	< 0.0001	1	0.9999	0.8228	1	1	1	0.9651	0.9999	0.9503
control T3 - S+L T3	< 0.0001	< 0.0001	< 0.0001	0.011	0.0521	0.0514	< 0.0001	< 0.0001	0.0114	0.0021	< 0.0001	< 0.0001
control T3 - litter T3	0.9857	1	1	0.6503	0.9942	0.9998	0.998	0.9997	0.9984	1	1	0.9961
control T3 - shading T3	< 0.0001	< 0.0001	< 0.0001	0.1955	0.3299	0.0031	0.003	0.0067	0.3434	0.0002	< 0.0001	< 0.0001
S+L T3 - litter T3	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0004	0.0017	0.0005	0.0001	0.295	0.0002	< 0.0001	< 0.0001
S+L T3 - shading T3	1	1	0.2586	0.9998	1	1	0.9487	0.7843	0.9968	1	1	1
litter T3 - shading T3	< 0.0001	< 0.0001	< 0.0001	0.0001	0.0082	0.0001	0.1381	0.1445	0.978	< 0.0001	< 0.0001	< 0.0001

Supplementary Table 3 – Post-hoc contrasts significance values (*P*-values) for time effect (T0, T1, T2 and T3) of Cerrado climber community. T0: before treatments implantation; T1: six months; T2: a year and a half; T3: two years and a half under treatments effect.

	Climbers		
	Richness	**Density**	**Cover**
T0 - T1	0.05	0.0779	0.131
T0 - T2	0.0215	0.0214	0.0331
T0 - T3	0.4664	0.1356	0.9808
T1 - T2	0.9894	0.9603	0.9416
T1 - T3	0.6625	0.9947	0.2741
T2 - T3	0.4664	0.8802	0.0868

CAPÍTULO 2

Aclimatação de espécies herbáceo-arbustivas de cerrado *sensu stricto* à redução da luminosidade e ao aumento da serapilheira impostos pelo adensamento

CAPÍTULO 2

Aclimatação de espécies herbáceo-arbustivas de cerrado *sensu stricto* à redução da luminosidade e ao aumento da serapilheira impostos pelo adensamento

Resumo As savanas têm apresentado uma tendência ao adensamento que, além de alterar a estrutura da vegetação em direção a um estágio florestal, vem causando uma série de modificações microambientais. Entre as modificações que se impõem nas áreas adensadas, podemos destacar a diminuição da luminosidade e o aumento da deposição de serapilheira. Assim, espécies típicas de savana precisam lidar com as novas condições ambientais geradas pelo adensamento, sendo que sua persistência irá depender da capacidade de ajuste a esses fatores. Isto posto, o presente estudo objetivou avaliar o efeito da diminuição luminosa e do aumento da camada de serapilheira sobre a fisiologia foliar de espécies de cerrado *sensu stricto* (CSS). Para tanto, foi feito um experimento em CSS simulando condições de sombreamento e de serapilheira encontradas em áreas adensadas; a partir disso foram medidos parâmetros fisiológicos de espécies representativas, a saber: teores de pigmentos foliares, área foliar específica, fluorescência da clorofila *a* e trocas gasosas. Como principais resultados temos que a serapilheira não exerceu efeito significativo nos parâmetros avaliados, colocando a diminuição luminosa como principal recurso limitante, pelo menos em um curto prazo, para as espécies de CSS. Sob o sombreamento, as espécies avaliadas apresentaram ajustes relacionados à melhora na captura luminosa, como o maior investimento nos teores de pigmentos fotossintetizantes e maiores valores de área foliar específica. Entretanto, elas não foram capazes de manter o seu desempenho fotossintético, tanto em termos fotoquímicos como na assimilação de carbono. Assim, os resultados levam a crer que a aclimatação a menores níveis de luz apresentada pelas espécies de CSS não é capaz de garantir sua sobrevivência em longo prazo em condição de adensamento.

Palavras-chave: atributos funcionais, fluorescência da clorofila *a*, fotossíntese, savana, tolerância ao sombreamento

Introdução

A plasticidade fenotípica refere-se à capacidade de um organismo – ou, mais precisamente de um mesmo genótipo - apresentar mudanças em suas características fenotípicas em resposta às variações ambientais (BRADSHAW, 1965). Essa capacidade plástica é essencial para as espécies vegetais que, por serem organismos sésseis, precisam de mecanismos para lidar com as variações ambientais (SULTAN, 2000; VALLADARES; NIINEMETS, 2008). As plantas estão constantemente expostas a mudanças ambientais. No caso das mudanças globais, o aumento do CO_2 atmosférico, mudanças no uso da terra e alterações nos regimes de distúrbio têm causado mudanças estruturais nas vegetações e alterado o funcionamento das espécies e dos ecossistemas ao redor do mundo (CRAMER et al., 2001; FRANCO et al., 2014; GARCÍA-CRIADO et al., 2020). No caso das savanas, o adensamento da vegetação devido ao aumento na densidade e na cobertura de indivíduos arbóreos, tem alterado as características ambientais e, consequentemente, causado prejuízos às comunidades de espécies típicas de ambientes abertos, bem como ao funcionamento do ecossistema (ABREU et al. 2017; HOFFMANN et al., 2012; NEWBERRY et al, 2020).

O aumento da cobertura do dossel e a maior deposição de serapilheira são importantes fatores que são alterados no processo de adensamento das savanas. Estas mudanças ambientais podem estar filtrando as espécies típicas de savana em ambientes adensados, principalmente aquelas do estrato herbáceo-arbustivo (ABREU et al., 2017; PILON et al., 2020; PINHEIRO; KOLB; ROSSATTO, 2016). Nesse sentido, a persistência dessas espécies irá depender da sua capacidade de ajustar-se às novas condições ambientais impostas pelo adensamento. Contudo, alguns estudos apontam para um padrão mais conservativo em relação aos atributos morfofuncionais foliares em espécies oriundas de ambientes com luz abundante e com solos pobres nutricionalmente (GOULART et al., 2011; POWER et al., 2019; VALLADARES et al., 2000a), o que é o caso das fisionomias savânicas do Cerrado, especialmente o cerrado *sensu stricto* (EITEN, 1972; RIBEIRO; WALTER, 2008).

Geralmente, as espécies herbáceo-arbustivas de cerrado *sensu stricto* apresentam atributos funcionais foliares típicos de espécies heliófitas, com maiores taxas de transpiração, condutância estomática e assimilação de carbono, maiores teores de clorofilas e carotenoides, e menores valores de área foliar específica quando comparadas com espécies típicas de ambientes florestados (CARLOS; ROSSATTO, 2018; PINHEIRO; KOLB; ROSSATTO, 2018; ROSSATTO et al., 2018). Mesmo assim, plantas do estrato rasteiro podem apresentar algum grau de plasticidade fenotípica nos atributos morfofuncionais frente à diminuição da luminosidade imposta pelo adensamento, contribuindo para a permanência destas espécies, ao menos em um curto prazo (MARRONI, 2019; PINHEIRO; KOLB; ROSSATTO, 2018). Adicionalmente, algumas espécies arbóreas apresentam plasticidade nos atributos morfofuncionais foliares que as permitem prosperar tanto em ambientes de floresta como em ambientes de savana (BARROS et al., 2012; MENDONÇA et al., 2020; ROSSATTO; KOLB, 2012). Assim, com o sombreamento, pode ocorrer a produção de novas folhas, com alterações morfológicas e funcionais para lidar com a menor disponibilidade de luz (BEDETTI et al., 2011; MARRONI, 2019).

A maior deposição de serapilheira em ambiente de Cerrado causa uma série de alterações no microambiente do solo. A serapilheira atua interceptando a luz solar e reduzindo as flutuações de temperatura no solo, bem como reduzindo a evaporação e aumentando a disponibilidade hídrica (ROSSATTO; RIGOBELO, 2016; VILLALOBOS-VEGA et al., 2011). Além disso, a serapilheira é um importante reservatório de matéria orgânica, que pode em médio prazo aumentar a disponibilidade nutricional dos solos (ROSSATTO; RIGOBELO, 2016). Assim, o aumento na deposição de serapilheira causado pelo adensamento oferece novas condições, com as quais as espécies herbáceo-arbustivas têm de lidar. Em um experimento com manipulação de serapilheira verificou-se alterações nos atributos foliares relacionados ao balanço hídrico em espécies lenhosas de cerrado *sensu stricto*, porém não chegando a afetar drasticamente as respostas fotossintéticas destas espécies (SCALON; ROSSATTO; FRANCO, 2014).

O presente estudo objetivou compreender as respostas de espécies herbáceo-arbustivas de cerrado *sensu stricto* frente às alterações de luminosidade e de serapilheira causadas pelo adensamento lenhoso. Para tanto, nós reproduzimos experimentalmente condições de sombreamento e de quantidade de serapilheira semelhantes à de áreas adensadas, e avaliamos ao longo de três anos as respostas funcionais de espécies herbáceo-arbustivas típicas do ambiente de cerrado *sensu stricto*. Os teores de pigmentos (clorofilas e carotenoides), a área foliar específica, e a fluorescência da clorofila *a* foram monitorados anualmente ao longo do experimento. Adicionalmente, foram avaliadas as trocas gasosas no final do experimento. Nós esperamos que as espécies herbáceo-arbustivas apresentem certo grau de aclimatação nos seus atributos foliares, principalmente aqueles relacionados a uma maior tolerância à sombra. Especificamente, esperamos encontrar maiores valores de área foliar específica e aumentos nos teores de clorofilas *a* e *b* para as espécies expostas ao sombreamento. Entretanto, mesmo que as espécies apresentem tais ajustes, esperamos uma diminuição da eficiência fotossintética tanto da etapa fotoquímica quanto da assimilação de carbono nestas espécies, de modo a não permitir sua permanência em condições sombreadas.

Métodos

Área de estudo

Este estudo foi realizado na Estação Ecológica de Assis (EEA), situada a 12 km da cidade de Assis, região sudoeste do Estado de São Paulo (22º33’20’’S e 50º21’27’’W). A vegetação da EEA apresenta várias fisionomias de Cerrado, porém nos últimos 50 anos as fisionomias abertas vêm sofrendo processo de adensamento, sendo que atualmente restam poucos fragmentos de cerrado *sensu stricto*, nos quais o experimento de campo foi realizado (PINHEIRO; DURIGAN, 2009). O clima nesta área é classificado como Cfa de acordo com a classificação de Köppen (ÁLVARES et al., 2013). A média de precipitação anual é cerca de 1.400 mm, com estação seca entre maio e setembro. Os solos são geralmente Latossolos arenosos, ácidos e nutricionalmente pobres (JUHÁSZ et al., 2006).

Experimento de campo

Para simular os efeitos do adensamento lenhoso sobre a comunidade herbáceo-arbustiva, foi realizado um experimento de campo em fragmentos de cerrado *sensu stricto* na EEA. Esse experimento consistiu de quatro tratamentos: sombreamento (S), adição de serapilheira (L), sombreamento e adição de serapilheira (S+L), e um controle, sem intervenção (C). Os tratamentos consistiram na manipulação da intensidade de luz e na quantidade de serapilheira, com o intuito de atingir valores semelhantes aos encontrados em áreas adensadas, a saber: 40-50 µmol $m^{-2}.s^{-1}$ de radiação fotossinteticamente ativa e 4 cm de espessura de serapilheira. Maiores detalhes sobre o experimento de campo foram apresentados no Capítulo 1.

Seleção de espécies

No início do experimento foram selecionadas algumas espécies com maior abundância na área de estudo para a análise de atributos fisiológicos. Estas espécies foram avaliadas preliminarmente para teores de pigmentos foliares e área foliar específica (T0, fevereiro de 2017). Elas foram acompanhadas de acordo com a evolução do experimento, sendo que 11 delas persistiram sob o efeito dos tratamentos (Tabela 1), propiciando a continuidade da realização das análises fisiológicas até o final do experimento.

Assim, o teor de pigmentos foliares e a área foliar específica foram avaliados em mais três ocasiões ao longo do experimento. Além disso, atributos de fluorescência da clorofila *a* também foram avaliados nestas ocasiões, a saber: com seis meses (T1), um ano e meio (T2) e dois anos e meio (T3) após a aplicação dos tratamentos. Todas as avaliações foram feitas durante a estação chuvosa, sempre em fevereiro. Os atributos foliares foram avaliados em um mínimo de três indivíduos e, preferencialmente, cinco indivíduos por tratamento. Esse número amostral menor, de três indivíduos, foi admitido em razão do desaparecimento de muitos indivíduos sob o efeito dos tratamentos, sendo que nem sempre foi possível a realização das medidas de fisiologia nos mesmos indivíduos ao longo dos anos, devido às mudanças na comunidade herbáceo-arbustiva. Em todas as análises, foram selecionadas folhas completamente expandidas do 3º ou 4º nó, sem sinais de doenças.

No final do experimento, já no fim da estação chuvosa (março de 2020), as trocas gasosas foram analisadas em nove espécies, escolhidas dentre as 11 mencionadas anteriormente, já que duas espécies (*Galactia decumbens* (Benth.) Chodat & Hassl. e *Moquiniastrum barrosoae* (Cabrera) G. Sancho) não foram encontradas em número suficiente (mínimo de três indivíduos) nas parcelas com tratamentos de sombra. As trocas gasosas foram avaliadas em no mínimo três indivíduos e, preferencialmente, cinco indivíduos, a partir de folhas do 3º ou 4º nó, completamente expandidas e sem sinais de doenças.

Tabela 1 – Espécies selecionadas para avaliação de atributos foliares no experimento simulando os efeitos do adensamento sobre a comunidade herbáceo-arbustivo de cerrado *sensu stricto* (n = 3-5). Forma de crescimento de acordo com Durigan et al. (2018) e Zappi et al. (2015).

Espécie	**Família**	**Forma de crescimento**
Andira humilis Mart. ex Benth.	Fabaceae	subarbusto
Banisteriopsis stellaris (Griseb.) B.Gates	Malpighiaceae	subarbusto
Byrsonima intermedia A.Juss.	Malpighiaceae	arbusto
Campomanesia adamantium (Cambess.) O.Berg	Mytaceae	arbusto
Chromolaena oxylepis (DC.) R.M.King & H.Rob.	Asteraceae	arbusto
Galactia decumbens (Benth.) Chodat & Hassl.	Fabaceae	subarbusto
Mascagnia cordifolia (A.Juss.) Griseb.	Malpighiaceae	trepadeira
Miconia fallax DC.	Melastomataceae	arbusto
Moquiniastrum barrosoae (Cabrera) G. Sancho	Asteraceae	arbusto
Rhynchospora exaltata Kunth	Cyperaceae	graminoide
Serjania lethalis A.St.-Hil.	Sapindaceae	trepadeira

Teor de pigmentos foliares e área foliar específica

Para mensuração do teor de pigmentos foliares (clorofilas *a* e *b* e carotenoides) foram obtidos discos foliares de $1cm^2$, os quais foram imediatamente colocados em tubos do tipo eppendorf na cor âmbar contendo 2 ml de dimetilformamida (PORRA; THOMPSON; KRIEDEMANN, 1989). Após a coleta, o material foi colocado em refrigerador (±10ºC, no escuro) para extração dos pigmentos foliares por 48 horas (WELLBURN, 1994). Os teores de clorofila e carotenoides foram determinados de acordo com as equações propostas por WELLBURN (1994) após a leitura a 667, 647 e 480 nm em espectrofotômetro Quimis, modelo Q898-DRMS.

Nas mesmas folhas em que foram obtidos os discos para extração dos pigmentos, outro disco foi retirado para o cálculo da área foliar específica (AFE, cm^2g^{-1}), que é calculada através da razão entre a área do disco e a massa seca foliar. A massa seca foi obtida após a secagem das folhas em estufa a 70°C por 3 dias. A massa seca foliar também foi considerada para a determinação dos valores dos teores de pigmentos foliares em base de massa ($\mu g.mg^{-1}$).

Fluorescência da clorofila a

Para as análises dos parâmetros fotoquímicos, foram feitas curvas rápidas de luz (WHITE; CRITCHLEY, 1999), através de um programa armazenado na memória de um equipamento MINI-PAM-II, Walz, Effeltrich, Germany (Software WinControl 3.0). As medidas foram tomadas entre 8 e 12 horas da manhã. As folhas foram aclimatadas ao escuro por um período mínimo de 30 minutos, utilizando os clipes de alumínio fornecidos juntamente com o MINI-PAM-II. Foram utilizados 12 níveis de intensidade luminosa (0 – 1500 $\mu mol\ m^{-2}\ s^{-1}$) para a realização da curva. A partir da curva foram obtidos os parâmetros: α = eficiência quântica da fotossíntese, ETR_{max} = máxima taxa de transporte eletrônico e Ik = irradiância mínima para saturação (JASSBY; PLATT, 1976). A seguinte função foi utilizada para determinar α e ETR_{max}:

$$ETR = ETR_m \ . \ \tanh(\alpha \ . \ PPFD / ETR_m)$$

Onde ETR = taxa de transporte de elétrons, ETR_m = taxa de transporte de elétrons potencial máxima em saturação de luz, e PPFD: densidade de fluxo de fótons fotossintéticos. α e ETR_{max} foram calculados por procedimento de ajuste (*fitting procedure*) e Ik foi calculado pela razão ETR_m/α.

Trocas gasosas

A assimilação de carbono ($A_{\text{área}}$), transpiração (E) e condutância estomática (g_s) em base de área foliar foram avaliados nas espécies selecionadas com um analisador de trocas gasosas LcProSD (ADC BioScientific Ltd., Hoddesdon, UK). Os dados foram obtidos com concentração de CO_2 ambiente (404-434 ppm). A intensidade luminosa usada foi entre 1300 e 1400 µmol m^{-2} s^{-1}, que é suficiente para saturar o aparato fotossintético da maioria das espécies de savana (ROSSATTO et al., 2018). A eficiência no uso da água (EUA) foi obtida pela razão entre $A_{\text{área}}$ e g_s.

Análise estatística

Os atributos relacionados à florescência da clorofila *a*, teor de pigmentos e AFE foram analisados por meio de modelos lineares mistos (LMM). Os tratamentos e o tempo foram fatores fixos e as espécies consideradas como fator aleatório. Os atributos de trocas gasosas também foram analisados por meio de LMM, considerando os tratamentos como fator fixo e as espécies como fator aleatório. Os modelos foram gerados usando o pacote *nlme* (PINHEIRO et al., 2020). Quando diferenças significativas foram detectadas, foi realizada análise *post-hoc* de contraste por meio da função *emmeans* do pacote *emmeans* (RUSSEL, 2019). Todas as análises foram realizadas no ambiente R (R DEVELOPMENT CORE TEAM, 2020).

Resultados

Teor de pigmentos foliares e área foliar específica

A AFE e todos os parâmetros relacionados aos pigmentos fotossintetizantes foram afetados pelos tratamentos, tempo, e interação entre estes fatores (Tabela 2). Os tratamentos com manipulação de sombreamento (S e S+L) tiveram teores de clorofilas e carotenoides mais elevados em relação aos tratamentos sem intervenção na luminosidade (C e L), já a partir do T1 (Figura 1 A-D). O teor de clorofila *a* foi aproximadamente 47% maior nos tratamentos com adição de sombreamento no T1 em relação ao controle e serapilheira (P = 0,0008; 0,002 e ≤ 0,0001); essa margem sobe para cerca de 90% no T3 ($P < 0{,}0001$; Figura 1A). Para o teor de clorofila *b*, os tratamentos de sombreamento e S+L têm aproximadamente 95% a mais que o controle no T1 (P = 0,036 e 0,0001; respectivamente); já no T2 e T3, o tratamento de serapilheira também se apresenta com mais teor de clorofila *b* em relação aos tratamentos com adição de sombreamento (Figura 1B; $P < 0{,}0001$). Essas alterações nas clorofilas *a* e *b* se refletem no teor total de clorofilas, sendo que no final do experimento os tratamentos com presença de sombreamento têm mais que o dobro de clorofilas em relação ao controle e serapilheira (Figura 1C; *P* < 0,0001). Embora em menor proporção, algumas alterações também puderam ser observadas na razão das clorofilas *a* e *b*, a qual foi maior para o tratamento de serapilheira em relação ao sombreamento e S+L no T2 (*P* = 0,008 e 0,02; respectivamente), e maior para o controle em relação ao sombreamento no T3 (Figura 1D; *P* = 0,03). O teor de carotenoides também se mostrou responsivo ao sombreamento, tendo, já no T1, aumentado cerca de 50% nos tratamentos de sombra e S+L em relação ao controle e serapilheira (*P* = 0,038; 0,0005; 0,027 e 0,004); essa margem sobe para aproximadamente 63% no T3 (Figura 1E; *P* < 0,0001).

A AFE seguiu um padrão semelhante ao dos pigmentos foliares. Os tratamentos de sombra e S+L apresentaram valores médios de AFE cerca de 58% mais elevados em comparação com o controle e a serapilheira no T1 (*P* = 0,007; 0,0005; 0,0013 e 0,0001). Já a partir do T2, os tratamentos com adição de sombreamento apresentaram valores de AFE por volta de duas vezes maiores em relação aos tratamentos não sombreados (*P* < 0,0001; Figura 1F).

Tabela 2 – Resultados para os modelos lineares mistos gerados para área foliar específica, parâmetros relacionados aos teores de pigmentos foliares e à fluorescência da clorofila *a* em espécies da comunidade herbáceo-arbustiva de cerrado *sensu stricto* submetidas à condição controle e a condições simuladas de adensamento lenhoso – sombreamento, adição de serapilheira e sombreamento mais serapilheira (para todos os contrastes *post-hoc* ver Tabela Suplementar 1). AFE: área foliar específica; α: eficiência quântica da fotossíntese; ETR$_{max}$: máxima taxa de transporte eletrônico e Ik: irradiância mínima para saturação.

Atributos		**F**	***P***	**Atributos**		**F**	***P***
Clorofila *a*	Tratamento	61.32	<0.0001	AFE	Tratamento	76.53	<0.0001
	Tempo	20.65	<0.0001		Tempo	45.38	<0.0001
	Interação	8.04	<0.0001		Interação	12.2	<0.0001
Clorofila *b*	Tratamento	58.63	<0.0001	α	Tratamento	30,03	<0,0001
	Tempo	6.36	<0.0001		Tempo	19,81	<0,0001
	Interação	7.11	<0.0001		Interação	2,4	0,03
Clorofilas totais	Tratamento	64.31	<0.0001	ETR$_{max}$	Tratamento	85,19	0,0001
	Tempo	12.32	<0.0001		Tempo	16,86	<0,0001
	Interação	8.16	<0.0001		Interação	1,85	0,095
Razão clorofila *a*:*b*	Tratamento	15.16	<0.0001	Ik	Tratamento	9,6	<0,0001
	Tempo	97.74	<0.0001		Tempo	17,54	<0,0001
	Interação	2.37	0.0157		Interação	0,82	0,56
Carotenoides totais	Tratamento	44.68	<0.0001				
	Tempo	42.67	<0.0001				
	Interação	6.64	<0.0001				

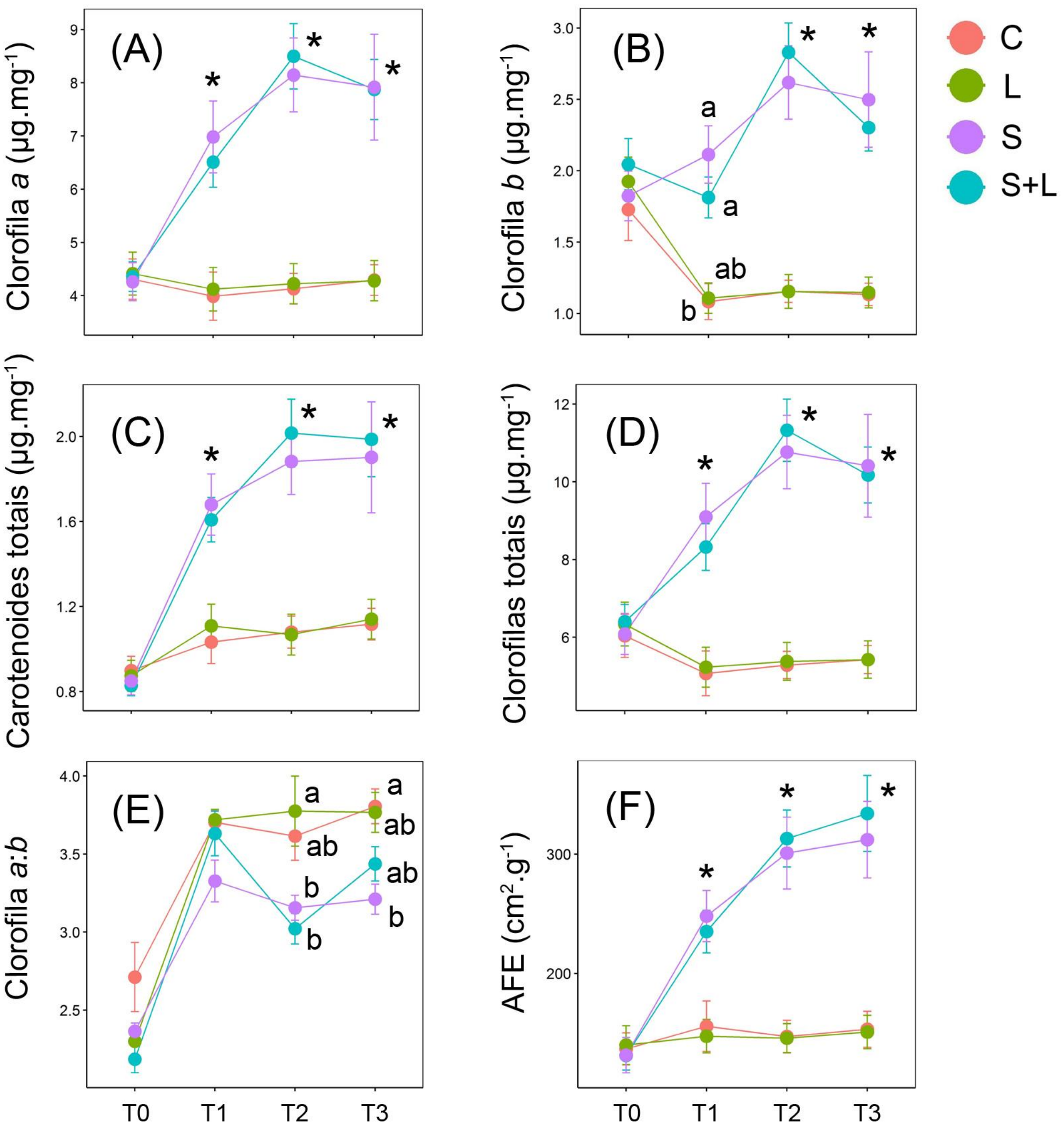

Figura 1 – Variação ao longo do tempo nos atributos relacionados aos teores de clorofila e carotenoides e na área foliar específica (AFE) (média ± erro padrão) em espécies herbáceo-arbustivas de cerrado *sensu stricto* submetidas à condição controle (C) e a condições simuladas de adensamento lenhoso – sombreamento (S), adição de serapilheira (L) e sombreamento mais serapilheira (S+L). T0: antes da implantação dos tratamentos; T1: seis meses; T2: um ano e seis meses; T3: dois anos e seis meses sob efeito dos tratamentos. Letras diferentes indicam diferenças significativas entre tratamentos e asteriscos indicam diferenciação entre tratamentos com e sem adição de sombreamento ($P < 0{,}05$).

Atributos de fluorescência da clorofila a

A eficiência quântica da fotossíntese (α) das espécies herbáceo-arbustivas foi afetada significativamente pelos tratamentos, pelo tempo e pela interação entres estes fatores (Tabela 2). Para todos os contrastes *post-hoc* ver TS2. Logo no T1, o tratamento de sombra (S) reduziu significativamente o valor de α, em torno de 34% em relação aos tratamentos controle, serapilheira e S+L ($P = 0{,}0006$; 0,047 e 0,0088; respectivamente). Já a partir do T2 houve uma completa diferenciação entre os tratamentos sombreados e os que não foram sombreados, sendo que no T2 foi encontrada a maior discrepância - cerca de 44% de redução nos tratamentos de sombra e S+L em relação ao tratamentos controle e serapilheira (Figura 2A) ($P = 0{,}004$; 0,0002; 0,0013 e 0,0001).

Tanto os tratamentos quanto o tempo exerceram influência significativa na taxa máxima de transporte de elétrons e na irradiância mínima para saturação (Tabela 2). No entanto, a interação entre tempo e tratamento não foi significativa, tanto para a ETR_{max} quanto para a Ik (Tabela 2). Considerando apenas o efeito dos tratamentos, tanto a ETR_{max} como a Ik foram mais elevadas nos tratamentos controle e de serapilheira em relação ao sombreamento e S+L (Figura 2B e C) ($P < 0{,}0001$ para ETR_{max}; $P = 0{,}0001$; 0,0009; 0,004 e 0,02 para Ik).

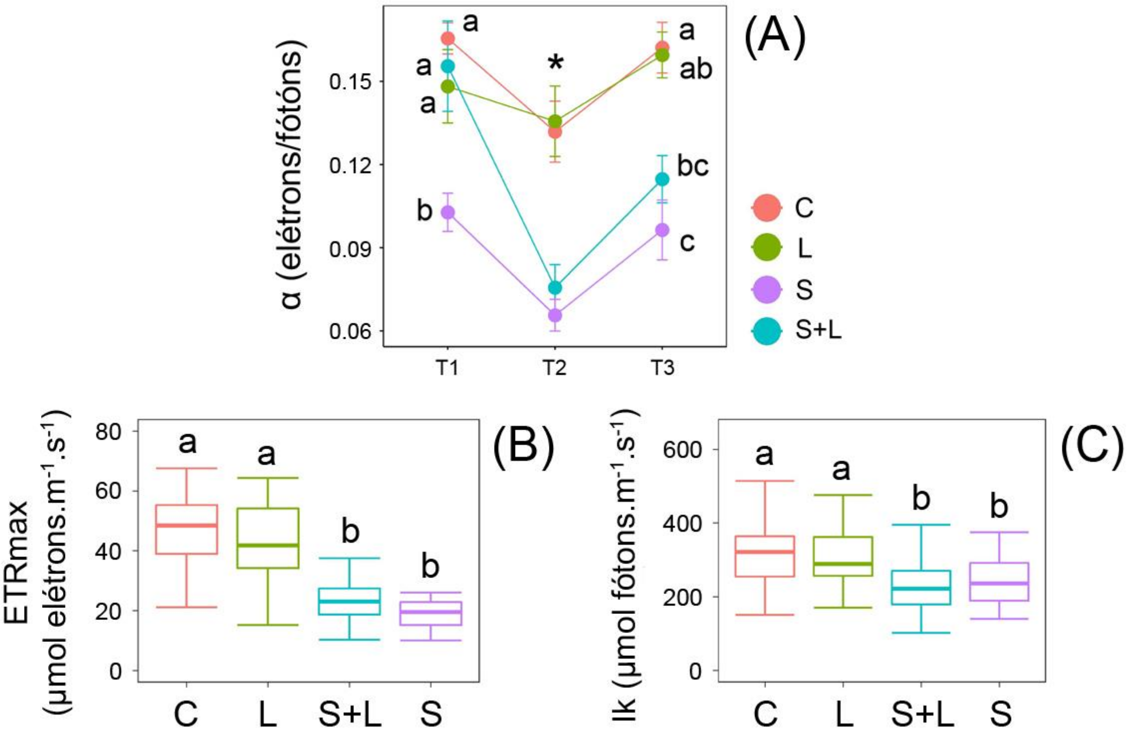

Figura 2 – Variação nos atributos relacionados à etapa fotoquímica da fotossíntese em espécies herbáceo-arbustivas de cerrado *sensu stricto* submetidas a condições controle (C) e a condições simuladas de adensamento lenhoso – sombreamento (S), adição de serapilheira (L) e sombreamento mais serapilheira (S+L). A. eficiência quântica da fotossíntese (α, média ± erro padrão); B. taxa máxima de transporte eletrônico (ETR_{max}); C. irradiância mínima para saturação (Ik). Os diagramas de caixas (*boxplots*) representam a mediana e os quartis. T1: seis meses; T2: um ano e seis meses; T3: dois anos e seis meses sob efeito dos tratamentos. Letras diferentes incidam diferenças significativas entre tratamentos e asteriscos indicam diferenciação entre tratamentos com e sem adição de sombreamento ($P < 0,05$).

Trocas gasosas

Com dois anos e meio de experimento houve efeito significativo dos tratamentos sobre a assimilação de carbono em base de área ($A_{área}$; F = 54,09; $P < 0,0001$), condutância estomática (g_s; F = 15,69; $P < 0,0001$) e transpiração (E; F = 24,04; $P < 0,0001$) das espécies da comunidade herbáceo-arbustiva. Tanto $A_{área}$ quanto a g_s foram negativamente afetadas pelo sombreamento, com redução de cerca de 70% nos tratamentos com sombreamento em relação aos tratamentos controle e serapilheira (Figura 3A e B) ($P < 0,0001$ para $A_{área}$; $P = 0,007$; 0,005 e $\leq 0,0001$ para g_s). Semelhantemente, os valores médios da E apresentaram redução de cerca de 54% nos tratamentos com adição de sombreamento em relação ao controle e serapilheira (Figura 3C; *P* < 0,0001). Por outro lado, a eficiência no uso da água não foi afetada pelos tratamentos (Figura 3D; F = 0,66; *P* = 0,58).

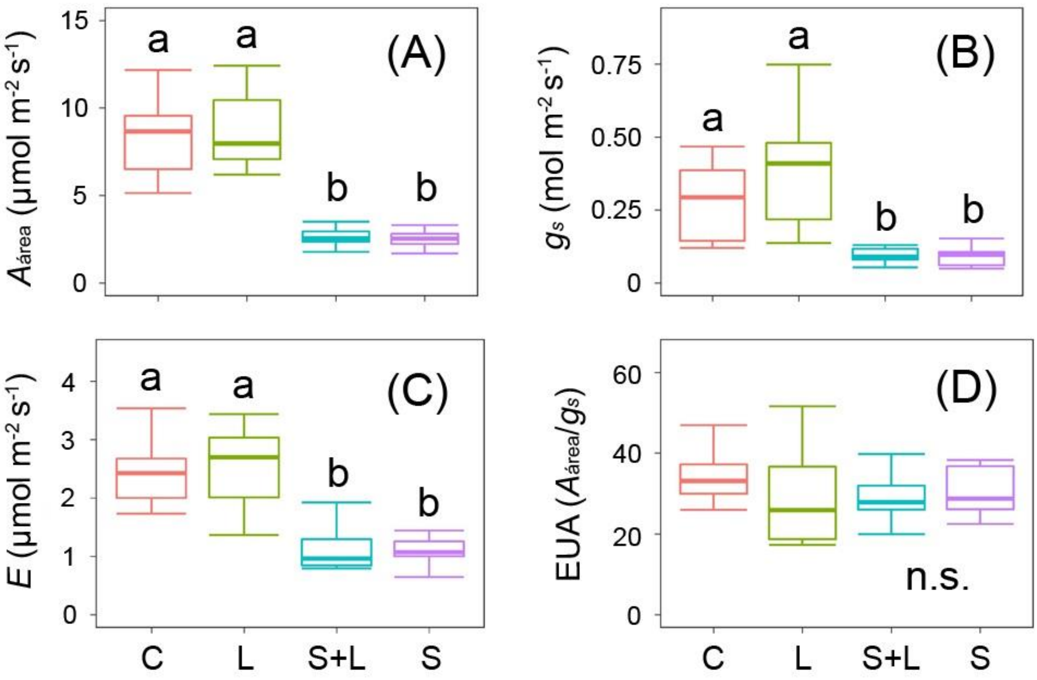

Figura 3 – Atributos de trocas gasosas em espécies da comunidade herbáceo-arbustivas de cerrado *sensu stricto* submetidas a dois anos e meio de condições controle (C) e de condições simuladas de adensamento lenhoso – sombreamento (S), adição de serapilheira (L) e sombreamento mais serapilheira (S+L). A. assimilação de carbono em base de área ($A_{\text{área}}$); B. condutância estomática (g_s); C. transpiração (*E*); D. Eficiência no uso da água (EUA). Os diagramas de caixas (*boxplots*) representam a mediana e os quartis. Letras diferentes incidam diferenças significativas entre tratamentos (*P* < 0.05); n.s.: diferenças não significativas entre os tratamentos (*P* > 0.05).

Discussão

Nós encontramos que espécies típicas do estrato herbáceo-arbustivo de cerrado *sensu stricto* são capazes de aclimatação de suas características foliares frente às condições de sombreamento impostas pelo adensamento lenhoso, alterando sua fisiologia foliar. Essas alterações envolvem o aumento nos teores de clorofila e carotenoides e, em certa medida, redução na razão de clorofila *a* e *b*, além de aumento nos valores de AFE. Essas alterações apontam na direção de maior tolerância ao sombreamento (GRATANI, 2014; VALLADARES; NIINEMETS, 2008). No entanto, esses ajustes não foram suficientes para a manutenção da eficiência quântica da fotossíntese e das taxas de assimilação de carbono.. Por outo lado, o aumento da camada de serapilheira não exerceu influência significativa sobre a fisiologia das plantas herbáceo-arbustivas, nem mesmo para os parâmetros relacionados ao balanço hídrico, diferentemente do que já foi relatado para espécies arbóreas de cerrado *sensu stricto* (SCALON; ROSSATTO; FRANCO, 2014). Estes resultados indicam a luz como principal recurso limitante para a sobrevivência destas plantas em condições de adensamento lenhoso.

A AFE representa a relação entre a área e a massa foliar, sendo que aumentos nos valores deste atributo representam uma tendência ao aumento na área foliar, propiciando maior interceptação luminosa, e à diminuição da espessura foliar, que pode facilitar a captura da luz difusa (McCLENDON; McMILLEN, 1982; GRATANI, 2014; VOGUELMANN, 1993). Altos teores de clorofilas são amplamente reconhecidos como estratégia para melhor aproveitamento luminoso em condições onde a luz é um recurso escasso (VALLADARES; NIINEMETS, 2008). A partição do investimento do nitrogênio foliar entre a produção de pigmentos fotossintetizantes e enzimas da fase bioquímica da fotossíntese (como a Rubisco) representa uma compensação (*trade-off*) entre a possibilidade de altas taxas fotossintéticas em altas quantidades de luz e a diminuição do ponto de compensação luminosa para um melhor desempenho em baixos níveis de luz (HENRY; AARSSEN, 1997; WALTERS; REICH, 1996). De fato, mesmo com os maiores teores de clorofilas, as espécies herbáceo-arbustivas aqui estudadas não foram capazes de realizar altas taxas de assimilação de carbono quando expostas a condições de sombreamento.

Plantas tipicamente de sol geralmente apresentam maiores teores de carotenoides em relação às plantas tolerantes à sombra, como mecanismo para evitar a fotoinibição, através da dissipação da energia excedente (FRANK; COGDELL, 1996; VALLADARES; NIINEMETS, 2008). Esse padrão foi inclusive observado para espécies herbáceo-arbustivas típicas de cerrado *sensu stricto* em comparação com espécies de sub-bosque comumente encontradas em áreas adensadas (ROSSATTO et al., 2018). No entanto, os carotenoides possuem outra função que é inclusive antagônica à proteção contra a fotoinibição, a de servir como pigmento acessório na coleta luminosa (HAVAUX; TARDY; LEMOINE, 1998; VALLADARES et al., 2000a). Nesse sentido, o aumento do teor de carotenoides nos tratamentos com adição de sombreamento aqui observado deve ser uma resposta para um melhor aproveitamento da luz. Estas alterações que visam o melhor aproveitamento da luz nos indivíduos em condições de sombreamento, no entanto, não foram capazes de garantir a manutenção de uma alta capacidade fotoquímica, como a encontrada nas plantas dos tratamentos controle e serapilheira. As reduções na eficiência quântica da fotossíntese, na taxa de transporte de elétrons e na irradiância mínima para saturação para as plantas nos tratamentos de sombreamento e S+L demonstram isso. Isso provavelmente se refletiu na baixa assimilação de carbono encontrada nessas plantas, o que pode comprometer o balanço de carbono nestas plantas.

Todavia, a realização de altas taxas fotossintéticas não é um requisito imprescindível para a tolerância ao sombreamento, uma vez que plantas adaptadas à sombra geralmente apresentam diminuição da respiração, como estratégia para compensar o menor ganho de carbono (GIVNISH, 1988); assim, muitas espécies típicas de sombra possuem menores valores de assimilação de carbono quando comparadas a espécies de sol (VALLADARES et al., 2000b). Porém, a diminuição das taxas de assimilação como resposta a baixos níveis de luz tampouco significa maior tolerância ao sombreamento. Ao contrário, VALLADARES et al. (2000b) submetendo duas espécies de *Quercus* nativas de ecossistema mediterrâneo ao sombreamento encontraram como resposta a manutenção dos valores de assimilação de carbono em base de área e, até mesmo, aumento na assimilação em base de massa, indicando que essas espécies teriam uma boa performance fotossintética mesmo em condições naturais de sombreamento.

Além disso, há que se considerar que espécies de Cerrado geralmente alocam a maior parte de sua biomassa na parte subterrânea, que muitas vezes atua como reserva de nutrientes e carboidratos (DE MORAES et al., 2016; SILVA et al., 2013). Assim, plantas herbáceo-arbustivas típicas de cerrado *sensu stricto* conseguem em curto prazo produzir folhas com características apropriadas para lidar com a menor disponibilidade de luz, mas isso possivelmente não traria uma assimilação de carbono suficiente para a manutenção destes sistemas subterrâneos. Assim, uma hipótese a ser considerada é que tais espécies passariam a utilizar as reservas alocadas nestes órgãos subterrâneos, para garantir a sobrevida e a tentativa de sucessivas rebrotas, levando ao exaurimento destas reservas e, em última análise, a mortalidade dos indivíduos (MARRONI, 2019; PINHEIRO; KOLB; ROSSATTO, 2016).

Em conclusão, a aclimatação ao sombreamento aqui encontrada para as espécies herbáceo-arbustivas de cerrado *sensu stricto* certamente não garante uma ampla tolerância ao sombreamento nestas espécies, haja vista as perdas na riqueza, densidade e cobertura deste componente, observadas ao longo do experimento (Capítulo 1). Para se compreender em detalhes essa questão, mais estudos seriam necessários, avaliando as espécies individualmente e levando em consideração outros parâmetros relevantes para o entendimento da tolerância ao sombreamento, como a respiração no escuro e mensuração dos pontos de compensação luminosa. Contudo, os ajustes fisiológicos aqui observados, em grande parte, podem ser atribuídos a uma resposta de sobrevivência em curto prazo frente à escassez de luz nos ambientes adensados.

Agradecimentos

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Material suplementar

Tabela suplementar 1 – Valores de significância (*P*) dos contrastes *post-hoc* para a interação entre tratamentos (CONT, SERA, SOMB e S+L) e tempo (T0, T1, T2 e T3) para os atributos funcionais de espécies herbáceo-arbustivas de Cerrado. AFE: área foliar específica; T0: antes da implantação dos tratamentos; T1: seis meses; T2: um ano e seis meses; T3: dois anos e seis meses sob efeito dos tratamentos; CONT: controle; SERA: serapilheira; SOMB: sombreamento, S+L: sombreamento mais serapilheira.

	Clorofila *a*	**Clorofila *b***	**Clorofilas totais**	**Razão clorofila *a*:*b***	**Carotenoides totais**	**AFE**
CONT T0 - SERA T0	1	0.9998	1	0.4549	1	1
CONT T0 - S+L T0	1	0.9725	1	0.1005	1	1
CONT T0 - SOMB T0	1	1	1	0.7374	1	1
CONT T0 - CONT T1	1	0.1175	0.9938	<0.0001	0.9998	0.9998
CONT T0 - SERA T1	1	0.1597	1	<0.0001	0.9738	1
CONT T0 - S+L T1	0.0076	1	0.1238	<0.0001	0.0001	0.0001
CONT T0 - SOMB T1	0.0002	0.8727	0.004	0.0196	<0.0001	<0.0001
CONT T0 - CONT T2	1	0.2689	1	<0.0001	0.9938	1
CONT T0 - SERA T2	1	0.2662	1	<0.0001	0.9969	1
CONT T0 - S+L T2	<0.0001	<0.0001	<0.0001	0.8711	<0.0001	<0.0001
CONT T0 - SOMB T2	<0.0001	0.0023	<0.0001	0.3251	<0.0001	<0.0001
CONT T0 - CONT T3	1	0.2147	1	<0.0001	0.9634	1
CONT T0 - SERA T3	1	0.2464	1	<0.0001	0.9167	1
CONT T0 - S+L T3	<0.0001	0.2646	<0.0001	0.0017	<0.0001	<0.0001
CONT T0 - SOMB T3	<0.0001	0.019	<0.0001	0.1555	<0.0001	<0.0001
SERA T0 - S+L T0	1	1	1	1	1	1
SERA T0 - SOMB T0	1	1	1	1	1	1
SERA T0 - CONT T1	1	0.0055	1	<0.0001	0.9985	1
SERA T0 - SERA T1	1	0.0086	1	<0.0001	0.9345	1
SERA T0 - S+L T1	0.0147	1	0.3137	<0.0001	<0.0001	0.0003
SERA T0 - SOMB T1	0.0005	0.9999	0.0176	<0.0001	<0.0001	<0.0001
SERA T0 - CONT T2	1	0.0192	1	<0.0001	0.979	1

	Clorofila *a*	**Clorofila *b***	**Clorofilas totais**	**Razão clorofila *a*:*b***	**Carotenoides totais**	**AFE**
SERA T0 - SERA T2	1	0.0189	1	<0.0001	0.9877	1
SERA T0 - S+L T2	<0.0001	0.0017	<0.0001	0.0018	<0.0001	<0.0001
SERA T0 - SOMB T2	<0.0001	0.0617	<0.0001	0.0001	<0.0001	<0.0001
SERA T0 - CONT T3	1	0.0135	1	<0.0001	0.9149	1
SERA T0 - SERA T3	1	0.0167	1	<0.0001	0.8387	1
SERA T0 - S+L T3	<0.0001	0.8886	<0.0001	<0.0001	<0.0001	<0.0001
SERA T0 - SOMB T3	<0.0001	0.267	<0.0001	<0.0001	<0.0001	<0.0001
S+L T0 - SOMB T0	1	0.9993	1	1	1	1
S+L T0 - CONT T1	1	0.0005	1	<0.0001	0.9799	0.9965
S+L T0 - SERA T1	1	0.0009	1	<0.0001	0.7807	1
S+L T0 - S+L T1	0.0104	0.9988	0.3707	<0.0001	<0.0001	<0.0001
S+L T0 - SOMB T1	0.0003	1	0.0237	<0.0001	<0.0001	<0.0001
S+L T0 - CONT T2	1	0.0022	1	<0.0001	0.8923	1
S+L T0 - SERA T2	1	0.0022	1	<0.0001	0.9233	1
S+L T0 - S+L T2	<0.0001	0.015	<0.0001	0.0001	<0.0001	<0.0001
S+L T0 - SOMB T2	<0.0001	0.2704	<0.0001	<0.0001	<0.0001	<0.0001
S+L T0 - CONT T3	1	0.0015	1	<0.0001	0.7426	0.999
S+L T0 - SERA T3	1	0.0019	1	<0.0001	0.621	0.9997
S+L T0 - S+L T3	<0.0001	0.9965	0	<0.0001	<0.0001	<0.0001
S+L T0 - SOMB T3	<0.0001	0.6746	<0.0001	<0.0001	<0.0001	<0.0001
SOMB T0 - CONT T1	1	0.0304	0.9901	<0.0001	0.9934	0.9968
SOMB T0 - SERA T1	1	0.0446	1	<0.0001	0.8692	1
SOMB T0 - S+L T1	0.0055	1	0.1453	<0.0001	<0.0001	<0.0001
SOMB T0 - SOMB T1	0.0002	0.9879	0.0051	<0.0001	<0.0001	<0.0001
SOMB T0 - CONT T2	1	0.0873	1	<0.0001	0.9467	1
SOMB T0 - SERA T2	1	0.0862	1	<0.0001	0.9652	1
SOMB T0 - S+L T2	<0.0001	0.0002	<0.0001	0.0081	<0.0001	<0.0001
SOMB T0 - SOMB T2	<0.0001	0.0127	<0.0001	0.0003	<0.0001	<0.0001
SOMB T0 - CONT T3	1	0.065	0.9999	<0.0001	0.8395	0.9991
SOMB T0 - SERA T3	1	0.0778	0.9999	<0.0001	0.736	0.9997
SOMB T0 - S+L T3	<0.0001	0.5798	<0.0001	<0.0001	<0.0001	<0.0001

	Clorofila *a*	**Clorofila *b***	**Clorofilas totais**	**Razão clorofila *a*:*b***	**Carotenoides totais**	**AFE**
SOMB T0 - SOMB T3	<0.0001	0.0796	<0.0001	0.0001	<0.0001	<0.0001
CONT T1 - SERA T1	1	1	1	1	1	1
CONT T1 - S+L T1	0.0008	0.036	0.0014	1	0.0038	0.0066
CONT T1 - SOMB T1	<0.0001	0.0001	<0.0001	0.6168	0.0005	0.0005
CONT T1 - CONT T2	1	1	1	1	1	1
CONT T1 - SERA T2	1	1	1	1	1	1
CONT T1 - S+L T2	<0.0001	<0.0001	<0.0001	0.0047	<0.0001	<0.0001
CONT T1 - SOMB T2	<0.0001	<0.0001	<0.0001	0.0704	<0.0001	<0.0001
CONT T1 - CONT T3	1	1	1	1	1	1
CONT T1 - SERA T3	1	1	1	1	1	1
CONT T1 - S+L T3	<0.0001	<0.0001	<0.0001	0.9596	<0.0001	<0.0001
CONT T1 - SOMB T3	<0.0001	<0.0001	<0.0001	0.171	<0.0001	<0.0001
SERA T1 - S+L T1	0.002	0.0523	0.0034	1	0.0272	0.0013
SERA T1 - SOMB T1	0.0001	0.0002	<0.0001	0.5412	0.0044	0.0001
SERA T1 - CONT T2	1	1	1	1	1	1
SERA T1 - SERA T2	1	1	1	1	1	1
SERA T1 - S+L T2	<0.0001	<0.0001	<0.0001	0.0032	<0.0001	<0.0001
SERA T1 - SOMB T2	<0.0001	<0.0001	<0.0001	0.0523	<0.0001	<0.0001
SERA T1 - CONT T3	1	1	1	1	1	1
SERA T1 - SERA T3	1	1	1	1	1	1
SERA T1 - S+L T3	<0.0001	<0.0001	<0.0001	0.9338	<0.0001	<0.0001
SERA T1 - SOMB T3	<0.0001	<0.0001	<0.0001	0.1332	<0.0001	<0.0001
S+L T1 - SOMB T1	1	0.9829	1	0.8865	1	1
S+L T1 - CONT T2	0.0022	0.1008	0.0045	1	0.0132	0.0012
S+L T1 - SERA T2	0.0042	0.0995	0.0072	1	0.0099	0.0009
S+L T1 - S+L T2	0.0284	0.0002	0	0.022	0.1785	0.0085
S+L T1 - SOMB T2	0.1726	0.0105	0.0681	0.2161	0.8064	0.0649
S+L T1 - CONT T3	0.0067	0.0756	0.0092	0.9995	0.0329	0.004
S+L T1 - SERA T3	0.0062	0.09	0.0091	1	0.0558	0.0026
S+L T1 - S+L T3	0.4651	0.5406	0.4314	0.9982	0.2834	0.0001
S+L T1 - SOMB T3	0.4103	0.0686	0.2311	0.4186	0.7178	0.01

	Clorofila *a*	**Clorofila *b***	**Clorofilas totais**	**Razão clorofila *a*:*b***	**Carotenoides totais**	**AFE**
SOMB T1 - CONT T2	0.0001	0.0006	0.0001	0.9242	0.0019	0.0001
SOMB T1 - SERA T2	0.0001	0.0006	0.0001	0.3098	0.0014	<0.0001
SOMB T1 - S+L T2	0.2835	0.0443	0.1484	0.8807	0.4905	0.0731
SOMB T1 - SOMB T2	0.73	0.4903	0.6184	1	0.9809	0.3247
SOMB T1 - CONT T3	0.0002	0.0004	0.0001	0.2095	0.0055	0.0003
SOMB T1 - SERA T3	0.0002	0.0005	0.0001	0.3403	0.0102	0.0002
SOMB T1 - S+L T3	0.9573	0.9999	0.9817	1	0.6467	0.0018
SOMB T1 - SOMB T3	0.9378	0.8747	0.9044	1	0.9578	0.0828
CONT T2 - SERA T2	1	1	1	0.9998	1	1
CONT T2 - S+L T2	<0.0001	<0.0001	<0.0001	0.0304	<0.0001	<0.0001
CONT T2 - SOMB T2	<0.0001	<0.0001	<0.0001	0.2678	<0.0001	<0.0001
CONT T2 - CONT T3	1	1	1	0.9985	1	1
CONT T2 - SERA T3	1	1	1	0.9999	1	1
CONT T2 - S+L T3	<0.0001	<0.0001	<0.0001	0.9994	<0.0001	<0.0001
CONT T2 - SOMB T3	<0.0001	<0.0001	<0.0001	0.4901	<0.0001	<0.0001
SERA T2 - S+L T2	<0.0001	<0.0001	<0.0001	0.0008	<0.0001	<0.0001
SERA T2 - SOMB T2	<0.0001	<0.0001	<0.0001	0.0181	<0.0001	<0.0001
SERA T2 - CONT T3	1	1	1	1	1	1
SERA T2 - SERA T3	1	1	1	1	1	1
SERA T2 - S+L T3	<0.0001	<0.0001	<0.0001	0.7789	<0.0001	<0.0001
SERA T2 - SOMB T3	<0.0001	<0.0001	<0.0001	0.0529	<0.0001	<0.0001
S+L T2 - SOMB T2	1	0.9996	1	1	0.9998	1
S+L T2 - CONT T3	<0.0001	<0.0001	<0.0001	0.0004	<0.0001	<0.0001
S+L T2 - SERA T3	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001
S+L T2 - S+L T3	0.9988	0.4101	0.9676	0.4363	1	0.9994
S+L T2 - SOMB T3	0.9995	0.9598	1	0.9986	1	1
SOMB T2 - CONT T3	<0.0001	<0.0001	<0.0001	0.0095	<0.0001	<0.0001
SOMB T2 - SERA T3	<0.0001	<0.0001	<0.0001	0.0213	<0.0001	<0.0001
SOMB T2 - S+L T3	1	0.9742	1	0.9342	1	0.9389
SOMB T2 - SOMB T3	1	1	1	1	1	1
CONT T3 - SERA T3	1	1	1	1	1	1

	Clorofila *a*	**Clorofila *b***	**Clorofilas totais**	**Razão clorofila *a*:*b***	**Carotenoides totais**	**AFE**
CONT T3 - S+L T3	<0.0001	<0.0001	<0.0001	0.6528	<0.0001	<0.0001
CONT T3 - SOMB T3	<0.0001	<0.0001	<0.0001	0.0298	<0.0001	<0.0001
SERA T3 - S+L T3	<0.0001	<0.0001	<0.0001	0.8082	<0.0001	<0.0001
SERA T3 - SOMB T3	<0.0001	<0.0001	<0.0001	0.0611	<0.0001	<0.0001
S+L T3 - SOMB T3	1	0.9999	1	0.9906	1	0.999

Tabela suplementar 2 – Valores de significância dos contrastes *post-hoc* para a interação entre tratamentos (CONT, SERA, SOMB e S+L) e tempo (T1, T2 e T3) para a eficiência quântica da fotossíntese (α) de espécies herbáceo-arbustivas de Cerrado. T0: antes da implantação dos tratamentos; T1: seis meses; T2: um ano e seis meses; T3: dois anos e seis meses sob efeito dos tratamentos; CONT: controle; SERA: serapilheira; SOMB: sombreamento, S+L: sombreamento mais serapilheira.

	α
CONT T1 - SERA T1	0.9804
CONT T1 - S+L T1	0.9998
CONT T1 - SOMB T1	0.0006
CONT T1 - CONT T2	0.3612
CONT T1 - SERA T2	0.5477
CONT T1 - S+L T2	<0.0001
CONT T1 - SOMB T2	<0.0001
CONT T1 - CONT T3	1
CONT T1 - SERA T3	1
CONT T1 - S+L T3	0.0143
CONT T1 - SOMB T3	0.0001
SERA T1 - S+L T1	1
SERA T1 - SOMB T1	0.0473
SERA T1 - CONT T2	0.9873
SERA T1 - SERA T2	0.9986

	α
SERA T1 - S+L T2	<0.0001
SERA T1 - SOMB T2	<0.0001
SERA T1 - CONT T3	0.9966
SERA T1 - SERA T3	0.9995
SERA T1 - S+L T3	0.3675
SERA T1 - SOMB T3	0.0111
S+L T1 - SOMB T1	0.0088
S+L T1 - CONT T2	0.8431
S+L T1 - SERA T2	0.9458
S+L T1 - S+L T2	<0.0001
S+L T1 - SOMB T2	<0.0001
S+L T1 - CONT T3	1
S+L T1 - SERA T3	1
S+L T1 - S+L T3	0.1194
S+L T1 - SOMB T3	0.0017
SOMB T1 - CONT T2	0.5877
SOMB T1 - SERA T2	0.3971
SOMB T1 - S+L T2	0.6892
SOMB T1 - SOMB T2	0.2213
SOMB T1 - CONT T3	0.0015
SOMB T1 - SERA T3	0.0031
SOMB T1 - S+L T3	0.9991
SOMB T1 - SOMB T3	1
CONT T2 - SERA T2	1
CONT T2 - S+L T2	0.0036
CONT T2 - SOMB T2	0.0002
CONT T2 - CONT T3	0.526
CONT T2 - SERA T3	0.6635
CONT T2 - S+L T3	0.9816
CONT T2 - SOMB T3	0.2818
SERA T2 - S+L T2	0.0013
SERA T2 - SOMB T2	0.0001

	α
SERA T2 - CONT T3	0.717
SERA T2 - SERA T3	0.8321
SERA T2 - S+L T3	0.9251
SERA T2 - SOMB T3	0.1571
S+L T2 - SOMB T2	0.9999
S+L T2 - CONT T3	<0.0001
S+L T2 - SERA T3	<0.0001
S+L T2 - S+L T3	0.1592
S+L T2 - SOMB T3	0.927
SOMB T2 - CONT T3	<0.0001
SOMB T2 - SERA T3	<0.0001
SOMB T2 - S+L T3	0.0213
SOMB T2 - SOMB T3	0.5027
CONT T3 - SERA T3	1
CONT T3 - S+L T3	0.0308
CONT T3 - SOMB T3	0.0002
SERA T3 - S+L T3	0.0545
SERA T3 - SOMB T3	0.0005
S+L T3 - SOMB T3	0.9696

CAPÍTULO 3

Woody plant encroachment constrains seed germination of ground layer species in a Neotropical savanna

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Abstract Woody plants have been encroaching into savannas on a global scale; particularly, many formerly open physiognomies of the Brazilian savanna have become encroached savannas over the course of a few decades. In this scenario, many typical savanna species are disappearing, possibly due to environmental filtering changes in these new sites of encroachment. Consequently, less incident light with lower red:far-red wavelength ratios, a reduced range of soil surface temperature, and greater litter deposition may hinder the regenerative capacity of typical savanna species. This study investigated the role of encroachment-induced environmental changes as filters for the recruitment ability of ground-layer savanna species. We examined the germination and seedling emergence of 12 species under controlled conditions, simulating natural aspects of encroached and unencroached sites of the Brazilian savanna regarding to temperature alternation, red:far-red ratio and litter presence. Based on our findings, the germination and/or seedling emergence of all the species were negatively affected by the simulated environmental filtering changes, wherein greater litter deposition produced the most widespread effect. Filtering changes caused by woody encroachment represent a bottleneck for the recruitment of ground-layer savanna species. This may be one of the causes for the disappearance of these species in sites undergoing encroachment and ultimately a facilitator for woody encroachment.

Keywords: crowding; environmental filtering; light requirements; shading; temperature requirements.

Introduction

Woody biomass and cover are increasing significantly in savannas and grasslands around the world, a phenomenon known as woody plant encroachment (ARCHER et al., 2017). Global climate changes and alterations in local conditions and resources as well as the disturbance regimes are considered to be the main drivers of this process (ARCHER et al., 2017; GARCIA-CRIADO et al., 2020; STEVENS et al., 2017; ROSAN et al. 2019). Among the world's savannas, woody encroachment is occurring more rapidly in Neotropical savannas, specifically in several grassland and savanna areas of the Cerrado (Brazilian savanna), where open areas have developed a forest physiognomy and structure in a very short time period (ABREU et al., 2017; DURIGAN; RATTER, 2006; STEVENS et al., 2017). Several environmental factors have been modified in these encroached savanna sites, compared to typical savanna sites: significantly less radiation reaches the understory; changes in the spectral composition of light; a more narrow range of understory temperatures; and increases in soil nutrients, humidity, and litter deposition (HOFFMANN et al., 2012a; PAIVA; SILVA; HARIDASAN, et al. 2015; PINHEIRO; KOLB; ROSSATTO, 2016; ROSSATTO; RIGOBELO, 2016; SALAZAR et al., 2012).

These shifts induced by woody encroachment seem to act as filters to the persistence of typical savanna species and may affect their survival since they may alter both resource availability (CARLOS; ROSSATTO, 2017; ROSSATTO et al., 2018) and aspects related to regenerative ability (ABREU et al., 2017; HOFFMANN; ORTHEN; FRANCO, 2004). As for the regeneration of savanna species, there are different regeneration strategies that might involve seed germination, vegetative spread, and/or resprouting ability (PILON et al., 2020; ZUPO et al., 2020). Particularly dependent on environmental filtering, seed germination and post-germination development traits can influence community assembly and contribute to population growth and species persistence or even disappearance in changing environments (DONOHUE et al., 2010; JIMÉNEZ-ALFARO et al., 2016; KEDDY, 1992). Based on the regeneration niche hypothesis, plant species have to fulfill a set of requirements so that a new individual may successfully replace another (GRUBB, 1977). Changes in environmental filtering triggered by woody encroachment in a encroached savanna may provide an inadequate environment for the regeneration of ground-layer savanna species.

Thus, shading caused by an increase in canopy closure may be an aspect that can hinder germination of savanna species in encroached environments (PINHEIRO; KOLB; ROSSATTO, 2016), since typical ground-layer savanna species are adapted to thrive under high radiation levels (ROSSATTO et al., 2018), and most present positive photoblasty

(MARQUES et al., 2014; ZAIDAN; CARREIRA, 2008). Additionally, canopy-filtered light as a result of encroachment reduces the availability of light and the red:far-red wavelengths ratio (SALAZAR et al., 2012). Both red and far-red light frequencies are involved in the photoconversion of phytochromes, wherein low red:far-red ratios promote phytochrome conversion in its inactive form, which inhibits germination in many species (CASAL; SÁNCHEZ, 1998; SMITH, 2000).

Temperatures and their alternation are also important factors in seed germination for many herbaceous savanna species, where regimes with a wide range of alternating temperatures may stimulate germination (DAIREL; FIDELIS, 2020; MUSSO et al., 2015; ZAIDAN; CARREIRA, 2008). In encroached sites, soil surface temperature can become more stable due to a greater canopy closure and a thicker litter layer covering the soil surface, which may inhibit germination in dormant species that require temperature fluctuations to break dormancy (DAIREL; FIDELIS, 2020; KOLB; PILON; DURIGAN, 2016). It is important to note that seeds can experience different levels of litter cover as litterfall is highly seasonal; thus, seeds located further below in the litter layer may also undergo shading (COSTA et al., 2020; SALAZAR et al., 2012). Aside from these effects on soil temperature and light availability, plant litter can act as a mechanical barrier for seedling emergence by constraining root or shoot growth based on the seed's location in the litter layer (FACELLI; PICKETT, 1991).

All of the abovementioned effects are likely to constrain the regeneration of typical savanna species and may be especially relevant to the herbaceous-shrub component. Herbaceous-shrub species make up most of the plant diversity of the Cerrado; specifically, for each tree species, there are about six to seven species that are not trees (MENDONÇA et al., 2008; ZAPPI et al., 2015). Moreover, herbaceous-shrub species are crucial to the savanna's ecology: the biomass of graminoid species is responsible for vegetation flammability, and fires are essential to maintaining the savanna's physiognomy and preventing woody encroachment (HOFFMANN et al., 2012a; b).

This paper sheds light on how environmental aspects of woody encroachment constrain the seed regeneration of herbaceous-shrub species of the Cerrado. We experimentally examined the effect of temperature alternation and red:far-red light ratio on the germination of 12 typical ground-layer savanna species. We also observed the litter layer's effect on seedling emergence. These experiments simulated the effects of woody encroachment on micro-environmental changes were based on field measurements and information gathered from the literature. We hypothesized that typical ground-layer savanna species would negatively respond to experimental treatments simulating encroached environmental conditions with a) low germination levels due to the incidence of light with low R:FR ratios and a narrow-ranging temperature regime, and b) low seedling emergence due to the litter layer's mechanical effects.

Methods

Selected species and area of study

We collected diaspores, hereinafter referred to as seeds, from at least 10 individuals of 12 typical herbaceous-shrub species of the savanna. We preferably chose species with high seed production, germination potential, and seed viability (Table 1). These species are commonly found in a typical savanna environment, but some can thrive under encroached conditions (PINHEIRO; KOLB; ROSSATTO, 2016). Samples of 100 seeds per species were soaked in 1% tetrazolium solution to assess seed viability (adapted from LAKON, 1949). The seeds were sorted: empty seeds were discarded (mainly among the grass species), and seeds with impermeable coats were mechanically scarified before the tetrazolium test and for use in the other experiments.

The seeds were collected from typical savanna areas at two ecological reserves located in southeastern Brazil: Assis Ecological Station (AES; 22° 33' 20" S, 50° 21' 27" W) and Santa Barbara Ecological Station (SBES; 22° 48' 59" S, 49° 14' 12" W). According to the Köppen classification, the climate in both reserves is classified as Cfa (ÁLVARES et al., 2013). Rainfall presents seasonality with a dry season between May and September and a wet season between October and April. Average rainfall is around 1,100 to 1,400 mm. The soil is predominantly dystrophic and has a high aluminum content; thus, it is classified as an oxisol (ABREU et al., 2017; MEIRA-NETO; MARTINS; VALENTE, 2007; JUHÁSZ et al., 2006).

Measurement of environmental variables

To determine the experimental treatments, we measured the range of the soil temperature regimes and litter layer thickness and its dry mass. We measured litter layer thickness and dry mass in 30 plots (1 x 1 m) haphazardly distributed across the encroached savanna of AES near the peak of the rainy season in December 2016. The minimum distance between plots was 1 km. Plant litter collected from each plot was dried in an oven at 70°C for three days and then weighed. Minimum and maximum soil surface temperatures (or under the litter layer, when present) were monitored once a month over the rainy season of 2017-2018 in 10 plots of typical savanna and in 10 plots of encroached savanna with an infrared thermometer. The rainy season was the time of year when most of the species in the study presented mature seeds (Table 1). The minimum soil temperatures were 19.36 ± 2.87°C in a typical savanna and 19.18 ± 2.26°C in a encroached savanna. The maximum temperatures were 30.86 ± 1.64°C in a typical savanna and 23.60 ± 0.71°C in a encroached savanna. The litter layer of a encroached savanna had a mean thickness of 3.93 ± 1.42 cm and a mean dry mass of 1.63 ± 0.49 Kg m^{-2}.

Table 1 – Ground-layer savanna species selected for seed collection and their seed viability. Growth form according to Durigan et al. (2004; 2018). Abbreviations: AES = Assis Ecological Station; SBES = Santa Barbara Ecological Station.

Species	**Family**	**Growth form**	**Locality and date of collection**	**Viable seeds (%)**
Andropogon leucostachyus Kunth	Poaceae	graminoid	AES; Feb/2019	98
Bidens gardneri Baker	Asteraceae	forb	AES; Mar/2018	99
Chamaecrista flexuosa (L.) Greene	Fabaceae	subshrub	AES; Mar/2018	96
Elephantopus biflorus (Less.) Sch.Bip.	Asteraceae	forb	SBES; Mar/2019	96
Eryngium juncifolium (Urb.) Mathias & Constance	Apiaceae	forb	SBES; Feb/2019	84
Eustachys distichophylla (Lag.) Nees	Poaceae	graminoid	SBES; Jan/2019	97
Gymneia interrupta (Pohl ex Benth.) Harley & J.F.B.Pastore	Lamiaceae	forb	SBES; Jan/2019	89
Gymnopogon foliosus (Willd.) Nees	Poaceae	graminoid	AES; Sep/2019	88
Hyptis campestris Harley & J.F.B. Pastore	Lamiaceae	forb	AES; Mar/2018	99
Miconia fallax DC.	Melastomataceae	shrub	AES; Jan/2019	48
Mimosa xanthocentra Mart.	Fabaceae	subshrub	AES; Mar/2018	99
Orthopappus angustifolius (Sw.) Gleason	Asteraceae	forb	AES; Feb/2019	98

Germination experiments – temperature and light

Based on the soil surface temperatures found, we established the experimental temperature regimes at 23-20°C for encroached savanna and 30-20°C for typical savanna conditions. Assays were performed in germination chambers (20W fluorescent lamps, around 60 μmol m^{-2} s^{-1}) in photoperiods of 12 hours of light/12 hours of darkness and in continuous darkness.

A second germination experiment was performed with seeds exposed to light with low R:FR ratios (0.1 and 0.5), simulating canopy-filtered light, and high R:FR ratios (7.2, i.e., no red light limitation). Low R:FR ratio treatments were conducted under incandescent lamps in a room with controlled temperatures; high R:FR ratio treatments were performed in germination chambers under fluorescent lamps. Both room and chamber temperatures were maintained at 30-20°C (based on field measurements) for a photoperiod of 12 hours of light/12 hours of darkness. To produce low R:FR ratios for the seed, germination boxes were wrapped with aluminum foil, and their covers were wrapped with plastic film (insulfilm®) (RIBEIRO; KOLB, 2016; SIMÃO; NAKAMURA; TAKAKI, 2008). We used the LI-1800 spectroradiometer (LI-COR U.S.A) to obtain the R:FR ratios.

For each species and both germination experiments, four replicates of 25 seeds were used in each treatment. Each replicate consisted of a germination box wherein seeds were placed on two layers of moistened filter paper. Each replicate was allocated to a different germination chamber to ensure replicate independence. Assays lasted until all treatments reached a stability of 15 days without any new germination. Germination was checked on a daily basis, where the criterion for seed germination was the appearance of the radicle. Germination was verified under a green light to maintain continuous darkness and low R:FR ratio treatments (all of the species had been previously tested, and green light did not affect final germination at the end of 30 days).

Seedling emergence – litter layer effect

We conducted a greenhouse experiment to evaluate how the litter layer affects seedling emergence; the experimental litter layer treatments were based on field conditions. Ten centimeters of Cerrado soil were deposited into transparent plastic recipients to ensure good root development (recipient height: 32 cm). Then, a litter layer of encroached savanna with a thickness of 4 cm was added on top of the soil. Two litter treatments were arranged: one with seeds on top of the litter layer and the other with seeds under the litter layer. For the seeds placed under the plant litter, the sides of the recipients were covered with aluminum foil so that light would not enter through the side. There was a control treatment with seeds placed on the soil surface without litter.

The treatments consisted of four replicates of 25 seeds for each individual species. Seedling emergence was verified every day, and the experiment was sprayed with water twice a day to prevent seed displacement. Holes had been drilled into the bottom of the plastic recipients previously to ensure the drainage of excess water. Due to differences in the seedling growth rate, the experimental period spanned between 30 and 90 days based on the tested species. The duration of the experiment was set for when all the treatments reached a stability of 15 days without seedling emergence and when the control seedlings were approximately 6 cm or more in height, ensuring enough time for the seedlings in the litter treatment to develop and eventually go through the litter layer. Seedling emergence constituted the appearance of the shoot (cotyledons, young leaves, or stem) above the substrate (litter or soil).

Statistical analyses

The percentages of germination and emergence were compared between experimental treatments by generalized linear models (GLM) with a binomial error structure and logit link function. Models were generated for each individual species. The experimental treatments were fixed factors, and Tukey's test was used to compare the R:FR ratio (0.1, 0.5, and 7.2) and plant litter treatments (on top, under, and no litter). When a treatment exhibited no germination or emergence, it was removed from the analysis. We adopted a significance level of 5% ($\alpha = 0.05$). The R 3.6.1 software, with the lme4, multcompView, and lsmeans packages, was used to perform all of the analyses, and the SigmaPlot 10.0 software was used to create the figures.

Results

Effects of temperature and R:FR ratio on seed germination

Seven species were negatively affected by the range of the temperature regime of the encroached savanna: the germination percentages of *A. leucostachyus*, *B. gardneri*, and *H. campestris* decreased regardless of light conditions ($P < 0.001$), while the germination percentages of *E. juncifolium*, *E. distichophylla*, *G. foliosus*, and *M. fallax* decreased only under continuous darkness ($P < 0.05$; Figure 1).

Light with low R:FR ratios had an even greater effect on species germination than the range of the temperature regime. The germination percentages of eight species (*A. leucostachyus, B. gardneri, E. biflorus, E. distichophylla, G. foliosus, H. campestris, M.*

fallax, and *O. angustifolius*) severely decreased with the R:FR ratio of 0.1 (P < 0.0001). Five of these species (*A. leucostachyus*, *B. gardneri*, *E. biflorus*, *G. foliosus*, and *O. angustifolius*) were even more sensitive as their germination percentages also decreased with the ratio of 0.5 in relation to the R:FR ratio of 7.2 (P < 0.05). The species *E. juncifolium* was the exception, with a germinability that enhanced with the ratio of 0.5 in relation to the R:FR ratio of 7.2 (P = 0.004; Figure 2).

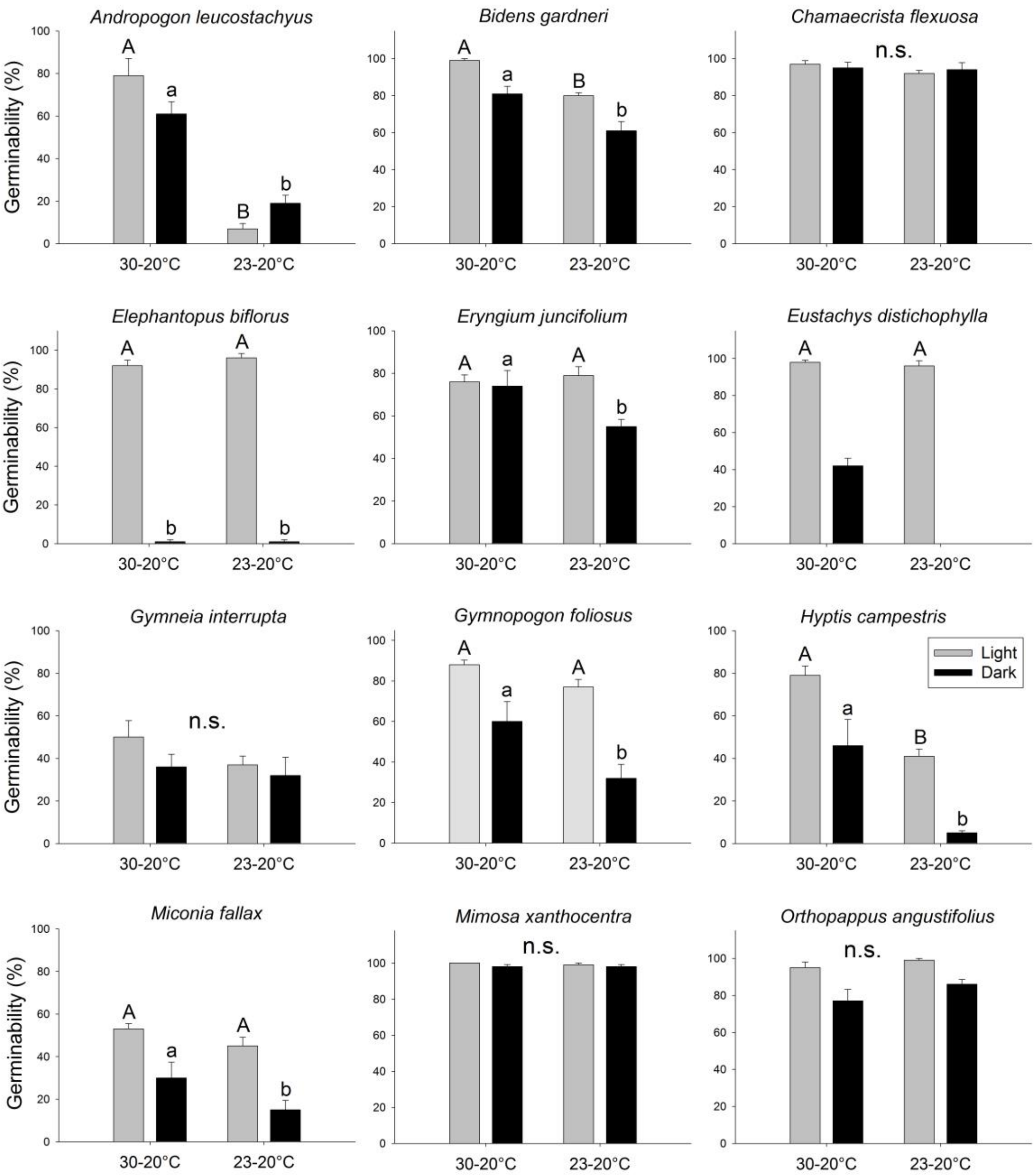

Figure 1 – Germination of ground-layer savanna species (mean ± standard error) under field-based temperature regimes of typical (30-20°C) and encroached savanna (23-20°C) conditions. Different upper- and lowercase letters represent significant differences ($P < 0.05$) between temperature regimes in a photoperiod of 12h light/12h dark (gray bars) and in continuous darkness (black bars), respectively, according to GLMs. Abbreviation: n.s. = no statistical differences.

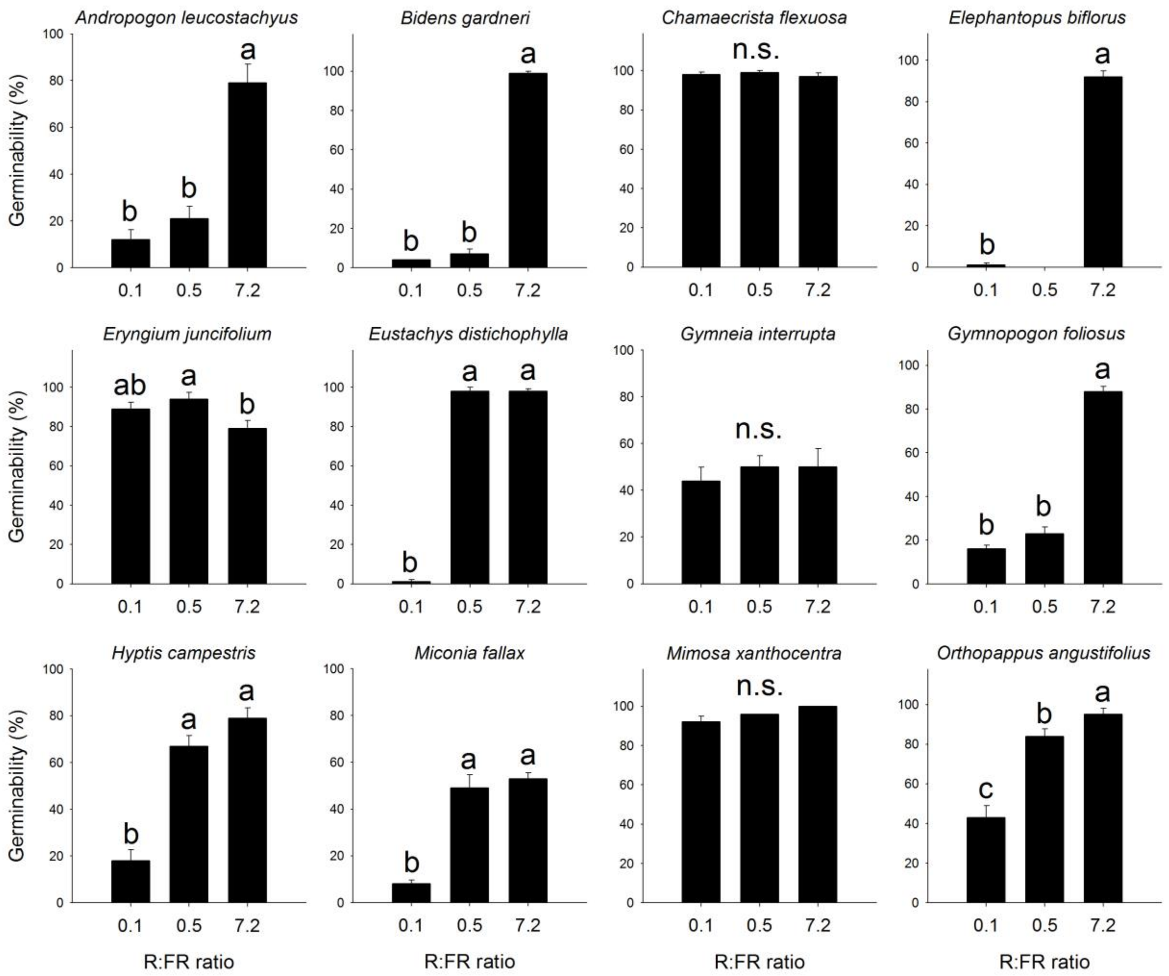

Figure 2 – The effect of red:far-red (R:FR) light composition on the germination of ground-layer savanna species (mean ± standard error). R:FR ratios of 0.1 and 0.5 represent canopy-filtered light, and 7.2 represents no red light limitation. Different letters represent significant differences ($P < 0.05$) between R:FR ratios according to adjusted Tukey post-hoc test. Abbreviation: n.s. = no statistical differences.

Litter layer's effect on seedling emergence

The species did not exhibit seedling emergence when their seeds were placed under the litter layer, except for *A. leucostachyus* and *B. gardneri*, which were capable of some emergence, but it was still much lower than their emergence in the control treatment ($P < 0.01$). As for the treatment where seeds were placed on top of the litter layer, only *E. distichophylla* was able to maintain the same seedling emergence as in the control treatment, but a high mortality was observed at the end of the experiment (88% compared to 36% found in the control treatment). The species *A. leucostachyus*, *E. biflorus*, *E. juncifolium*, *G. foliosus*, and *O. angustifolius* presented a minor decrease in seedling emergence (43% to 70%

less than the control; $P < 0.05$) compared to the other species (> 80% in relation to the control; $P < 0.01$) (Figure 3).

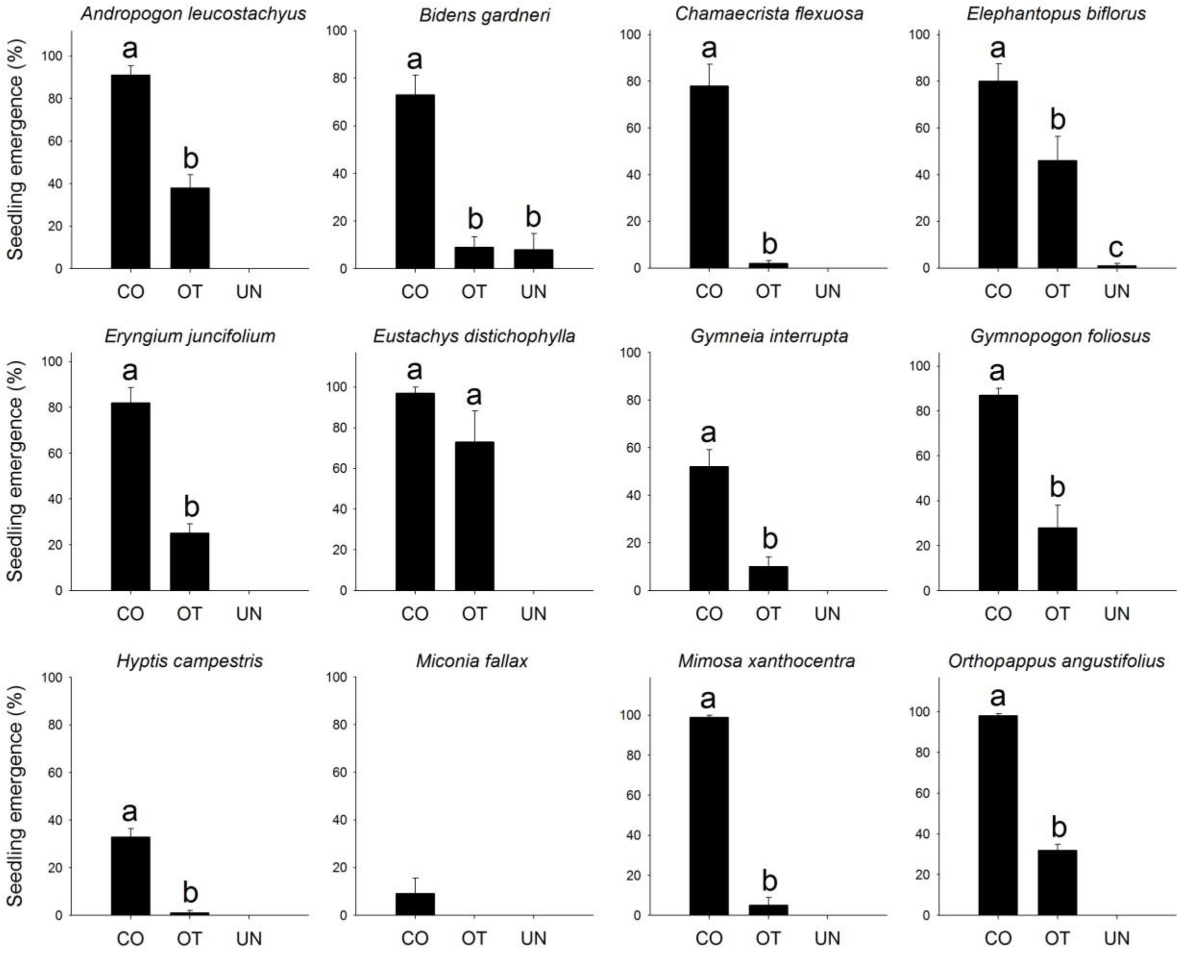

Figure 3 – Plant litter's effect on seedling emergence of ground-layer savanna species (mean ± standard error). Different letters represent statistically significant differences ($P < 0.05$) between litter treatments according to adjusted Tukey post-hoc test. Abbreviations: CO = control treatment without litter; ON = seeds placed on top of the litter layer; UN = seeds placed under the litter layer.

Discussion

We demonstrate that woody plant encroachment and its subsequent alterations on environmental aspects may constrain the seed germination and seedling establishment of ground-layer savanna species. As expected, the simulated encroached savanna conditions (narrower temperature regime, low R:FR ratios, and litter layer presence) negatively affected the seed germination and seedling emergence of these species. More precisely, half of the species in the study were negatively affected at least to some degree by each of the three environmental filtering changes tested in the experiment (*A. leucostachyus*, *B. gardneri*, *E. distichophylla*, *G. foliosus*, *H. campestris*, and *M. fallax*). The presence of a litter layer was the factor with most widespread effect since seedling emergence had decreased for all of the species under this condition. Meanwhile, the decrease in the R:RF ratio and the more narrow temperature regime negatively affected the seed germination of 67% and 58% of the species, respectively.

Plant litter's role in seedling emergence

The combination of factors such as the absence of light, a more narrow temperature range, and mechanical effects may explain why there was a significant decrease in seedling emergence or no emergence at all for most of the species when the seeds were subjected to litter cover, at least when they were placed under the litter (FACELLI; PICKETT, 1991; VÁZQUEZ-YANES; OROZCO-SEGOVIA, 1993). This explanation is corroborated by the results from the germination experiments, which showed significant decreases in germination in a narrow-ranging temperature regime and in the absence of light, thus indicating a cumulative effect of these factors with the mechanical effect of litter. However, no seedling emergence was observed for three species (*C. flexuosa*, *G. interrupta*, and *M. xanthocentra*) when their seeds were under litter cover, but emergence did not decrease in a more narrow temperature regime or in the absence of light, which demonstrates that mechanical aspects of litter cover may be the most important factor in these cases. For seeds placed on top of the litter layer, it may act as a mechanical barrier for root penetration and consequently reduce seedling emergence. Furthermore, litter can act as an inadequate substrate for germination and initial seedling growth. Some studies reported that litter decomposition and leaching may release phytotoxic compounds which inhibit germination and/or seedling development (BONANOMY et al., 2011; FACELLI; PICKETT, 1991).

There is little information about the litter layer's effects on the regeneration of typical savanna species. Most studies are focused on tree species regeneration (HOFFMANN; ORTHEN; FRANCO, 2004; GEIGER et al., 2011). For ground layer species, plant litter's role in regeneration remains practically unknown. Here, we observed that the effects of litter on the seedling emergence of ground-layer species were even more comprehensive than those reported for woody plant species (SALAZAR et al., 2012). Non-woody plants tend to have smaller seeds (DÍAZ et al., 2016), which implies less energy storage for survival in unfavorable conditions and less vigorous growth; therefore, the presence of litter may hamper the seedling emergence of these species (VÁZQUEZ-YANES; OROZCO-SEGOVIA, 1993).

Moreover, seedling emergence constrained by the litter layer in this study highlights the role of vegetation gaps for seed recruitment in a typical savanna environment. Although there is much less litter in typical savanna areas than in encroached savanna, much of the ground in typical savanna physiognomies is covered by plant litter and by an almost continuous layer of herbaceous grass (COSTA et al., 2020; RIBEIRO; WALTER, 2008). Fire serves to remove litter and consume the layer of herbaceous and grasses, which favors the appearance of bare soil patches (FIDELIS et al., 2012; PILON et al., 2020). There is evidence that these gaps provide safety for seed survival during fire events and that they can facilitate dormancy breaking in some species by the increase in temperature oscillation (DAIBES et al., 2017; 2018; DAIREL; FIDELIS 2020).

Light, temperature, and seed germination

As mentioned before, a considerable number of species was negatively affected by the light conditions of the encroached savanna. Understory R:FR ratios are spatially and temporally variable in forest environments; values between 0.4 and 0.5 are frequently found, but R:FR ratios can even reach minimums of 0.1 (CAPERS; CHAZDON, 2004; GOLDMAN; FATTORI; JANUÁRIO, 1989). Specifically, Salazar et al. (2012) found a R:FR average near 0.4 in the understory of a encroached savanna. In this sense, five species in this study surely would have difficulty in germinating and establishing themselves in a shady encroached savanna environment, given their low germination in the 0.5 R:FR ratio treatment. The more narrow temperature regime of the encroached savanna also hampered the germination of a large number of species, corroborating other studies with ground-layer savanna species that benefited from a wide-ranging temperature regime (DAIREL; FIDELIS, 2020; KOLB; PILON; DURIGAN, 2016; MUSSO et al., 2015). A curious finding is that a larger number of species was negatively affected by the more narrow temperature regime in constant darkness than in a light-dark photoperiod – seven against three species – suggesting a cumulative effect between temperature and light availability (VÁZQUEZ-YANES; OROZCO-SEGOVIA, 1993).

Perspectives for savanna management and conservation

The encroachment-induced environmental effects on seed regeneration ability indicate some degree of recruitment limitation in ground-layer savanna species due to establishment limitation; in other words, the failure in plant recruitment driven by germination and establishment ability and the availability of niches (GRUBB, 1977; MULLER-LANDAU et al., 2002). Additionally, there is evidence that the recruitment limitation in savanna vegetation undergoing woody encroachment can also arise from seed limitation, as seeds of fire-tolerant savanna species were rarely found in seed rain (MARIANO; REBOLO; CHRISTIANINI, 2019). Seed limitation can be defined as the failure in plant recruitment in all suitable sites driven by limitations in seed production and dispersal (MULLER-LANDAU et al., 2002).

Therefore, we can hypothesize that both seed and establishment limitations in savanna species may play a role in savanna woody encroachment. Fire suppression already has a well-known effect in the forest species colonization of typical savanna areas by preventing the fire trap (HOFFMANN et al., 2012b). However, as encroachment along with environmental filtering changes occur, it becomes more challenging for typical savanna species to disperse and establish themselves in formerly open savanna areas. The rate at with which this is happening cannot be overlooked; Pilon (2019) found that tree and generalist species increased among regenerants while monitoring woody encroachment over a period of four years.

In conclusion, our study shows that the regenerative ability of ground-layer savanna species is constrained by environmental changes caused by woody encroachment. Considering the ground layer, restoration of degraded, old-growth grassy biomes remains a challenging issue, and in advanced forested conditions (BUISSON et al., 2019; STEVENS et al., 2017), conservation efforts that involve fire management and/or the reestablishment of adequate environmental conditions for the regeneration of typical savanna species are highly recommended for mitigating woody encroachment and its negative effects on typical savanna biodiversity.

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CONSIDERAÇÕES FINAIS

Ao testar experimentalmente os efeitos do adensamento lenhoso da savana sobre a comunidade herbáceo-arbustiva de cerrado *sensu stricto*, nós pudemos comprovar empiricamente o quanto este componente da vegetação é sensível frente ao distúrbio que a supressão do fogo representa para as savanas. Em especial, pudemos verificar o sombreamento como principal fator causando o desaparecimento de espécies, ao menos em termos de suas partes aéreas. Já o efeito do adensamento no que diz respeito aos sistemas subterrâneos é uma questão que merece ser melhor explorada em estudos futuros, tendo em vista a importância da estratégia de rebrota via banco de gemas na resiliência das plantas de Cerrado.

O conhecimento gerado por esta tese também nos permite compreender melhor os meandros das perdas das partes aéreas de espécies da comunidade herbáceo-arbustiva com o avanço do processo do adensamento lenhoso. Isso envolve tanto a incapacidade de um ajuste fisiológico de espécies de cerrado *sensu stricto* às condições de sombreamento, como a dificuldade na germinação e, possivelmente, na regeneração frente às alterações ambientais causadas pelo adensamento. Abaixo pontuo alguns dos principais achados deste estudo:

- o sombreamento foi o principal fator afetando negativamente a diversidade e a estrutura da comunidade herbáceo-arbustiva de cerrado *sensu stricto*. Além disso, a riqueza funcional também foi reduzida em condição de sombreamento;

- o sombreamento também foi o principal fator afetando a fisiologia foliar das plantas herbáceo-arbustivas, sendo que estas espécies apresentam limitada capacidade de se ajustar a baixa disponibilidade de luz;

- o adensamento lenhoso também faz com que as condições ambientais fiquem inadequadas ao nicho de germinação de várias espécies de cerrado *sensu stricto*. Em contraposição ao que foi encontrado para a dinâmica e a fisiologia do componente herbáceo-arbustivo, a presença de uma camada mais espessa de serapilheira impactou severamente a emergência de todas as espécies estudadas, possivelmente afetando a capacidade de regeneração destas em áreas adensadas.

É até irônico pensar que a supressão de um distúrbio, no caso o fogo, representa outro distúrbio que, em longo prazo, é deletério para o ecossistema como um todo. Explico: o fogo é amplamente reconhecido como um distúrbio natural para as savanas ao redor do mundo, fazendo parte da ecologia deste ecossistema. Muitas são as formas que as plantas de savana lidam com a influência do fogo e há, inclusive, evidência de que estas estratégias representam adaptações à presença dos regimes de fogo e, deste modo, as plantas de savana teriam evoluído sob a pressão deste distúrbio. Deste modo, a supressão dos regimes de fogo representa um distúrbio - antrópico, neste caso - para essas plantas adaptadas a este fator. Os achados deste trabalho quanto ao estrato herbáceo-arbustivo corroboram esta hipótese, sobretudo quanto ao efeito indireto da ausência de fogo, via adensamento lenhoso.

Considerando a rapidez que o processo de adensamento lenhoso vem acontecendo nas savanas, principalmente na América do Sul, é imprescindível que estratégias de manejo levem em consideração a manutenção ou o restabelecimento das condições ambientais necessárias para o pleno funcionamento do ecossistema, mantendo assim a diversidade de espécies. Assim, o manejo de fogo torna-se imprescindível para as áreas de Cerrado que ainda não passaram por adensamento lenhoso contribuindo, assim, para a manutenção das fisionomias abertas, com alta heterogeneidade ambiental e com as condições microambientais adequadas para a prosperidade da comunidade herbáceo-arbustiva. Por outro lado, estudos ainda são necessários em áreas já adensadas para se compreender a possibilidade de restauração destas áreas tanto em termos de estrutura, como de diversidade e funcionamento ecossistêmico.